ENZYMES OF NUCLEOTIDE METABOLISM: THE SIGNIFICANCE OF SUBUNIT SIZE AND POLYMER SIZE FOR BIOLOGICAL FUNCTION AND REGULATORY PROPERTIES

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I. INTRODUCTION

Historically, the first to perceive the possible importance of subunit structure in protein polymers was A. V. Hill,[1] who proposed in 1910 that the binding of oxygen by hemoglobin reflected different states of polymerization. Additional work led to the proposal of cooperativity arising from interaction between subunits in hemoglobin.[2] With the discovery of cooperativity in the enzyme aspartate carbamoyltransferase (*Escherichia coli*),[3] and subsequent models[4,5] for cooperativity defining allosteric, or conformational, changes as a mechanism for communicating regulatory effects, the importance of, and search for, quaternary structure were steadily expanded. By the early 1970s, a literature survey by Klotz et al.[6] listed more than 500 proteins that existed as polymers, although for the great majority of these it was not known if any regulatory significance accompanied this polymeric state.

In the present survey, I examine enzymes in a central area of metabolism, the synthesis and interconversion of nucleotides, with the purpose of assessing the importance of subunit size, polymer size, and their relation to activity or regulatory properties. Since this area of metabolism is extensive and diverse (72 separate and distinct enzyme activities are included in the present survey), it may reflect a cross section of enzymes in general. Perhaps the analysis obtained from this sampling may then have wider applicability.

Recent reviews in this area of metabolism have focused on the enzymes for *de novo* pyrimidine synthesis,[7] and nucleotide enzymes and nucleotide compartmentation.[8,9] The best source book for detailed data on many enzymes covered here remains Volume 51 of *Methods in Enzymology*.[10]

II. ASSUMPTIONS

A. Selection of Data Set

Enzymes were included if they function in the synthesis, interconversion, or catabolism of nucleotides, and if subunit M_r values were available for any enzyme from at least one source. No restriction was made for the number of entries in the data set for any given enzyme; even multiple reports for the same enzyme from the same tissue were included, since such reports did not always agree with each other. The data set thus reflects the focus of research in the field, and may have some bias when considered as a whole.

B. Assignment of Molecular Weight Values or Polymer Sizes

Not all publications reported M_r values; some reported $S_{20,w}$ values from sedimentation

studies. In order to include such data, the reported $S_{20,w}$ values were converted to apparent M_r values using the relationship:

$$\frac{S_{20,w\ (X)}}{S_{20,w\ (Hb)}} = \frac{[M_{r\ (X)}]^{2/3}}{[M_{r\ (Hb)}]^{2/3}}$$

where X refers to any given enzyme, while Hb refers to the reference protein, hemoglobin. All values calculated in this fashion are shown in Tables A1 to A5 in parentheses (see Appendix). Also, on the assumption that subunit M_r values are reasonably consistent for the same enzyme from different sources, I have computed polymer sizes in cases where authors published a native M_r, but no subunit M_r, if the subunit M_r for that enzyme was available from some other source. Again, such interpreted values are shown in parentheses.

Some caution is necessary in accepting values for polymer sizes. It is, in fact, not difficult to compute incorrect polymer sizes if the observed M_r values for the subunit and native size are incorrect by as little as 10%. Consider this sample illustration. A tetrameric enzyme has an absolute native M_r of 120,000 and a subunit M_r of 30,000. If the experimental value for the subunit M_r is 10% low (27,000) and that for the native molecular weight is 10% high (132,000), then the enzyme could be interpreted as a pentamer (132,000/27,000 = ~5). If measurements deviate in the other direction by 10%, the enzyme could be designated a trimer (108,000/33,000 = ~3).

Variation in observed M_r or polymer size may occur in the presence of ligands that alter the equilibrium mixture of a dissociating enzyme (e.g., monomer-dimer or dimer-tetramer). As an example: sedimentation studies of UMP synthase by Reyes and Guganig[11] yielded a native M_r of 65,000 to 71,000. However, these studies had been done with the enzyme in 50 m*M* phosphate buffer, and included a substrate, P-Rib-PP at 1 m*M*.[11] Later studies showed that UMP synthase, with a subunit M_r of 51,000,[12] could have a native M_r anywhere between 51,000 and 102,000 as the concentration of phosphate was increased,[13] and the enzyme was titrated from being predominantly monomeric to being predominantly dimeric; P-Rib-PP also caused the enzyme to dimerize.[14] Since stabilizing ligands such as substrates or analogs are frequently but not consistently used, observed M_r values may easily differ from true subunit or polymer values.

C. Regulatory Enzymes

Enzymes were included in this category if they showed complex kinetics, with positive or negative cooperativity on Lineweaver-Burk plots. Enzymes were also included if they gave other types of evidence of being interconvertible between active and inactive conformations. One out of seven enzymes in this survey shows the ability to dissociate reversibly under nondenaturing conditions. Since such dissociation implies conformational changes, the possibility of a regulatory mechanism will be entertained until disproved by additional studies. Several authors have developed theoretical frameworks for enzyme regulation by reversible dissociation.[15-17]

III. RESULTS AND DISCUSSION

A. The Data Set: Size and Structure of Enzymes

1. Subunit Molecular Weights

The enzymes surveyed are tabulated, in an Appendix, under separate categories of monomer, homopolymer, heteropolymer, and multifunctional proteins. Although the last term is sometimes used to designate an enzyme with broad substrate specificity, here it will mean a single protein that contains two or more separate and different catalytic activities.

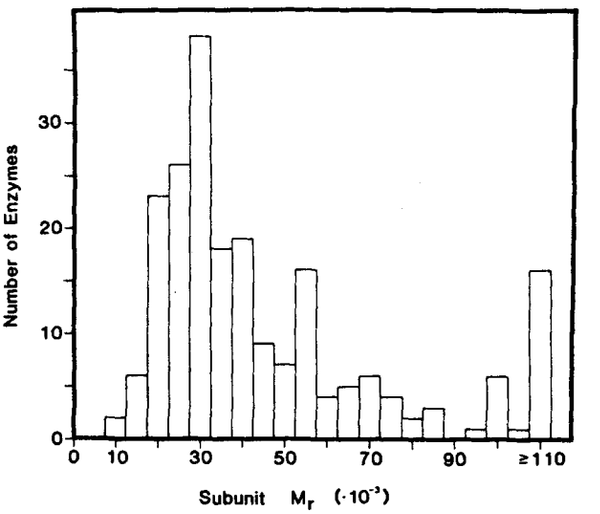

FIGURE 1. Frequency distribution of subunit molecular weights for enzymes in nucleotide metabolism. The average molecular weight for all protein subunits is 47,900; if synthetases and multifunctional proteins are omitted, the average molecular weight for single enzymes is 32,800.

Table 1
PROTEIN SUBUNIT SIZES

	Definition of sample set	n	Average M_r ($\cdot 10^{-3}$)	Median M_r ($\cdot 10^{-3}$)	Ref.
1.	Total proteins (HeLa cells)	Unknown	31.7	<20	18
2.	Total proteins (*E. coli*)	Unknown	24	20	18
3.	Soluble proteins (human liver)	(~200)	54.6	41	19
4.	Polymeric proteins (eukaryotic)	153	51.5	42	20
5.	Polymeric proteins (prokaryotic)	207	49.6	40	20
6.	Human enzymes	99	45.8	41	21
7.	Enzymes of nucleotide metabolism (all species)	228	47.9	33.3	This survey

Figure 1 shows the distribution of enzymes by their subunit molecular weight. The distribution is quite comparable to frequency histograms of subunit sizes made for other data sets.[18-21] For the present survey, the modal M_r was around 30,000, while the mean M_r was 47,900.

Table 1 compares the values obtained with the present data set to values obtained for other sample sets. All the sample sets have some type of bias. The first two entries in Table 1 were obtained by measuring the steady-state incorporation of radioactive amino acids into total cellular proteins, partly separated (as a continuous distribution) on sodium dodecyl sulfate (SDS) gel electrophoresis. Since total radioactivity was used as a measure of protein, these two data sets will therefore be skewed by the more abundant proteins in the cell. The third data set analyzed the same sample (total liver protein) on a variety of SDS gels (varying in percent acrylamide) which resulted in 200 discrete bands. The least abundant proteins might not be detectable by staining, and since a tissue would be expected to have as many as 2000 proteins,[22] each of the 200 bands detected in this experiment must have represented

different numbers of discrete proteins of common molecular weight. Data sets 4 and 5 were taken from the compilation of polymeric proteins,[6] and therefore lack monomers.

Data sets 6 and 7 exclude nonenzymatic proteins (receptors, regulatory subunits, transport proteins, structural proteins, signaling/hormone proteins, etc.). Considering the rapid discovery and characterization of such proteins, it is possible that eventually the number of identified nonenzymatic proteins will equal or exceed the number of enzymes. Data set 6 is further biased towards only human proteins, but has the feature of including any given enzyme only once, while data set 7 represents all available sources, but permits multiple sampling of the same enzyme. A final caveat about evaluating subunit sizes: depending on how many, and which, reference proteins are used, an error of 2000 to 5000 (or greater) in determination of molecular weight is quite possible. When the sample set is obtained from many different laboratories (Table 1, sets 4 to 7), such errors may cancel out. When the sample set is from a single laboratory (sets 1 to 3), the entire sample set may be skewed.

With all the above cautions in mind, it is then interesting that sample sets 3 to 7 give a comparable range of values for both the average and median molecular weights of subunits. Sample sets 1 and 2 have considerably smaller values, perhaps reflecting an abundance of smaller proteins in HeLa and *E. coli* cells. Ribosomal proteins are quite abundant, and only 4 of over 50 ribosomal proteins in *E. coli* have subunit M_r greater than 25,000.[23] The enzymes of nucleotide metabolism produce molecular weight values that appear to be within the range of sample sets 3 to 6 (Table 1), although the values are clearly at the lower end of this range. The unweighted averages across sample sets 3 to 7 produce an average subunit molecular weight of 49,900 and an average median molecular weight of 39,500.

2. Quaternary Structure

An analysis of the polymer size, or quaternary structure, is shown in Table 2. On the possibility that some simple evolutionary pattern might be detectable, the set of enzymes was divided into three broad categories: prokaryotes, simple eukaryotes (mainly single-celled organisms such as fungi and protists), and higher eukaryotes (multicellular, and in the present case overwhelmingly represented by mammals and birds). About 80% of all the proteins exist in their native form as polymers, the majority as homopolymers (76%), and far fewer as heteropolymers (4%). A few enzymes count in both categories since they are formed by two types of homopolymers (e.g., aspartate carbamoyltransferase from *E. coli* is a heteropolymer containing six catalytic subunits and six regulatory subunits). The term heteropolymer, as used here, means that a defined enzyme activity (it may be simple or complex) requires more than one type of subunit for proper activity. Heteropolymers in this sense are distinct from multienzyme complexes, which are discussed in Section III.C.2.

Only about one in five enzymes appears to exist as a monomer under native conditions. This can easily be an overestimate since many enzymes attain a polymeric structure in the presence of an appropriate physiological ligand as already described for UMP synthase, which exists as a monomer when measured only in buffer, but readily dimerizes in the presence of micromolar concentrations of OMP.[13,14] An additional source of error which favors detection of monomers is the diluted concentration of enzyme used in many studies. For polymeric proteins, dilution favors dissociation, especially in the absence of effector ligands. In this context, it is noteworthy that myoglobin, routinely used as an example of a monomeric protein in contrast to the tetrameric hemoglobin, will dimerize under conditions of increased protein concentration.[24]

Examination of polymer size (Table 2) shows that dimers are most common, consistent with an earlier survey.[6] At a much lower frequency can be found tetramers and hexamers. Trimers are much rarer, as can be seen for prokaryotes and simple eukaryotes. The value for higher eukaryotes is considerably skewed by the eight entries for purine nucleoside phosphorylase. Pentamers are very rare in the general literature; no examples were found

Table 2
QUATERNARY STRUCTURE OF ENZYMES IN NUCLEOTIDE METABOLISM (72 DISTINCT ENZYME ACTIVITIES)

POLYMERS

	Total*	av Mr (kDa)	allosteric		MFP		Dissociating		Homo polymer		Hetero-polymer	
			#	%	#	%	#	%	#	%	#	%
Prokaryote	68	44	22	34	1	2	7	11	52	80	4	6
Simple eukaryote	35	46	5	16	8	26	3	10	18	58	1	3
Higher eukaryote	158	52	47	36	9	7	24	18	104	79	4	3
All enzymes	261	48	74	32	18	8	34	15	174	76	9	4

POLYMER SIZE

	One		Two		Three		Four		Five		Six		Eight		Ten		Sixteen	
	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%
Prokaryote	9	14	31	48	5	8	10	15	1	2	9	14	1	1	1	2	0	0
Simple eukaryote	7	23	14	45	0	0	1	3	1	3	2	6	1	1	0	0	0	0
Higher eukaryote	37	28	71	54	15	11	16	12	0	0	9	7	2	2	0	0	0	0
All enzymes	53	22	116	48	20	8	27	11	2	1	20	8	4	2	1	0.4	1	0.4

* Since some enzymes are heteropolymers, the total number of proteins are greater than the number of enzymes.

in the survey by Klotz et al.[6] Therefore, the two examples in the current set deserve special attention (see Sections III.B.2.c and III.B.2.d).

Although interchain disulfide bonds are known to link subunits for some extracellular enzymes (e.g., acetylcholinesterase),[304] such bonds are not routinely observed in the reducing intracellular environment. Therefore, cytosolic polymeric enzymes should all be able to dissociate to subunits. However, it appears that the majority of enzymes exists as very stable polymers, since only about one out of six polymeric enzymes has been shown to undergo reversible dissociation. Such changes in polymerization indicate that the enzyme exists in two (sometimes more) differing conformations that are in equilibrium. Many factors affect such an equilibrium: (1) concentration of enzyme; temperature; pH; ionic strength; and (2) specific regulatory ligands. The first group (1) includes variables that are easily manipulated in vitro, whether or not by design, and can at least give preliminary indications that a particular enzyme undergoes reversible changes in polymerization. Whether such dissociation/reassociation processes imply a mechanism for regulation of enzyme activity is not clear, since these variables in vitro (pH, temperature, etc.) are much more constant in vivo.

Enzymes that dissociate or reassociate in response to appropriate ligands at physiological concentrations are much better candidates for consideration as true regulatory enzymes. For dissociation to be a regulatory mechanism, the distinct polymeric states must differ in intrinsic activity, so that the regulatory ligand acts to shift the equilibrium between the active and less active (or inactive) forms of the enzyme. Even when papers report that a substrate or product alters the native molecular weight of an enzyme, experiments to demonstrate a concomitant change in enzyme activity are not routinely done.

In our own studies on dissociating enzymes, we have observed that substrates can convert UMP synthase[25] and uridine kinase[26] from an inactive/less active form to a fully active form. Such experiments started with the enzyme preincubated under conditions that produced the

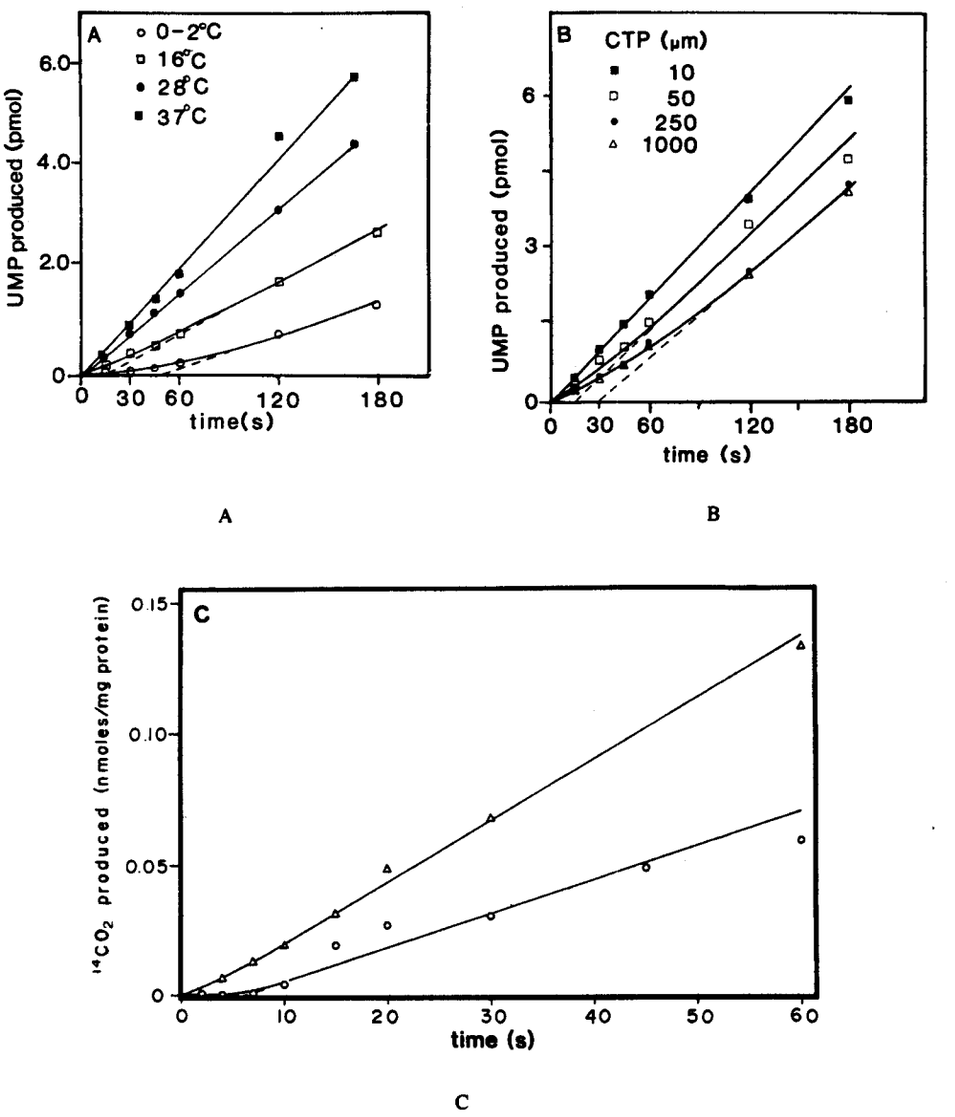

FIGURE 2. Initial velocity studies with uridine kinase and UMP synthase. The appearance of a lag in the progress curve was measured with uridine kinase as a function of temperature (A), or as a function of inhibitor concentrations at 4°C (B), and with UMP synthase as a function of enzyme concentration at 4°C (C) at 1.5 mg protein per milliliter (○) or 4.5 mg protein per milliliter (△). (Reprinted with permission from *J. Biol. Chem.*, copyright 1986, American Society of Biological Chemistry (Figure 2A and 2B); and reprinted with permission from *Biochemistry*, copyright 1980, American Chemical Society (Figure 2C)).

monomer, and then rapidly measured the appearance of product after the addition of the substrate known to produce association: OMP for UMP synthase, or ATP for uridine kinase. Examples of such kinetic experiments are shown in Figure 2. If the enzyme is intrinsically active at the beginning of the experiment, then the progress curve will be linear and will go through the origin. However, if the substrate converts the enzyme to a more active species, the progress curve will be nonlinear initially. Extrapolation of the linear steady-state part of the curve to the abscissa gives the time required for the conversion of less active enzyme to fully active enzyme. This time interval is commonly designated as the lag time.

As illustrated in Figure 2, the observation of a lag time is dependent on temperature (Figure 2A); at higher temperatures, the reassociation of enzyme subunits occurs more rapidly, and the lag time becomes smaller and more difficult to measure. Where dissociation to the less active form requires a negative regulatory ligand, as with uridine kinase, then increasing the concentration of the inhibitor during the preincubation should shift more of the enzyme to the less active form and this is accompanied by an increased lag time (Figure 2B).

The lag time also varies with enzyme concentration: at a more dilute concentration of UMP synthase, the lag time is much greater (Figure 2C). Because enzymes may be more active as polymers, and since increased concentrations of enzyme subunits favor association to the polymeric form, this explains why many enzymes have more activity during purification, or after storage, if maintained in a concentrated form. Additionally, this suggests caution in extrapolating laboratory results to the in vivo state when protein concentrations used are far from the physiological value.

It is indicated in Table A1 if enzymes exist in different polymeric states, and if such polymeric forms are readily interconvertible, and finally if such changes in quaternary structure appear to be of regulatory significance. Researchers have concluded for one enzyme that dissociation is not involved in regulation — dCMP deaminase from human spleen[27] or donkey spleen[28] — although the same enzyme from chick embryo does undergo reversible dissociation.[241] Enzymes that are probably regulated (in vivo) by dissociation/reassociation include cytidine triphosphate (CTP) synthase,[29-31,311,312] phosphoribosylpyrophosphate (PRPP) synthase,[32-35] deoxythymidine kinase from *E. coli*,[36-38] UMP synthase,[23,25] and uridine kinase.[26,39] Many additional candidates for this type of regulation are indicated in Table A1, and further studies may clarify whether dissociation is of regulatory significance for these.

3. Multifunctional Proteins and Ligases (Synthetases)

Nineteen enzyme activities, over one fourth of the total, are found in multifunctional proteins (MFPs) in one or more species,[12,40-61] and are listed in Table A3. Nomenclature presents a minor, but continuing, source of difficulty. Most enzymes are easily identified since they are named for their action plus a substrate or product of the reaction. Such useful names have not generally evolved for MFPs, and they are frequently identified as a complex of their constituent enzyme activities; this approach becomes impractical for the more complex members of this class. For the MFP containing the first three activities of the *de novo* pyrimidine pathway, the acronym CAD is widely used; while succinct, it has the disadvantage of being unrecognizable to enzymologists outside this area of research. Jones[7] introduced the designations *pyr 1-3* for this same MFP, as well as *pyr 5,6* for the MFP that contains the last two activities for UMP biosynthesis. This nomenclature is succinct, unambiguous, and even may convey the covalently linked nature of the enzyme activities. This nomenclature works well for the *de novo* pyrimidine and purine pathways (and is used in Tables A1 to A5), but becomes difficult to apply in a number of cases (e.g., the putative MFP containing deoxyguanosine kinase and deoxyadenosine kinase) since it is not evident which gene, or metabolic pathway, should contribute the numbers to be used. In the majority of cases, the MFP could be named as a synthase for its final product. Thus, *pyr 1-3* or CAD becomes dihydroorotate synthase, *pyr 5,6* is UMP synthase, *pyr 2,3,5* is phosphoribosylaminoimidazole synthase, and *pur 9,10* is IMP synthase. Only the appellation UMP synthase has been used extensively. To aid in identifications, all the above terminology is used in the tables.

While only a few MFPs are being characterized extensively, it is generally assumed that they have evolved by fusion of the genes that coded for the separate enzyme activities. For most of the MFPs, there are examples of species containing the same enzyme activities on separable and distinct proteins. For the cases in which subunit molecular weight data are

Table 3
CORRESPONDENCE OF SUBUNIT SIZE IN MULTIFUNCTIONAL PROTEINS TO THE SUM OF SUBUNIT SIZES FOR THEIR SEPARATE ENZYMES

Multifunctional protein	Average subunit M_r ($\cdot 10^{-3}$)	Separate enzymes[a]	Average subunit M_r ($\cdot 10^{-3}$)	
UMP synthase (*pyr 5,6*)	51.5	Orotate PRTase	22	49
		OMP decarboxylase	27	
dTMP synthase/dihydrofolate reductase	56	dTMP synthase	35	53
		Dihydrofolate reductase	18	
d·guo kinase/d·ado kinase	56	d·guanosine kinase	29	69
		d·adenosine kinase	40	
Tetrahydrofolate synthase (formyl-methenyl-methylene tetrahydrofolate synthase combined)	102	5,10-Methylenetetrahydrofolate dehydrogenase	29	119
		5,10-Methylenetetrahydrofolate cyclohydrolase	30	
		10-Formyltetrahydrofolate synthase	60	
Dihydroorotate synthase (CAD or *pyr 1-3*)	232	Glutaminase	43	244
		Carbamoylphosphate synthase	120	
		Aspartate carbamoyltransferase	35	
		Dihydroorotase	46	

[a] Values are from enzymes in bacteria or yeast.

available for both the MFP and for the corresponding separate enzymes, the concept of gene fusion is well supported (Table 3). The examples in Table 3 span the range from the smallest to the largest MFP, and the subunit molecular weight of each MFP appears to correspond appropriately to the number and type of catalytic activities contained in it.

Multifunctional proteins are found at all levels of evolution, from bacteria to birds and mammals, and in many branches of metabolism.[62] In the biosynthesis of the aromatic amino acids in bacteria, 10 of the 17 enzymes are found in the MFP in at least 1 species.[63-66] Since prokaryotes have many MFPs in amino acid metabolism, it is interesting that only one has yet been found in the nucleotide metabolism of these organisms. In the great majority of cases, MFPs contain activities that are consecutive in a metabolic sequence: *pyr 1-3, pyr 5,6, pur 6,7, pur 9,10,* dTMP synthase/dihydrofolate reductase. There are also examples where activities are from the same metabolic sequence, but are not consecutive: *pur 2,3,5.* In one case, an MFP contains two parallel reactions: deoxyguanosine kinase/deoxyadenosine kinase.

The tetrahydrofolate synthase protein encompasses two distinct routes for the formation of 10-formyl-H_4 folate. These activities, along with the other enzymes of folate metabolism, are shown, and numbered, in Figure 3. Thus, five of the enzyme activities may occur in two separate MFPs (enzymes 1 to 3 plus 7 and 8). In addition, the tetrahydrofolate synthase MFP forms a complex with the two formyltransferase activities plus serine hydroxymethyltransferase.[47,56] Since each of the formyl transferases is itself part of the larger MFP, the complex observed is in fact composed of three different MFPs. This represents a considerable amount of organization: thus, *pur 9,10* is an MFP containing two enzymes, and forms a homopolymer (Table A1), which in turn associates with tetrahydrofolate synthase (also an MFP and a homopolymer), etc.

a. Are Ligases (Synthetases) Multifunctional Proteins?

Synthetases (EC class 6) are enzymes that carry out complex ligase reactions, always requiring ATP or another nucleoside triphosphate. Because of confusion in the literature, they must be clearly distinguished from synthases, a name that may be applied to any type

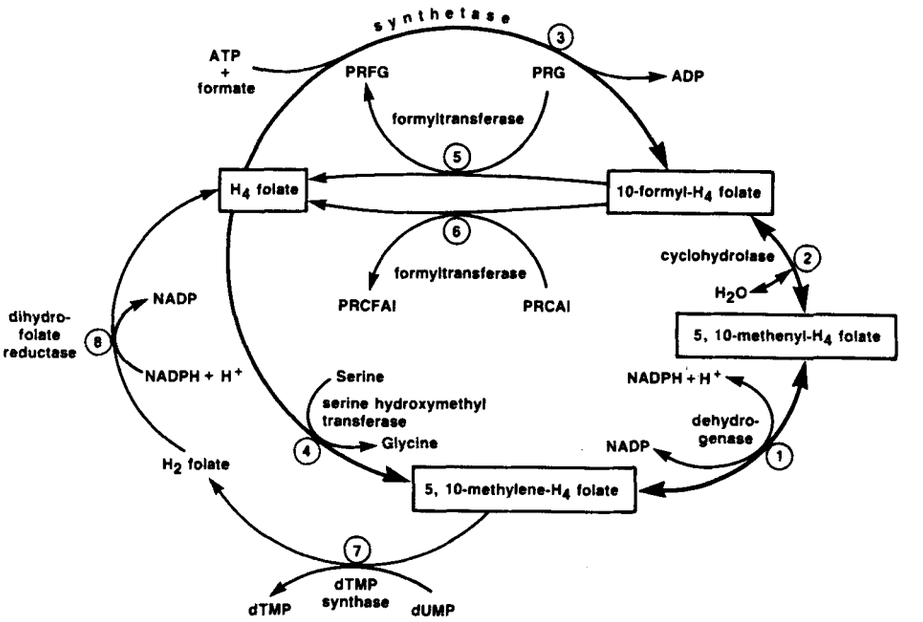

FIGURE 3. Enzymes of folate metabolism. Enzymes 1, 2, and 3 are frequently contained on a single multifunctional protein, as are enzymes 7 and 8. In some cases, enzymes 4 and 5 are tightly associated with MFP 1-3, and enzyme 6 may be loosely associated with this complex. Enzyme 5 is part of *pur 2,3,5* and enzyme 6 is part of *pur 9,10* (IMP synthase). PRG, 5′-phosphoribosylglycinamide; PFRG, 5′-phosphoribosyl-*N*-formylglycinamide; PRCAI, 5′-phosphoribosyl-4-carboxamide-5-aminoimidazole; PRCFAI, 5′-phosphoribosyl-4-carboxamide-5-formamidoimidazole.

of enzyme (EC classes 1 to 6) where formation of the product is to be emphasized. As an illustration: despite examples in the literature to the contrary, EC 2.7.6.1 should be designated PRPP *synthase*, and EC 2.1.1.45 should be deoxythymidylate *synthase* (or dTMP synthase). Because of past misinterpretation, the current edition of *Enzyme Nomenclature (1984)*[313] discourages the use of *synthetase*, and prefers *ligase* for all class 6 enzymes. For many of the class 6 enzymes in nucleotide metabolism, *synthase* is now also recommended (e.g., CTP synthase and carbamoylphosphate synthase).

Ligases (synthetases) normally have at least three substrates, and may have two (or more) catalytic sites at which sequential reactions occur leading to the final products. With many ligases it appears that ATP is used to activate a second substrate, by phosphorylation or adenylylation, which is then ligated to the third substrate. With aminoacyl tRNA ligases, these two partial reactions are easily measured independently,[67,68] and appear to be associated with different sites on the enzyme.[67,68] Not all the ligases in the present survey have been characterized extensively, but CTP synthase and carbamoylphosphate synthase can be used as examples.

CTP synthase has a glutaminase activity, in addition to the other reactions that are required for joining the amino group to UTP,[29-31,69,70] as outlined in Table 4. By considering the size of enzyme subunit required for each of these three separate reactions, a composite CTP synthase could be assembled as a multifunctional protein with a subunit molecular weight in the range of 54,000 to 80,000. By comparison, the measured subunit molecular weights are 52,000 (*E. coli*) and 66,000 (Ehrlich ascites cells), as shown in Table A1.

The comparable (but mechanistically different) sequence of reactions by which IMP is aminated to form AMP involves two separate enzymes: a ligase that joins aspartate to IMP (adenylosuccinate synthase), plus a lyase that cleaves the amino nitrogen free from its donor

Table 4
REACTIONS OF TWO PYRIMIDINE LIGASES

Reaction	Activity	Average subunit M_r for activity ($\cdot 10^{-3}$)	Smallest subunit for activity ($\cdot 10^{-3}$)
CTP synthase			
1. Glutamine + $H_2O \rightarrow$ glutamate + NH_3	Glutaminase	32	20
2. UTP + ATP $\rightarrow$ 4-P-UTP + ADP	Kinase	25	16
3. 4-P-UTP + $NH_3 \rightarrow$ CTP + P_i	Aminotransferase	23	18
Total		80	54
Carbamoylphosphate synthase			
1. Glutamine + $H_2O \rightarrow$ glutamate + NH_3	Glutaminase	32	20
2. HCO_3^- + ATP $\rightarrow$ $^-OOC\sim P$ + ADP	Kinase	25	16
$^-OOC\sim P + NH_3 \rightarrow H_2NCOO^- + P_i$	Aminotransferase	23	18
4. H_2NCOO^- + ATP $\rightarrow$ $H_2NCO\sim P$ + ADP	Kinase	25	16
Total		105	70

(adenylosuccinate lyase).[71-73] The combined subunit molecular weight for these two enzymes (Table A1) is 75,000, and thus is comparable to the single CTP synthase.

Even when separate, sequential reactions are very similar in nature, they may still be catalyzed at distinct and separate sites on the ligase. As an example, carbamoylphosphate synthase (*E. coli*) uses 2 ATP in the formation of carbamoylphosphate. Studies have shown that there are two distinct ATP binding sites, one for carboxyphosphate formation, and a second for phosphorylation of carbamate.[74] The separate reactions for this enzyme (Table 4) would require an average assembly with an M_r of about 105,000. The enzymes from *E. coli* and *Salmonella typhimurium* are in fact heteropolymers (Table A2) with a small subunit of 42,000 or 45,000 containing glutaminase activity, and a large subunit of 130,000 or 110,000 which is required for the other three reactions.

It is shown in Section III.A.4 that ligases are generally larger than other enzymes, and from the examples above, it appears that ligases may generally have two (or more) separate reactions. This leads to the suggestion that ligases, as a class, are multifunctional proteins containing sequential enzyme reactions. One property of MFPs is that different catalytic centers are usually on separate domains. This has been studied by proteolytic digestion to isolate separate activities with dihydroorotate synthase (*pyr 1-3* or CAD)[75-77] and with UMP synthase.[78] This leads to the prediction that comparable studies with ligases should lead to the isolation of domains with separate activities.

4. Relation of Subunit Size to Enzyme Function

Does the range of protein subunit sizes shown in Figure 1 merely reflect a stochastic process for generating many different catalytic functions, or is the size of an enzyme related to its biological functions? As an approach to answering this, the data on subunit molecular weight are shown in Figure 4A, separated according to major types of enzyme reaction: reductases, transferases, deaminases, etc. Examination of Figure 4A shows that initially there appears to be no specific size for reductases, transferases, etc.: all show quite a broad range of enzyme sizes. The apparent exceptions — lyases and isomerases — contain only a few entries.

Ligases and multifunctional proteins (MFPs) contain far fewer members with small molecular weights, being predominantly over 50,000. For MFPs, the increase in subunit size corresponds to the additional catalytic functions identified for these proteins. If ligases are also multifunctional proteins, then the same correlation would be expected for this class of enzymes. But for the "simple" enzymes (classes 1 to 5), why should there be such variation

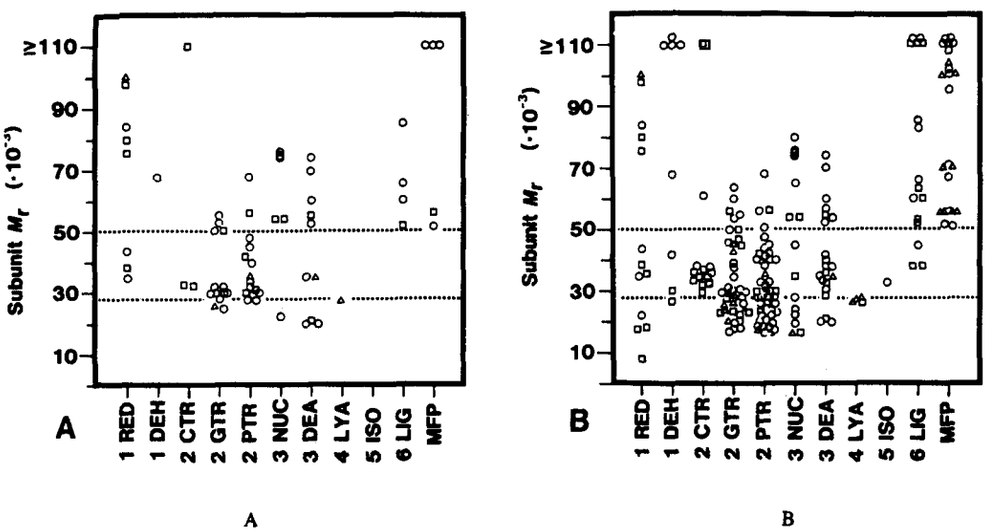

FIGURE 4. Subunit molecular weights for enzymes as a function of reaction mechanism. Numerals designate categories assigned by the Enzyme Commission. (□) Prokaryotes; (△) simple eukaryotes (e.g., fungi, protozoa); (○) higher eukaryotes. (A) All enzymes surveyed. (B) Enzymes manifesting cooperative kinetics or other regulatory properties. RED, reductases; DEH, dehydrogenases; CTR, one-carbon transferases; GTR, glycosyltransferases; PTR, phosphotransferases; NUC, nucleosidases and nucleotidases; DEA, deaminases; LYA, lyases; ISO, isomerase; LIG, ligases (synthetases); MFP, multifunctional proteins.

in size? Clearly, there are many examples of kinases or nucleotidases in the 20,000 M_r range. Why then are some two or three times as large?

A possible answer is that size correlates with functions (as already shown for MFPs and ligases), and regulation by allosteric mechanisms may be an extra attribute of larger proteins. Figure 4B shows the distribution of enzymes that give evidence of regulatory properties. Generally, at least one enzyme in each subset shows regulatory features, but of more interest is the fact that a greater proportion of large enzymes shows regulatory properties. To make a more appropriate comparison between size and total number of ligand binding functions, we can define as complex those enzymes that contain additional allosteric or catalytic sites. A working assumption will be that all enzymes that show cooperative kinetics may have an allosteric site, though this has not been demonstrated in all cases. As shown in Table 5, the percent of proteins that are complex increases with molecular weight, clearly suggesting a strong correlation between protein subunit size and biological function, where the latter is quantified by the number of catalytic and regulatory sites. This is suggested in a simpler manner by the horizontal lines in Figure 4.

Analyzing the data in Figure 4, no strong evolutionary trends are evident. Somewhat comparable ranges for subunit size are to be seen for prokaryotes, simple eukaryotes, and higher eukaryotes. Evidence for regulatory properties was found in 34% of enzymes from prokaryotes, in 16% from simple eukaryotes, and in 36% from higher eukaryotes. The lower values for simple eukaryotes may not be significant since the sample size for this category was much smaller. If we consider the proportion of complex enzymes, as defined above, then overall 43% of all enzymes are in this category.

Enzymes with subunit molecular weights >50,000 are predominantly complex, while those <27,000 are not. These partitions were chosen in part to include about one third of the sample set in each division, and may be useful as approximate guidelines.

a. Modules

I have previously proposed a correspondence between protein subunit size and function

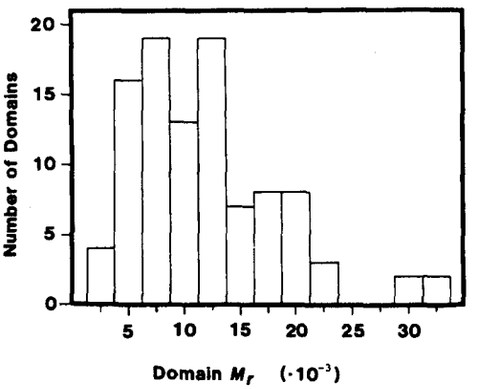

FIGURE 5. Frequency distribution of domain molecular weights. Domains were identified in a variety of crystallized proteins; these data were taken from a review by Janin and Wodak.[314]

suggesting that proteins are formed of modules[79] where modules have the following properties: an average M_r of 5000 (range: 2500 to 8000); independent tertiary structure; and a specific ligand binding function, where ligands include substrates, cofactors, metal ions, allosteric effectors, other macromolecules (to form homopolymers and/or heteropolymers), and cellular structural proteins or membranes (for enzymes that partition between soluble and bound forms). As reviewed previously,[79] there are some proteins where a correspondence exists between exons in the gene coding for a protein and functional portions of the protein. Gilbert[80] and Blake[81,82] both have favored the concept that proteins were formed by assorting and recombining exons to generate the diversity of proteins needed. There are also examples (cited in Reference 79) where no correspondence could be observed between exons and functional/structural portions in the protein; the fact that introns are deleted from some genes[83] may account for such discrepant examples.

Domains, as generally identified in the literature, have a much wider size range. In a review[314] of 101 protein domains, the range was from 2.9 kdalton to 32.5 kdalton with an average size of about 12 kdalton (illustrated in Figure 5). While the smallest domains have the same size as modules, most domains would be expected to contain two, three, or more modules. In fact, the size range of domains[314] overlaps with the size range of simple enzymes as identified in Figure 4 and Table 5.

The suggested size range for modules was based on several factors: this size range has been shown to correspond with the smallest regions of tertiary structure, obtained after proteolysis;[84] this size range was found as the increment in molecular weight per ligand binding function for a limited survey of enzymes with different functions, and from various organisms;[79] and this size range corresponds to the amount of protein coded for by most exons for a survey of 116 different genes.[85] Figure 6 shows the frequency distribution for the amount of protein per ligand binding function (i.e., putative modules), where the latter is defined as previously described.[79] The distribution is centered on M_r 7,500 and is skewed toward the larger values; the range is from 2,100 to >25,000.

The average molecular weight per ligand binding function is about 50% larger than previously found[79] or suggested by the size distribution of exons.[85] Possible reasons for this discrepancy include (1) the modular size range expected is in error; (2) enzymes in nucleotide metabolism are not adequately characterized; and (3) in larger proteins, some binding sites become masked. If the second reason is correct, then additional ligand binding functions may yet be observed for a significant proportion of the enzymes in this survey. While almost

Table 5
COMPLEXITY OF ENZYME FUNCTIONS IN RELATION TO SUBUNIT SIZE

M_r range ($\cdot 10^{-3}$)	Total enzymes	Regulatory (%)	Complex[a] (%)
<20	19	5	5
20—29	55	24	24
30—39	48	31	35
40—49	21	24	33
50—59	19	68	79
60—69	11	36	91
70—99	16	69	94
≥100	16	13	81

[a] Complex designates enzymes that may have other ligand binding sites in addition to the catalytic site; this category includes all ligases (synthetases) and multifunctional proteins, as well as enzymes that show cooperative kinetics or other regulatory features.

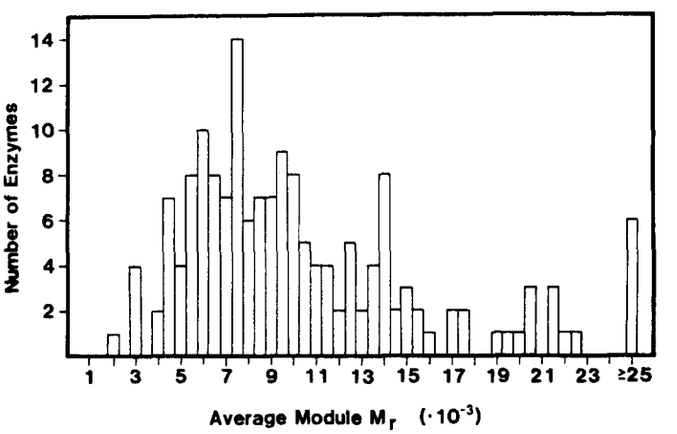

FIGURE 6. Frequency distribution of the average subunit molecular weight per ligand binding function for all the enzymes in nucleotide metabolism. The molecular weight values calculated in this manner are an estimate of the size of protein modules, as discussed in the text.

all these enzymes have been purified to homogeneity in at least one laboratory, the fact that enzymes in nucleotide metabolism are generally less abundant in cells or tissues means that investigators often do not have enough purified protein for extensive studies. The increased use of cloning may overcome this difficulty. The third possibility is discussed in Section IV.

B. The Enzymes

1. Oxidoreductases

Both reductases and dehydrogenases may be small proteins with a molecular weight ≤30,000.[86-91] Others are much larger,[92-98] especially the ribonucleoside diphosphate reductases and ribonucleoside triphosphate reductases.[99-111] Where they have been well charac-

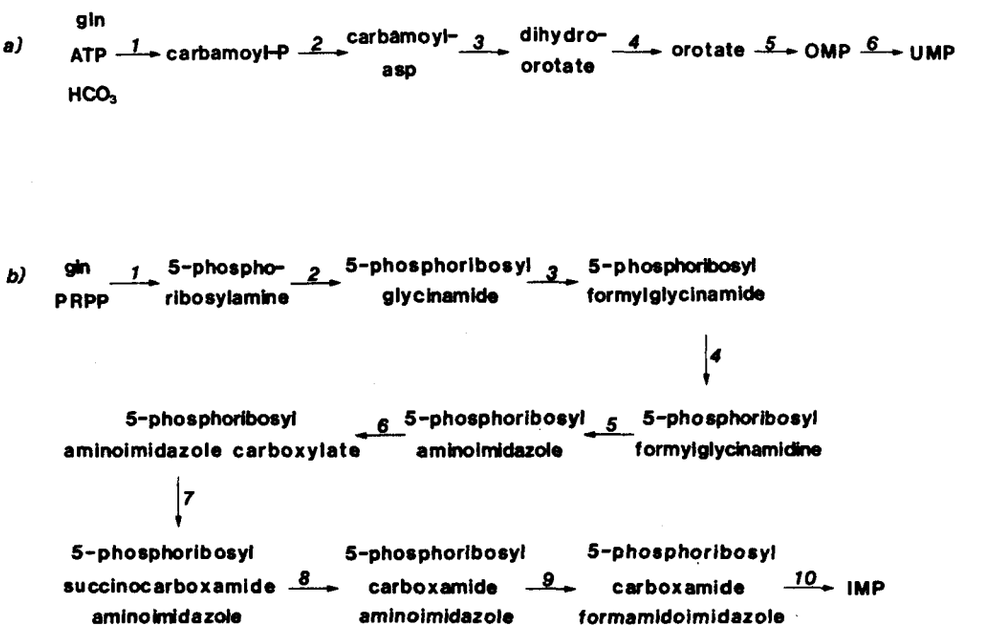

FIGURE 7. Metabolic pathways in nucleotide metabolism. (a) The *de novo* synthesis of UMP (pyrimidines). The enzyme activities are 1, carbamoylphosphate synthase; 2, aspartate carbamoyltransferase; 3, dihydroorotase; 4, dihydroorotate dehydrogenase; 5, orotate PRTase; 6, OMP decarboxylase. (b) The *de novo* synthesis of IMP (purines). The enzyme activities are 1, phosphoribosyltransferase; 2, phosphoribosylamine-glycine ligase; 3, phosphoribosylglycinamide formyltransferase; 4, phosphoribosylformylglycinamidine synthase; 5, phosphoribosylformyl-glycinamidine cyclo-ligase; 6, phosphoribosylaminoimidazole carboxylase; 7, phosphoribosylaminoimidazole-succinocarboxamide ligase; 8, adenylosuccinate lyase; 9, phosphoribosylaminoimidazole-carboxamide formyltransferase; 10, IMP cyclohydrolase.

terized, these enzymes appear to be regulated in a rather complex fashion by the various dNTPs.[104,108-110] The reductases in *Euglena gracilis*[105] and *Corynebacterium*[106] are homopolymers, while the same enzyme in *Lactobacillus*[107] is a monomer.

Best characterized is the *E. coli* ribonucleotide reductase which, like the mammalian enzyme, is a heteropolymer containing a dimer of subunit B1 and a dimer of subunit B2.[99,104,111] Subunit B1 has one substrate binding site plus two effector binding sites,[104] while B2 contains a nonheme iron plus a tyrosine radical necessary for the catalytic reduction of substrates.[111] The full catalytic site is therefore formed by the interface of subunits B1 and B2.

Equally interesting for its small subunit molecular weight is the dihydrofolate reductase contained in R-plasmid strains of *E. coli*.[86] With an M_r of 8500 it is among the smallest enzymes known, and is half the size of most other dihydrofolate reductases.[86,87] However, there is only one binding site for NADPH per two subunits, so that it takes the same amount of total protein to form a functional catalytic unit.[87]

Among the dehydrogenases, dihydropyrimidine dehydrogenase has an unusually large M_r of 110,000.[97] No allosteric or regulatory properties were reported for this purified enzyme, but the anomalous subunit size suggests that additional characterization of this enzyme could yield more ligand binding functions. A crude preparation of this enzyme from pig leukocytes was reported to have positive cooperativity, as well as altered polymer forms in the presence of substrates.[315] The enzymes from rat and mouse liver and from a human cancer line showed hysteretic kinetics.[112]

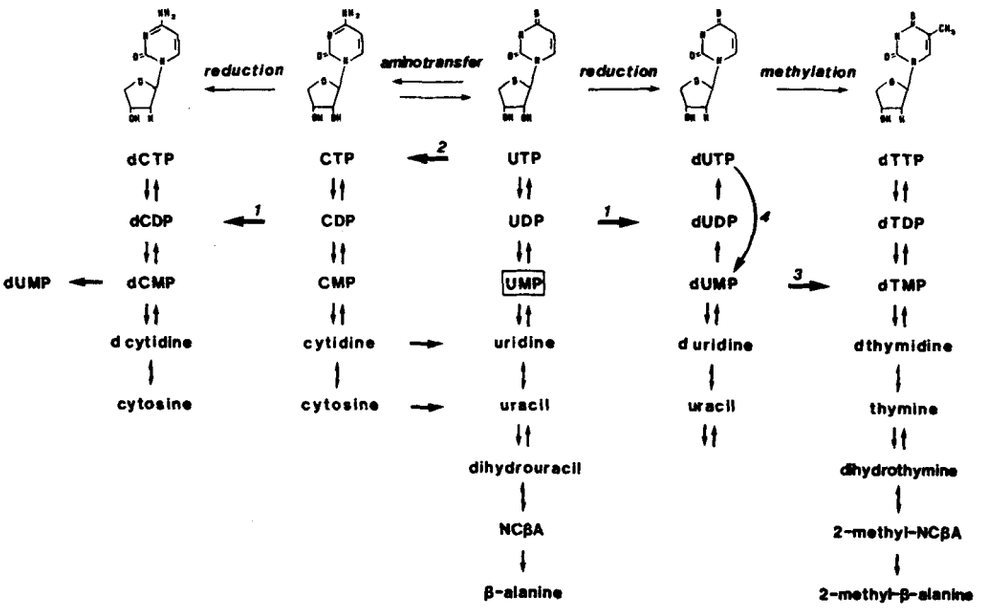

FIGURE 8. Metabolic pathways in nucleotide metabolism: the interconversion of pyrimidine nucleotides. Modifications of the pyrimidine base, or of the ribose, are represented horizontally; changes in phosphorylation, nucleoside cleavage, or catabolism of the base are represented vertically. Key enzymes are 1, ribonucleotide reductase; 2, CTP synthase; 3, dTMP synthase; 4, dUTPase. The identity of other enzymes can generally be determined from the step catalyzed. The boxed nucleotide, UMP, is the product of the *de novo* pathway. Easily reversible reactions are represented by a double arrow. NCβA is *N*-carbamoyl-β-alanine.

2. *Transferases*

a. Phosphoribosyltransferases

The majority of these enzymes[113-126] are smaller than average (Table A5). Amido phosphoribosyltransferase (PRTase) is twice as large and is regulated in a complex fashion by positive and negative effectors.[127-131,316] Since a subunit molecular weight of about 23,000 is sufficient to produce a variety of phosphoribosyltransferase activities, the greater M_r of amido PRTases (50,000 to 56,000) should provide additional functions. These include a separate glutaminase activity, comparable to CTP synthase (discussed above), and therefore the amido PRTase could also be considered a multifunctional protein. This was implied by Zalkin,[130] who compared the *E. coli* amido PRTase to anthranilate synthase, a heteropolymer having one subunit with glutaminase activity and a separate subunit with a transferase activity. Since a deaminase activity requires a subunit of at least M_r 20,000 (Table A5), then the M_r for amido PRTase appears to be appropriately larger than for simple phosphoribosyltransferases. In addition, the complex allosteric regulation by multiple ligands[127-131] suggests that this enzyme may have two allosteric sites.

These enzymes are also generally homopolymers, although adenine PRTase from rat liver has been described as a monomer,[118] while the same enzyme is a dimer in human erythrocytes[113] or *E. coli*.[116] While hypoxanthine-guanine phosphoribosyltransferases (HGPRTases) are all homopolymers, there is no agreement on the number of subunits in the native enzyme. Polymers of two,[120,125,132] three,[122] and four[123] subunits have been reported. However, since the enzyme from human lymphoblasts can form dimers or tetramers,[124] the observed trimers may reflect a hybrid population of dimers and tetramers in rapid equilibrium. It was described above how errors of only 10% in the measurement of subunit and polymer M_r can lead to erroneous polymer values. The other parameter that contributes to incorrect polymer deter-

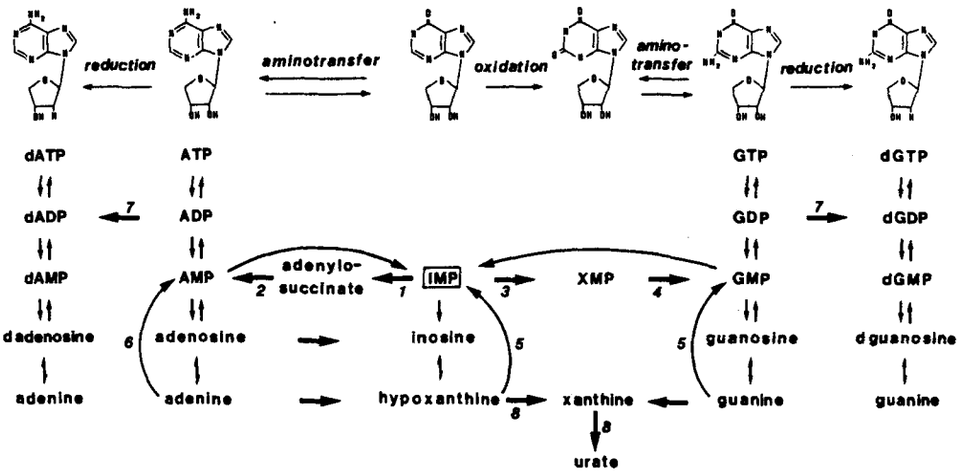

FIGURE 9. Metabolic pathways in nucleotide metabolism: the interconversion of purine nucleotides. Modifications of the purine base, or of the ribose, are represented horizontally; changes in phosphorylation, nucleoside cleavage, or catabolism of the base are represented vertically. Important enzymes are 1, adenylosuccinate synthase; 2, adenylosuccinate lyase; 3, IMP dehydrogenase; 4, GMP synthase; 5, hypoxanthine-guanine PRTase; 6, adenine PRTase; 7, ribonucleotide reductase; 8, xanthine oxidase (dehydrogenase). The identity of other enzymes can generally be determined from the step catalyzed. The boxed nucleotide, IMP, is the product of the *de novo* pathway.

mination is the ability of an enzyme to exist as a mixture of two polymeric forms. When this equilibrium is sensitive to ionic strength, or the presence of stabilizing ligands, it becomes easy to observe a variety of apparent native molecular weights, leading to a variety of polymer sizes. We see additional examples of this below.

The recently purified HGPRTase from *Schistosoma mansoni*[132] is more than twice as large as other HGPRTase enzymes (Table A5), and larger even than the complex regulatory amido PRTase. No regulatory properties have been reported for this enzyme, but the large subunit size suggests that further studies may be warranted.

b. Kinases

Similar to phosphoribosyltransferases, most kinases have a subunit M_r below 30,000.[134-179,331,332] While most kinases form homopolymers,[26,36,39,136,139,144,147,154-173,331] one example of AMP kinase from rat liver has been reported as a heteropolymer,[175] and it is interesting that adenosine kinase[174,176-179] and most nucleoside monophosphate kinases are almost all monomers: AMP kinase,[145,146] GMP kinase,[140-143] dTMP kinase,[148] and UMP kinase;[149-153] the only exceptions are AMP kinase from rat liver,[147,175] and dTMP kinase from Yoshida sarcoma.[150]

Deoxythymidine kinase from various sources is a homopolymer, most commonly a dimer (Table A1). The enzyme from most sources has a reported subunit M_r of about 45,000.[36,164-167] The majority of papers show the enzyme to have positive cooperativity vs. ATP, or negative cooperativity vs. deoxythymidine.[36-38,163,167,168,170,171] Deoxythymidine kinase from herpes virus (type I) also accepts dTMP as a substrate; this was initially interpreted as evidence for two catalytic sites and a multifunctional protein.[165,180,181] Additional kinetic studies suggested that there may be only one catalytic site at which both deoxythymidine and dTMP are phosphorylated.[182]

Based on subunit size, the small vaccinia virus enzyme (M_r 19,000)[331] would appear to be different from the enzyme in herpes or eukaryotic sources, (average M_r ~45,000, Table A1). Recently determined genes for these enzymes point to a different interpretation. The gene for deoxythymidine kinase from herpes codes for proteins of about 42,000,[334,335] while

the other genes for the enzyme code for proteins of about 21,000 (vaccinia),[331,332] or 25,000 in chicken,[336,337] human,[338] and hamster.[339] Furthermore, sequence comparisons show high homology among the genes from vaccinia, chicken, hamster, and human, and no homology for any of these with the deoxythymidine kinase gene from herpes. This points out that the subunit values for the eukaryotic enzyme (Table A1) are generally much too large (especially the value of 68,000 for the lymphocyte enzyme[168]), and suggests that the herpes enzyme is genetically different from the others, and also almost twice as large. It is therefore possible that the herpes enzyme does in fact have two catalytic sites for the separate phosphorylation of deoxythymidine and dTMP as originally suggested.[165,180,181]

MFP status has also been proposed for the enzyme that contains both deoxyguanosine kinase and deoxyadenosine kinase.[40] Since the reactions, and the two nucleoside substrates, are so similar, it would appear that a single catalytic site could mediate both enzyme activities. The enzyme shows very complex kinetics that are consistent with, though not total proof of, two separate sites.[40] However, the subunit size (M_r 56,000) does not correspond as well to the sum of separate kinase domains for d·adenosine and d·guanosine (Table 3), and is only a little larger than the M_r 51,000 adenosine kinase from rabbit liver.[176] Additional studies may clarify the MFP status of this protein. Similar to other purine nucleoside kinases, the enzyme is a monomer.

As exemplified by uridine kinase, the size of the enzyme subunit can become altered in mutagenized or transformed cells. The enzyme in normal mouse tissues has a subunit M_r of 28,000;[305] for mouse tumors, this value changes to 32,000 in Ehrlich ascites cells, and to 26,500 for a mouse lymphoma line.[305] Only the uridine kinase from Ehrlich ascites cells has been characterized extensively,[26,39,161] so it is not clear if the above variations in subunit M_r have any functional significance. However, a derivative of the mouse lymphoma line has lowered uridine kinase activity,[306] and a subunit M_r of 25,000.[305] In human lymphocytes, uridine kinase has a subunit M_r of 30,000;[305] human lymphoma have the same subunit M_r for the enzyme, but a lymphoma line that was mutagenized has a subunit M_r of 21,000[305] and almost total loss of enzyme activity.[305,307]

c. Phosphorylases

This category includes the various phosphorylases,[183-207,324-327] of which purine nucleoside phosphorylase (PNP) has received the most study. There is little agreement about the polymer size, since PNP has been reported as a hexamer in bacteria,[183,184] a pentamer in *Plasmodium lophurae*,[185] a trimer in bovine spleen,[188] Chinese hamster fibroblasts,[191] human erythrocytes,[193,194] bovine and chicken liver,[196,197] and human placenta.[198] PNP has also been reported to be a dimer in rabbit liver,[192] rabbit brain[200] and bovine brain,[199] and a monomer in rabbit liver.[201]

Since hexamers may readily be formed from trimers, the prevalent values of hexamer in prokaryotes and trimer in eukaryotes suggest a structural homology that has been conserved. A preliminary report on the crystal structure of the human erythrocyte PNP states it to be a trimer.[202] The report of a pentamer[185] is highly questionable because this polymer bears no relation to the other values, and because pentamers are exceedingly rare. Reported polymer values of dimer and monomer are almost all from the same laboratory,[192,195,199-102] as are the highest values of 34,500,[200] 38,000,[199] and 39,000[201] for the subunit M_r. If purine nucleoside phosphorylase were a dissociating enzyme, then all the reports of trimer, dimer, or monomers could be correct, with the enzyme having a different native M_r as a function of the enzyme concentration or the buffer in which it was observed.

For eukaryotic PNP, it is noteworthy that almost all reports of the enzyme as a trimer also report that PNP has negative cooperativity vs. one or more of its substrates.[188,189,191,193,196,197] By comparison, there is only one report for negative cooperativity with the dimeric species,[195] and no cooperativity with the monomer.[201] These results would

also be consistent with a dissociating enzyme if the monomer had full activity while the trimer had only partial activity.

Preliminary studies in our laboratory with the enzyme from calf spleen give some support for dissociation of this enzyme.[322] Dissociation occurs when the enzyme is diluted to concentrations below 10 μg protein per milliliter, and is facilitated by the presence of inorganic phosphate. At 100 m*M* Pi, the enzyme is predominantly dissociated to monomers, as measured by gel permeation chromatography. As the enzyme undergoes dissociation, there is a corresponding increase in specific activity, supporting the interpretation that the monomer has greater intrinsic activity than the trimer. However, the dissociated enzyme is unstable and activity decays rapidly in Tris buffer ($t_{1/2}$ = 30 min), but more slowly in the presence of 50 m*M* Pi ($t_{1/2}$ > 3 hr). Whether this type of dissociation is physiologically important is not clear; by extrapolating from the extent of purification for the enzyme from various sources, it appears that the concentration of enzyme in cells or tissues is 10 to 20 μg/mℓ or greater.

PNP is one example of an enzyme that has a smaller subunit M_r in prokaryotes and simple eukaryotes (average 23,700) than in higher eukaryotes (average 31,800). The enzyme from chicken liver has been observed to undergo apparent proteolytic cleavage from a subunit of 30,000 to 24,000,[197] comparable in size to the prokaryotic PNP. The smaller M_r enzyme no longer showed cooperativity.[197]

Uridine phosphorylase had been reported as a tetramer from several sources,[203,204] and also as an octamer.[327] Recent work on the crystal structure of the enzyme from *E. coli*, in agreement with newer determinations of subunit M_r (27,500) and native M_r (165,000), supports the interpretation that this enzyme, like the bacterial PNP, is also a hexamer.[324]

d. Other Transferases

Among the other transferases,[32-35,47,208-223] both dTMP synthase and P-Rib-PP (PRPP) synthase are well characterized and have similar subunit sizes in the neighborhood of 35,000.[32-35,208-213] PRPP synthase is an allosteric dissociating enzyme. The active form of the human erythrocyte enzyme is associated with polymers containing 16 or 32 subunits, while negative feedback effectors produce dissociation of the enzyme to less active dimers and tetramers.[34,35] The bacterial PRPP synthase is also an allosteric dissociating enzyme.[32,33] The dissociated polymer size has been reported as a pentamer;[32] in addition to the one report of a PNP pentamer,[185] this is the only other report of a pentamer for all the enzymes in nucleotide metabolism.

The aspartate carbamoyltransferase from *E. coli* contains homopolymers that are trimers (catalytic subunits) and dimers (regulatory subunits) to form a $(C_3)_2(R_2)_3$ heteropolymer.[214] Opposing regulatory ligands act at the same regulatory site.[215] This last feature has been proposed as a general case for allosteric regulation by opposing ligands,[79] and other enzymes where this has been demonstrated include mammalian glycogen phosphorylase[216] and phosphofructokinase from *Bacillus subtilis*.[217] A less rigorous example is uridine kinase, where kinetic studies suggest that a single allosteric site mediates the opposing effects of ATP and CTP.[26]

While there are six catalytic sites per native heteropolymer, each catalytic site in aspartate carbamoyltransferase is formed by parts of two adjacent subunits.[218] This latter feature suggests how very slight conformational movements in the catalytic subunits could readily alter the binding of substrates, leading to corresponding changes in activity.

The aspartate carbamoyltransferase from *B. subtilis* has a comparable subunit M_r, and like the *E. coli* catalytic subunits, associates to form trimers. Lacking a regulatory subunit, the enzyme from *B. subtilis* also lacks allosteric regulation.[317] Aspartate carbamoyltransferase from *S. foecalis* is a homopolymer with the same subunit molecular weight[219] as the catalytic subunit for the *E. coli* enzyme,[214] while the enzyme from *Pseudomonas fluorescens*, also a

homopolymer, has an unusually large molecular weight of 180,000.[220] These enzymes also show cooperativity and allosteric regulation. The *S. foecalis* enzyme was reported to be a tetramer, but the native size is also consistent with the assignment of a trimer for this enzyme.[318] The latter interpretation is more consistent with the trimeric structure for the enzymes from *E. coli* and *B. subtilis*.

3. Hydrolases

a. Nucleosidases and Nucleotidases

Based on subunit size, these enzymes are readily separated into two groups of about 23,000[223-231] and 65,000 average molecular weight.[232-239,308] Many of the enzymes in the group with larger subunits have additional functions such as allosteric regulation and association with membranes.[32-235,237] No special regulatory features have been reported for the smaller enzymes. Enzymes in this group are monomers or polymers, but there is no special pattern as with the kinases.

b. Deaminases

These enzymes are also readily divided into two groups, having a small subunit of about 32,000[28,209,243-251,309,310] or a larger subunit of about 60,000.[27,252-255] Four of the five larger enzymes have allosteric regulation. However, so does the smaller dCMP deaminase. This interesting enzyme has a subunit M_r of about 20,000 from most sources,[28,209,243] but has also been reported with a subunit of 53,000 from human spleen.[27] The latter value has been challenged by Maley et al.[209] dCMP deaminase is the smallest enzyme that shows allosteric regulation. With a native M_r of about 120,000, it is a hexamer in phage,[243] birds,[209] or mammals.[28] The human enzyme has the same native M_r, but with a measured subunit M_r of 53,000 it was reported to be a dimer.[27]

There are only a few cyclohydrolases,[256-260] and there are no general patterns except that they are all polymeric. For the two different dihydroorotases, only the larger one has given evidence of allosteric regulation.[257,258]

c. Hydrolases

The third and final enzyme in the catabolism of uracil and thymine is *N*-carbamoyl-β-alanine (NCβA) amidohydrolase (also called β-alanine synthase and β-ureidopropionase). The enzyme from rat liver has a native M_r of 235,000[340] and a subunit M_r in the range of 60,000 to 68,000. It thus appears to be larger than other amidohydrolases which are ≤30,000. The rat liver NCβA amidohydrolase is a dissociating enzyme, regulated by the product β-alanine and the substrate NCβA.[340] It is somewhat surprising that the final enzyme in a pathway should be allosterically regulated; it has been suggested that the enzyme may be the beginning of a biosynthetic pathway, since β-alanine is a precursor for the synthesis of carnosine.[340]

4. Lyases

The members in this group are all small, and polymeric.[72,73,261-263] Adenylosuccinate-AMP lyase is noteworthy in that this enzyme, presumably having but one catalytic site, catalyzes two separate reactions in the *de novo* biosynthesis of AMP: reaction 8 (phosphoribosylsuccinocarboxamide-aminoimidazole → phosphoribosylcarboxamide-aminoimidazole + fumarate) and reaction 12 (adenylosuccinate → AMP + fumarate). The two reactions are sufficiently similar that the same catalytic site could perform both.

OMP decarboxylase is an individual enzyme in *E. coli*[263] and *Saccharomyces cerevisiae*.[261,262] In mammals, it is part of the multifunctional protein UMP synthase (*pyr 5,6*).[12]

5. Isomerases

The only member of this class is phosphopentomutase.[221]

6. Ligases (Synthetases)

These enzymes[29-31,69,71,264-274] have already been discussed as a group in Section A.3. As a rule, they are larger enzymes, and it was proposed above that their size corresponds quite well to the number of total functions. It can be seen (Table A5) that for different enzyme classes, simple enzymes are commonly found with a subunit size of about 20,000. The smallest ligases are at least twice as large (38,500 to 43,000), consistent with the proposal that these enzymes are also multifunctional proteins having at least two catalytic centers.

Ligases occur frequently in the *de novo* synthesis of AMP, at reactions 2, 4, 5, 7, and 11. Four of these enzymes have comparable subunit sizes from 38,500 to 52,000 (Table A5). An interesting exception is phosphoribosylformylglycinamidine synthase, a monomeric enzyme with a reported M_r of 133,000 from chicken liver[272,273] and 135,000 from *E. coli*.[274] Like other ligases that involve transfer of a nitrogen (e.g., carbamoylphosphate synthase, CTP synthase), this enzyme has a separate glutaminase activity. However, since no allosteric regulation has been observed with phosphoribosylformylglycinamidine synthase, the subunit M_r of 133,000 is unusually large for its currently known functions.

7. Multifunctional Proteins

Some of the largest proteins are found in this group, since these enzymes combine two or more catalytic domains. It is interesting how well organized the pathways for *de novo* purine and pyrimidine synthesis have become in higher eukaryotes. In the synthesis of UMP, five of six enzyme activities are on two MFPs.[12,49-52,75,275,276] In the synthesis of IMP, 7 of 10 enzyme activities are on 3 MFPs;[42-47] if the folate enzymes are included,[42,56-61] then 10 of 13 enzymes are on 4 MFPs. The MFP containing dTMP synthase and dihydrofolate reductase is widespread in simple eukaryotes.[53-55]

C. Special Regulatory Features

1. Covalent Modification

a. Phosphorylation

The only mammalian protein in nucleotide metabolism reported to undergo phosphorylation is dihydroorotate synthase (CAD or *pyr 1-3*) isolated from rat ascites hepatoma[279] or from Syrian hamster kidney cells.[50] In vitro, the protein can be phosphorylated by cAMP-dependent protein kinase at about 0.5 mol Pi per mole subunit[279] or >1.5 mol Pi per mole subunit.[50] The latter report gave evidence for two phosphorylation sites; one of these was modified by autophosphorylation.[50] Phosphorylation had no effect on carbamoylphosphate synthase activity with the hepatoma enzyme,[279] but increased activity twofold for the kidney cell enzyme, and made it less sensitive to inhibition by UTP.[50]

Deoxythymidine kinase from *Physarum polycephalum* is also phosphorylated.[162] The phosphorylated enzyme is less sensitive to inhibition by dTTP, and phosphorylation also alters the K_m for deoxythymidine. This suggests that phosphorylation may be physiologically important for regulating activity.

b. Proteolysis

Purine nucleoside phosphorylase from chicken liver has been reported to undergo proteolysis, with a reduction in subunit M_r from 30,000 to 24,000.[197] The proteolytically clipped enzyme no longer showed cooperativity, but still had a native M_r of 90,000, suggesting that the two fragments only came apart when examined by denaturing electrophoresis.[197] Since the observation of proteolytic cleavage was dependent on the type of purification protocol,[197] this may reflect an artifact of purification.

Proteolysis has also been observed during the purification of phosphoribosylaminoimidazole synthase (*pur 2,3,5*), leading to a subunit M_r of 50,000 to 55,000,[42] which was about half the normal size. Phosphoribosylglycinamide formyltransferase activity was readily meas-

ured despite this proteolysis; the other two activities of this MFP were not assayed. In a later paper,[43] it was shown that in vitro proteolysis resulted in two fragments, one having phosphoribosylamine-glycine ligase activity (*pur 2*) and the other having phosphoribosylglycinamide formyltransferase activity (*pur 3*). While these data support the interpretation of separate functional domains in this protein, it is not likely that the observed proteolysis has a physiological function. The above results also explain how a low M_r (67,000) was obtained in earlier studies when only the formyltransferase activity was measured.[47]

2. Multienzyme Complexes

A variety of enzymes has been described as heteropolymers and is listed in Table A2. For some of these, a single enzyme activity appears to require two types of dissimilar subunits. For carbamoylphosphate synthase,[268,269] aspartate carbamoyltransferase,[214] and ribonucleoside diphosphate reductase,[99,104] separate functions are known for the separate subunits. In the case of adenosine deaminase, an enzyme active as a monomer or a homopolymer may also bind to another cellular protein. For the enzymes AMP kinase,[175] nucleoside phosphotransferase,[223] and uracil PRTase,[281] no separate functions are assigned to the different subunits. Since for each of these examples there are similar enzymes active as homopolymers (Table A1, A4), it is possible that the results reported[175,223,281] represent an artifact due to proteolysis, or to a nonfunctional contaminating protein.

In addition to the covalently assembled enzyme activities found in MFPs (Table A3), separate enzymes may also associate into multienzyme complexes,[47,56,282-293] or into multiprotein complexes, as illustrated by nucleoside diphosphate kinase binding to tubulin.[280] Where such complexes bind tightly and are very stable during purification (e.g., pyruvate dehydrogenase, or the ribosomal subunits), they have been readily observed. Multienzyme complexes in nucleotide metabolism are apparently less stable, and have only been detected in recent years.[282-293] Figure 10 outlines enzyme sequences involved in the formation of pyrimidine dNTPs. Complexes containing different combinations of these enzymes have now been identified in both prokaryotic and eukaryotic sources (Table 6).

Infection of *E. coli* with T4 phage results in the formation of an enzyme complex containing at least one virus-coded enzyme (dCMP hydroxymethylase) plus several *E. coli*-coded enzymes (see Figure 10 and Table 6). The enzyme complex has been demonstrated by the cosedimentation[283-285] of the separate enzymes through sucrose gradients, but otherwise has not been purified very much.

Comparable complexes have been found to cosediment in a membranous fraction from rat liver,[286] or by cosedimentation of a soluble fraction from CHEF 18 cells[287-289] or the coelution of a soluble fraction from human lymphoblastoid cells during gel permeation chromatography.[290,291] There are some consistent differences between the prokaryotic and eukaryotic complexes: the T4 phage system does not include DNA polymerase or dihydrofolate reductase,[283-285] while the eukaryotic complexes all contain DNA polymerase,[286-291] and in some cases also dihydrofolate reductase.[287,288] It is not clear, however, if the dihydrofolate reductase was looked for in some of the eukaryotic systems.[286,290,291]

The majority of the reports have interpreted kinetic studies as providing a functional role for such complexes: the proximity of sequential enzymes leads to kinetic coupling or channeling such that distal precursors are more effectively converted into the final product. Such kinetic studies have sometimes been done with permeabilized cells,[287,288] although subsequent work with the isolated complex gave comparable results.[289] The pattern of uptake or channeling studies is indicated in Table 6.

There are also reports that are inconsistent with the participation of ribonucleotide reductase in such a complex.[341,342] In order to channel ribonucleotides into DNA, it is necessary for the active complex to be in the nucleus. However, careful examination of CHO cells throughout the cell cycle showed that ribonucleotide reductase activity was only associated with the

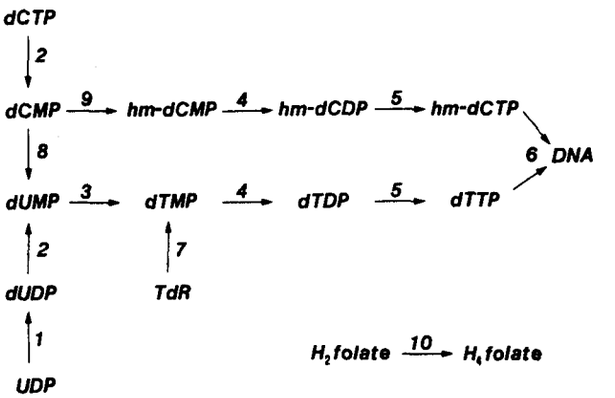

FIGURE 10. Enzymes in deoxynucleotide metabolism. Multienzyme complexes containing different combinations of these enzymes have been isolated from various sources (Table 6). The enzymes are 1, ribonucleotide reductase; 2, nucleotidase; 3, dTMP synthase; 4, pyrimidine deoxynucleoside monophosphate kinase; 5, nucleoside diphosphate kinase; 6, DNA polymerase; 7, deoxythymidine kinase; 8, dCMP deaminase; 9, dCMP hydroxymethylase; 10, dihydrofolate reductase. The same enzyme may not catalyze the separate reactions shown for enzyme 2 or enzyme 4.

Table 6
MULTIENZYME COMPLEXES SYNTHESIZING DEOXYNUCLEOTIDES

System	Enzymes in complex[a]	Kinetic coupling or channeling	Ref.
Prokaryotic			
T4 phage: *E. coli*	1, 2, 3, 4, 5, 8, 9	dUMP → dTTP	283
		dUMP → dTTP	284
		UDP → dTTP	285
Eukaryotic			
Rat liver	1, 4, 5, 6, 7		286
CHEF 18 cells	3, 4, 5, 6, 7, 10	NDP → DNA	287
	1, 3, 4, 5, 6, 7, 10	NDP → DNA	288
		CDP → DNA	289
Human lymphoblastoid cells	3, 4, 5, 6, 7	TdR → DNA	290
		dTMP → DNA	290
		TdR → DNA	291

[a] See Figure 7 for identification of individual enzymes.

cytoplasm,[341] and immunocytochemical studies with two different antibodies to subunit M1 showed an exclusive cytoplasmic localization of ribonucleotide reductase in both MDBK and mouse 3T6 cells, as well as in cells from various rat tissues.[342]

The enzyme complex from T4 phage-infected *E. coli* lacks DNA polymerase. However, these cells appear to have a second enzyme complex involved in DNA synthesis, containing DNA polymerase plus accessory replication proteins.[292,293] Perhaps the two complexes interact in vivo for an overall coordinated function.

The inclusion of dihydrofolate reductase in the mammalian enzyme complex is interesting. It has already been described that the tetrahydrofolate synthase MFP is part of a larger multienzyme complex involved in *de novo* purine synthesis (Figure 3). Additionally, dihydrofolate reductase is part of an MFP in many simple eukaryotes (Table A3).

An important aspect about all the complexes described in Table 6 is that usually only a fraction of the total activity for a constituent enzyme is part of the complex; the remainder is free. The exact stoichiometry of the constituent enzymes is not yet known for any complex, because they have not been purified sufficiently. It is also worth noting that the prokaryotic enzyme complex contains proteins specified by the host cell, as well as by the phage.

Several reviews have detailed studies on the compartmentation of metabolites,[8,9] suggesting that in vivo the type of organization illustrated by the multienzyme complexes (Table 6) may be much more common. While the physical demonstration of such integrated enzyme assemblies may continue to be difficult, our current understanding of enzymes and metabolism suggests that such a coordinated architecture may be inferred as inevitable in the continuum of structural hierarchy from simple enzyme polymers to entire cells.

IV. IMPLICATIONS FOR THE EVOLUTION OF ENZYMES

Examination of the data allows some general conclusions and interpretations:

1. The same enzyme tends to have a fairly constant subunit M_r from whatever source. Twenty two enzymes in this survey had subunit values from three or more sources. When the standard deviation in M_r was considered as a percent of the subunit M_r, then for only 6 enzymes did the standard deviation exceed 15% of the average subunit M_r. For these six enzymes, in each case a single exception was responsible for a larger standard deviation (some of these unusual examples have already been discussed).
2. Most simple enzymes in all classes (except ligases) have subunit sizes of <30,000. Since structural domains defined in proteins tend to have the same size range, it may be that most simple enzymes are formed as single domains.
3. Ligases (synthetases), multifunctional proteins, and most complex enzymes have subunit sizes of 40,000 or greater. Such proteins may then contain two or more domains.

When sequence data become available for all these proteins, then searches for homology should permit a more definitive analysis of how diverse enzyme activities originated. As an example, comparison of sequence data for three enzymes (human HGPRTase, *S. typhimurium* ATP PRTase, and *E. coli* glutamine PRTase) suggests a common dinucleotide binding fold.[294] This structure, at which PRPP would bind, was found by comparison of smoothed plots of amino acid physical characteristics. The impressive result is that phosphoribosyltransferases, differing in their acceptor substrates, and in their current evolutionary origin, still show such good homology for a binding site.

In the meantime, what suggestions can we get from the data available? There are two processes that have been suggested to account for the emergence of novel enzymes/proteins. The exon recombination hypothesis[80-82] suggests that exons, coding for units of protein structure (e.g., ligand binding modules) could be shuffled and recombined to create new enzymes. Thus, to create a new type of kinase, it would only be necessary to assemble a gene containing the exon for the module that would bind the new acceptor ligand to be phosphorylated. While this exon might be randomly recombined anywhere in the gene, selection would favor only that arrangement of exons which produced a protein where the modules were in the right spatial configuration for catalytic activity. The most favorable aspect of such a mechanism is that a new ATP binding site would not need to be developed for each different kinase; a few successful ATP binding modules could be used to assemble all possible kinases.

An alternative process suggests that existing proteins are altered slightly to generate new variations. The existing gene would be duplicated, and one of the two copies could drift, via mutations, until it acquired a different activity, which would then be stabilized by

selection. Strong support for this mechanism exists in the many examples of isozymes. This process also has the advantage that the tertiary structure is already established in the functioning protein; mutation would only substitute one type of substrate binding activity for another.

The process of mutation may have limits. It is quite plausible that a hexokinase (phosphorylating at C6 of hexose) could be modified to a uridine kinase (phosphorylating at C5 of the ribose); depending on the orientation of the acceptor substrate in binding at the catalytic site, a few mutations could alter the affinity for one substrate into preferential affinity for the second. But could mutational drift lead to binding of a radically different substrate, as required for a protein kinase, for example?

It is worth noting that these two evolutionary processes need not be seen as mutually exclusive. The exon recombination process would appear to be more powerful, and perhaps more likely, during prebiotic conditions, leading to an assortment of many simple enzymes. Once replicating cells were established, gene duplication would provide an acceptable means to develop additional structures or functions. Doolittle[295,296] has noted, however, that many serum proteins (i.e., more recently evolved) have many common modular regions, as would be expected for the assortment/recombination process, suggesting that both processes functioned in the evolution of mammalian proteins.

An additional mechanism that could operate in conjunction with either of the above is gene fusion, leading to larger proteins. Examples suggesting such a process are provided by the various multifunctional proteins. When combined with gene duplication, gene fusion leads to proteins containing two or more domains having a common structure and function. Examples of such proteins include ovomucoid[297] with 3 domains, calmodulin[298] with 4 calcium binding "domains" (these domains are really the size of modules), ubiquitin with 7 domains/modules,[299] and collagen with about 50 repeating structural segments.[300] Such proteins would therefore also be MFPs, in the structural sense, though the separate domains/modules all have a similar function. The above examples suggest that selection may favor the formation of binding proteins or structural proteins by such a process of duplication plus fusion.

Evidence for gene duplication plus fusion has also been presented for the leucyl and isoleucyl tRNA ligases from *E. coli*,[319,320] and for the leucyl, methionyl, and valyl tRNA ligases from *B. stearothermophilus*.[321] For enzymes, this process can also lead to expanded regulatory functions. It has been proposed that the mammalian phosphofructokinase was produced in this manner (being twice the size of the bacterial enzyme), with the extra catalytic centers becoming modified into allosteric regulatory sites.[301]

The current data set is consistent with all the above mechanisms for enzyme divergence. As an example: kinases that phosphorylate the different nucleoside monophosphates all have a fairly common subunit M_r, and also in general do not form polymers. Such high functional homology combined with constant size supports the mechanism of gene duplication plus mutation to generate multiple specific enzymes from a common ancestor. However, these mechanisms would appear inadequate to account for all the kinases, since sizes begin to vary, and many form polymers. This in no way proves that they were formed by exon reassortment, but such a mechanism would be more plausible. There is also considerable evidence for gene fusion with the many MFPs, and perhaps also with ligases (synthetases) and the larger complex enzymes.

Figure 11 summarizes possible mechanisms involved in the generation of the types of enzymes discussed in this review. While aspects of this figure are still conjecture, it does outline conceptually how the more complex enzymes could have evolved, and also underscores the relationship between size (whether measured in modules or domains) and function. While the current data set is consistent with the scheme in Figure 11, it probably will take only 10 years before the accumulation of sequences will make evolutionary comparisons more meaningful, and allow the more speculative aspects to be substantiated or discarded.

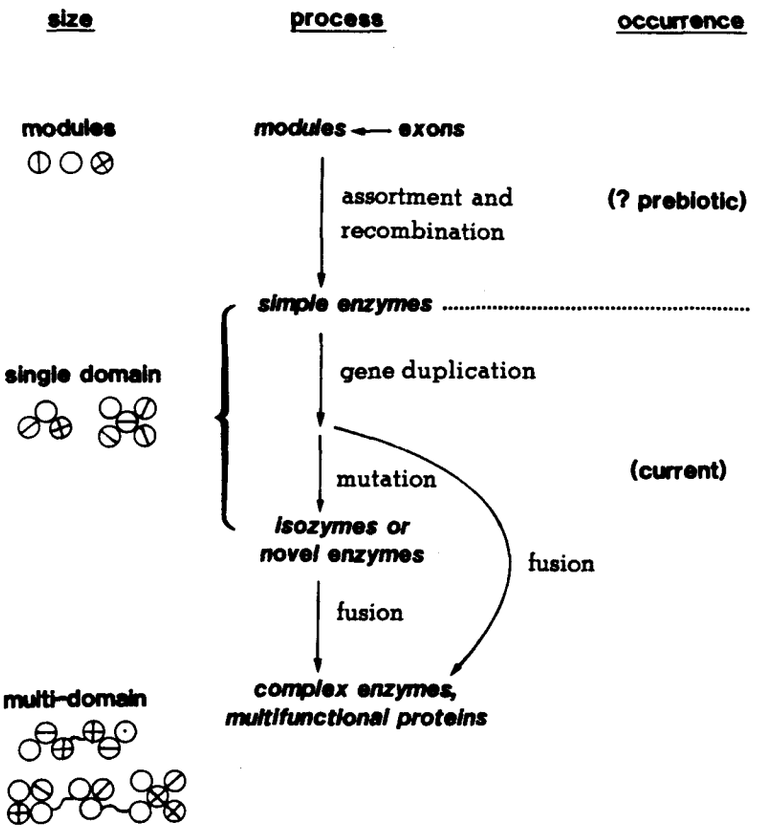

FIGURE 11. Mechanisms for the evolution of enzymes.

If larger proteins are formed by fusion of domains, then this may explain how some binding functions could become lost or masked. Let us consider polymerization (subunit to subunit) sites. The multifunctional UMP synthase forms dimers Table A1), as do the two enzymes (orotate PRTase and OMP decarboxylase) that are equivalent to the two domains in UMP synthase (Table A1). Thus, at least one set of polymer-forming sites has become masked (i.e., while both domains in UMP synthase may participate in subunit association, we can only deduce one such site in the absence of a defined crystal structure). In a similar case, dihydroorotate synthase (CAD, *pyr 1-3*) forms trimers and hexamers, and the proteolytically isolated domains that have aspartate carbamoyltransferase and dihydroorotase activities separately form trimers or higher aggregates and dimers, respectively.[75,275,276,302,303] Here, again, the authors conclude that all the potential bonding domains are not seen in the native protein.[302] This may explain why many larger proteins do not seem to have enough ligand binding functions for their size, so that they appear to have much larger modules (Figure 6).

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APPENDIX

As described in the text, all values that appear in parentheses in the following tables represent recalculated values, not reported as such in the original papers, or values that were not rigorously defined. Under the heading of native molecular weight, such values are recalculated from $S_{20,w}$; under the heading of subunit molecular weight, such values were not obtained under denaturing conditions; under the heading of polymer size, such values are obtained by comparison to the subunit M_r for the same enzyme from another source. An asterisk indicates that the enzyme has regulatory properties.

As much as possible, terminology recommended in *Enzyme Nomenclature (1984)* has been used, even though these recommended names sometimes differ significantly from those used by researchers. The most serious discrepancies are found for enzymes in the *de novo* synthesis of purines. These are specifically defined below.

Reaction	Recommended name	Common trivial name
pur 2	Phosphoribosylamine-glycine ligase	Phosphoribosylglycinamide synthetase, GAR synthetase
pur 3	Phosphoribosylglycinamide formyltransferase	Glycinamide ribonucleotide transformylase, GAR transformylase
pur 4	Phosphoribosylformylglycinamidine synthase	Formylglycinamidine synthetase, FGAM synthetase
pur 5	Phosphoribosylformylglycinamidine cyclo-ligase	Phosphoribosylaminoimidazole synthetase, AIR synthetase
pur 9	Phosphoribosylaminoimidazolecarboxamide formyltransferase	Aminoimidazolecarboxamide ribonucleotide transformylase, AICAR transformylase

Table A1
HOMOPOLYMERS

Enzyme	Source	Subunit size ($M_r \cdot 10^{-3}$)	Native polymer (n)	Regulation of 4° structure	Ref.
Adenine phosphoribosyl transferase	Human erythrocytes	18	2	Yes	113, 114
	Human erythrocytes	19.5			115
	Escherichia coli	20	1, 2	?	116
	Human erythrocytes	(18)	1, 2	?	117
Adenosine deaminase	Human intestine	36	3		247
d-adenosine kinase	Rat liver*	(~40)	2		174
Adenylosuccinate-AMP lyase	*Neurospora**	27.6	2 or 8	?	72, 73
Adenylosuccinate synthase	Rabbit liver	45	2		71
	Rabbit muscle	53	2		71
Amido phosphoribosyltransferase	*Bacillus subtilis**	50	2 or 4	?	127, 316
	Pigeon liver*	50	2 or 4	Yes	128
	Chicken liver*	53	4		129
	Escherichia coli	56.4	4		130
	Human placenta*		(2) or (4)	?	131
AMP deaminase	Rat skeletal muscle*	60	4		252
	Rabbit skeletal muscle*	(~70)	4	Yes	253
	Human erythrocytes*	74	(~4)		254
AMP kinase	Rat liver III	23.4	2 or 3	?	147
AMP nucleosidase	*Escherichia coli**	54	6		237
	*Azotobacter vinelandii**	54	6		237
Aspartate carbamoyltransferase	*Streptococcus foecalis**	32.5	4 (or 3)		219, 318
	Bacillus subtilis	33.5	3		317
	*Pseudomonas fluorescens**	180	2		220
	Mouse spleen		(2)		246
d-cytidine kinase	Human spleen	28	2		154
	Calf thymus*		(2)		155
	Human leukemic granulocytes		(2)		156
d-cyt/d-guo kinase	*Lactobacillus acidophilus**	30	2		157
dCMP deaminase	Chick embryo*		(2) or (6)	Yes	241
	Chick embryo*	~20	6		209
	T2 phage*	21	6		242
	Donkey spleen*	20	6	No	28
	Human spleen*	53	2	No	27
CTP synthase	*Escherichia coli**	52	2,4	Yes[a]	29, 311, 312
	Ehrlich ascites* cells	66	2		69
	Bovine liver*		(2) or (4)	Yes[a]	30, 31
Dihydrofolate reductase	*Escherichia coli*	17.5	2		87
	Escherichia coli R-plasmid	8.5	4		86
Dihydroorotase	*Escherichia coli*	38.4	2		256
	*Clostridium oroticum**	55	2		257, 258
Dihydroorotate dehydrogenase	Bovine liver	42	(2)		92
Dihydroorotate synthase (CAD, *pyr 1-3*)	Syrian hamster fibroblasts*	220 240	3 or 6 6	?	51 275, 276
Dihydropyrimidinase	Bovine liver	56.5	4		259

Table A1 (continued)
HOMOPOLYMERS

Enzyme	Source	Subunit size ($M_r \cdot 10^{-3}$)	Native polymer (n)	Regulation of 4° structure	Ref.
Dihydropyrimidine dehydrogenase	Rat liver	110	2		97
	Porcine leukocytes*			?	112
Formyltetrahydrofolate synthase	*Clostridium thermoaceticum*	60	4		264
GMP kinase	*Escherichia coli*		(4)		144
GMP reductase	*Escherichia coli*	36			93
	Human erythrocytes		(~4)		94
	*Leishmania donovani**				95
GMP synthase	*Escherichia coli*	63	2		265
Guanine phosphoribosyltransferase	*Giardia lamblia*	29	2		126
d-guanosine kinase	Human placenta*	29	2		158
	Mouse skin*		(2)		159
	Pig skin		(2)		160
	Lactobacillus acidophilus	30	2		157
Hypoxanthine-guanine phosphoribosyltransferase	Chinese hamster brain	25	3		122
	Human erythrocytes	24	4		123
	Human erythrocytes	26	~3		122
	Human lymphoblasts*		2 or 4	Yes	124
	Saccharomyces cerevisiae	26	2		125
	*Saccharomyces cerevisiae**	26	2	Yes	120
	Shistosoma mansoni	64	2		132
IMP dehydrogenase	Yoshida sarcoma*	68	2 or 4	?	96
IMP synthase (*pur 9,10*)	Chicken liver	67	2		42, 46
	Chicken liver	71	2		47
Methylenetetrahydrofolate cyclohydrolase	Bovine liver	(30)	(2)		260
Methylenetetrahydrofolate dehydrogenase	*Clostridium thermoaceticum*	27.5	2		89
	Clostridium formicoaceticum	30	2		90
	Clostridium cylindrosporum		2		91
Nucleoside diphosphate hydrolase	Rat liver microsomes	65	2		238
Nucleoside diphosphate kinase	Pig heart	17	6		136
	Saccharomyces cerevisiae	17.3	6		137
	Beef brain	17.7	6		138
	Beef brain	18	6		139
Nucleoside triphosphate hydrolase	Rabbit liver		(2)		241
	Rabbit erythrocytes		(2)		241
5′-Nucleotidase	Rat heart*	74	2		233, 234
	Human placenta*	76	2		235
	Sheep brain		(2)		236
OMP decarboxylase	*Saccharomyces cerevisiae*	26	2		260
	Saccharomyces cerevisiae	27.5	2		262
	Escherichia coli	27	2		263
OMP nucleotidase	Mouse liver		(2)		240

Table A1 (continued)
HOMOPOLYMERS

Enzyme	Source	Subunit size ($M_r \cdot 10^{-3}$)	Native polymer (n)	Regulation of 4° structure	Ref.
Orotate phosphoribosyl transferase	*Saccharomyces cerevisiae*	20	2		119
	Saccharomyces cerevisiae	24	2	Yes	120
	Escherichia coli	23.3			121
Phosphopentomutase	Rat liver	32.5	2		221
Phosphoribosylamino-imidazole synthase (*pur 2,3,5*)	Chicken liver	110	3		41
Phosphoribosylglycinamide formyltransferase (*pur 3*)	Chicken liver	61	2		47
Phosphoribosylformylglycinamidine (AIR) cycloligase (*pur 5*)	*Escherichia coli*	38.5	2		271
Phosphoribosylformylglycinamide succinocarboxamide synthase (*pur 6,7*)	Chicken liver	52	~6		45
PRPP synthase	*Salmonella typhimurium**	31	5 or 10	Yes	32, 33
	Human erythrocyte*	33.2	16 or 32	Yes[a]	34, 35
	Rat liver*	40	Large		208
Purine nucleoside phosphorylase	*Salmonella typhimurium*	23.5	6		183
	Salmonella typhimurium	23.7	6		184
	Escherichia coli	23.7	6		184
	Plasmodium lophurae	23.9	5		185
	Plasmodium falciparum		(6)		186
	Escherichia coli	25—28	3 and 6		329
	Bacillus cereus		(3) and (6)	?	187
	Bovine spleen*	28	3	Yes	188, 189, 322
	Human erythrocytes	29.7	3		190
	CHO fibroblasts*	30	2 and 3		191
	Rabbit liver	30.5	2		192
	Human erythrocytes*	30	3		193
	Human erythrocytes	31.6	3		194
	Human erythrocytes*	32	2		195
	Bovine liver*	30	3		196
	Chicken liver*	30	3		197
	Human placenta	31	3		198
	Rabbit brain	34.5	2		199
	Bovine brain	38	2		200
	Rabbit brain	34.5	2		199
Bovine brain	38	2		200	

Table A1 (continued)
HOMOPOLYMERS

Enzyme	Source	Subunit size ($M_r \cdot 10^{-3}$)	Native polymer (n)	Regulation of 4° structure	Ref.
Ribonucleoside diphosphate reductase	*Corynebacterium nephridii**	98	2		106
Ribonucleoside diphosphate reductase					
M1	Calf thymus*	84	2 or 4	Yes	99
M2	Calf thymus*	44	2		100—102
B1	*Escherichia coli**	80	2		103, 104
B2	*Escherichia coli**	39	2		103, 104
Ribonucleoside triphosphate reductase	*Euglena gracilis**	100	4		105
Tetrahydrofolate synthase	Chicken liver	95	2		47
	Porcine liver	100	2		57
	Saccharomyces cerevisiae mitochondria	100	2		59
	Saccharomyces cerevisiae	104	2		60
	Sheep liver	108.5	2		61
	Rabbit liver		2		277
d-thymidine kinase	Vaccinia virus	19	4		331, 332
	Human fibroblast*	28	2	Yes	163
	Herpes virus I	42	2		164, 165
	Herpes virus	44			333
	*Escherichia coli**	42	2	Yes[a]	36—38
	Escherichia coli	46.5			330
	Human placenta	44	2		166
	Human liver mitochondria*	48	2 or 8	?	167
	Human lymphocytes*	68	2	Yes	168
	Regenerating rat liver		(2)		169
	Human myelocytic leukemia cells*		(2)		170, 171
	Human myelocytic leukemia mitochondria		(2)		170
	Walker carcinoma		(2) or (≥16)	Yes	172
	Novikoff hepatoma		(≥16)		173
d-thymidine phosphorylase	*Escherichia coli*	45	2		206
	Escherichia coli	46	2		325
	Salmonella typhimurium	47	2		207
	Human platelets	60	2		326
dTMP kinase	Yoshida sarcoma		(2)		150
dTMP synthase	*Escherichia coli*	29.5	2		209
	T_2 phage	32	2		209
	Lactobacillus casei	35	2		210
	Human leukemia cells	36	2		211

Table A1 (continued)
HOMOPOLYMERS

Enzyme	Source	Subunit size ($M_r \cdot 10^{-3}$)	Native polymer (n)	Regulation of 4° structure	Ref.
	Lactobacillus casei	36.5	2		209
	Lactobacillus casei	37	2		211
	Mouse L1210 cells	38.5	2		212
	Ehrlich ascites cells	38.5	2		213
dTMP synthase/dihydrofolate reductase	*Leishmania tropica*	56	2		55
dTTPase	Human serum	24	2		230
UMP synthase	Ehrlich ascites cells*	51.5	2	Yes*	12, 13, 24, 25
Uridine kinase	Ehrlich ascites cells*	32	4	Yes*	26, 39, 161
	Rat liver	27.5			305
	Mouse liver	28			305
	Mouse lymphoma S-49	26.5			305
	Mouse lymphoma AU-11	25			305
	Human lymphocytes	30			305
	Human lymphoma 6410	30			305
	Human lymphoma 6410 MP/DU	21			305
Uridine nucleosidase	*Saccharomyces cerevisiae*	16.5	2		224
Uridine phosphorylase	*Escherichia coli*	22	8		327
	Escherichia coli	27.5	6		324
	Escherichia coli	29	4		204
	Rat liver	26	4		203
	Novikoff hepatoma		(2)		205
dUTPase	*Escherichia coli*	16	4		226
	HeLa*	22.5	2	Yes	227
	HeLa		(~3)		228
	Human lymphocytic leukemia		(2)		229
Xanthine oxidase (dehydrogenase)	Chicken liver	120	2		98
	Drosophila	135	2		98
	Bovine milk	150	2		98

Table A2
HETEROPOLYMERS

Enzyme complex	Source	Subunits ($M_r \cdot 10^{-3}$)	Activity/function	Ref.
Adenosine deaminase	Kidney*	38 (2)	Adenosine deaminase	247
		106 (2)	Binding protein	
AMP kinase	Rat liver	11 (1)	Not defined	175
		13 (2)		
Aspartate carbamoyltransferase	*Escherichia coli**	35 (6)	Catalytic subunit	214
		16.5 (6)	Regulatory subunit	
Carbamoylphosphate synthase	*Escherichia coli**	130 (1)	Carbamoylphosphate synthase	268
		42 (1)	Glutaminase	
	*Salmonella typhimurium**	110 (1)	Carbamoylphosphate synthase	269
		45 (1)	Glutaminase	
Nucleoside phosphotransferase	Morris hepatoma*	110 (1)	Not defined	223
		130 (1)		
Ribonucleoside diphosphate reductase	Calf thymus*	84 (2)	M1 effector site	99
		44 (2)	M2 reduction	
	*Escherichia coli**	80 (2)	B1 effector site	104
		39 (2)	B2 reduction	
Uracil phosphoribosyltransferase	*Saccharomyces cerevisiae*	27 (1)	Not defined	281
		58 (1)		

Note: Values in parentheses indicate the number of proteins per functional complex.

Table A3
MULTIFUNCTIONAL PROTEINS

Enzyme (complex)	Source	Subunit ($M_r \cdot 10^{-3}$)	Enzyme activities	Ref.
Carbamoylaspartate synthase (*pyr 1,2*)	*Neurospora*		Carbamoylphosphate synthase	48
			carbamoyltransferase	
Dihydroorotate synthase (CAD, *pyr 1-3*)	*Drosophila*	220	Glutaminase	49
	Syrian hamster fibroblasts	235	Carbamoylphosphate synthase	51, 52
		240	Aspartate carbamoyltransferase	50
			Dihydroorotase	
d·guanosine/d·adenosine kinase	*Lactobacillus acidophilus*	56	d·guanosine kinase	40
			d·adenosine kinase	
IMP synthase (*pur 9,10*)	Chicken liver	67	Phosphoribosylaminoimidazolecarboxamide formyltransferase	42
		71		46, 47
			IMP cyclohydrolase	
Phosphoribosylaminoimidazole synthase (*pur 2,3,5*)	Murine lymphoma	102	Phosphoribosylamine-glycine ligase	278
	Chicken liver	110	Phosphoribosylglycinamide formyltransferase	41—43
	Drosophila	150	Phosphoribosylformylglycinamidine cycloligase	44
Phosphoribosylaminoimidazolesuccinocarboxamide synthase (*pur 6,7*)	Chicken liver	52	Phosphoribosylaminoimidazole carboxylase	45
			Phosphoribosylaminoimidazolesuccinocarboxamide ligase	
Tetrahydrofolate synthase	Chicken liver	95	Methylenetetrahydrofolate dehydrogenase	42, 56
	Pig liver	100	Methylenetetrahydrofolate cyclohydrolase	57, 58
	Saccharomyces cerevisiae mitochondria	100	Formyltetrahydrofolate synthase	59
	Saccharomyces cerevisiae	104		60
	Sheep liver	108.5		61
dTMP synthase/dihydrofolate reductase	*Crithidia fasciculata*	56.7	dTMP synthase	53
			Dihydrofolate reductase	
	Crithidia fasciculata	56		54
	Leishmania tropica	56	Dihydrofolate reductase	54, 55
	Leishmania mexicana	56		54
	Plasmodium falciparum	70		54
	Plasmodium lophurae	70		54
	Eimeria tenella	100		54
UMP synthase	Ehrlich ascites cells	51.5	Orotate phosphoribosyltransferase	12, 13
			OMP decarboxylase	

Table A4
MONOMERS

Enzyme	Source	Subunit size ($M_r \cdot 10^{-3}$)	Native size ($M_r \cdot 10^{-3}$)	Ref.
Acid nucleotidase	Rat liver		79.5	239
Adenine phosphoribosyltransferase	Rat liver	17.5	22	118
Adenosine deaminase	Human lymphoblasts	40	38	248
	Human erythrocytes	41.7	37.6	249
	Calf intestine		38	250
	Escherichia coli		29	251
Adenosine kinase	Rabbit liver	51	34.5	176
	Leishmania donovani	38.2	37.7	328
	Human placenta	40.7	37.3	177
	Saccharomyces cerevisiae		40	178
	Mouse L1210 cells		56	179
d-adenosine kinase	Rat liver mitochondria		40.7	175
AMP kinase	Bovine heart I	22		145
	Bovine heart II	30		145
	Human erythrocyte	23	23	146
	Escherichia coli	23.6	23	323
Cytosine deaminase	*Saccharomyces cerevisiae*		34	244
Dihydrofolate reductase	*Escherichia coli* R-plasmid	18		87
	Chicken liver	22.5		88
Dinucleoside tetraphosphatase	Ehrlich ascites cells		19.8	225
GMP kinase	Rat liver		20.5	140
	Vero cells		22	141
	Hog brain		19	142
	Human erythrocytes		18.4 and 24	143
	Sarcoma 180		20	143
GMP synthase	Rat hepatoma		83	266
	Ehrlich ascites cells*		85	267
Guanine deaminase	Rabbit liver	54	56	255
d-guanosine/d-adenosine kinase	*Lactobacillus acidophilus**	56	50	40
Nicotinate PRTase	*Saccharomyces cerevisiae*	45	43	125
	Saccharomyces cerevisiae	43		133
Nucleoside phosphotransferase	Carrot	38	44	222
5′-Nucleotidase	Human erythrocytes		28	231
	Bovine retinal rod*	75	79	232
Phosphoribosylamine-glycine ligase (*pur 2*)	*Klebsiella pneumoniae*		(38)	270
Phosphoribosylaminoimidazole synthase (*pur 2,3,5*)	Murine lymphoma L5178Y	102	125	278
Phosphoribosylformylglycinamidine synthase (*pur 4*)	Chicken liver	133		272
	Chicken liver	133		273
	Escherichia coli	135		274
Purine deoxynucleoside kinase	Human T lymphocytes	19	25	135
Purine nucleoside kinase	*Trichomonas vaginalis*		16	134
Purine nucleoside phosphorylase	Rabbit liver	39	46	201
Ribonucleoside triphosphate reductase	*Lactobacillus leichmanii**	76	76	107, 108
d-thymidine kinase	*Physarum polycephalum**	35	40	162
dTMP kinase	*Saccharomyces cerevisiae*	25	25	148
UMP kinase	Rat liver		17	149
	Yoshida sarcoma cells		26	150
	Rat bone marrow		26	151
	Human leukemic blast cells		28	152

Table A5
SUBUNIT MOLECULAR WEIGHTS OF ENZYMES ARRANGED BY ENZYME CLASS

	Subunit M_r ($\cdot 10^{-3}$)
1. Oxidoreductases	
Methylene tetrahydrofolate dehydrogenase	27.5, 30
Dihydroorotate dehydrogenase	42
IMP dehydrogenase	68
Dihydropyrimidine dehydrogenase	110
Dihydrofolate reductase	8.5, 17.5, 18, 22.5
GMP reductase	36
Ribonucleoside triphosphate reductase	76, 100
Ribonucleoside diphosphate reductase M_1	84
Ribonucleoside diphosphate reductase M_2	44
Ribonucleoside diphosphate reductase B_1	80
Ribonucleoside diphosphate reductase B_2	39
Ribonucleoside diphosphate reductase	98
Xanthine oxidase (dehydrogenase)	120, 135, 150
2. Transferases	
A. One-carbon transferases	
Aspartate carbamoyltransferase	32.5, 33.5, 35, 180
dTMP synthase	29.5, 32, 35, 36, 36.5, 37, 38.5, 38.5
Phosphoribosylglycinamide formyltransferase	61
B. Glycosyltransferases	
Adenine PRTase	17.5, 18, (18), 19.5, 20
Orotate PRTase	20, 23.3, 24
Hypoxanthine-guanine PRTase	24, 25, 26, 26, 26
Guanine PRTase	29
Uridine phosphorylase	22, 26, 27.5, 29
Purine nucleoside phosphorylase	23.5, 23.7, 23.7, 23.9, 28, 29.7, 30, 30, 30, 30, 30.5, 31, 31.6, 32, 34.5, 38, 39
Nicotinate PRTase	43, 45
d·thymidine phosphorylase	45, 46, 47, 60
Amido PRTase	50, 50, 53, 56.4
Hypoxanthine-guanine PRTase	64
C. Phosphotransferases	
Purine nucleoside kinase	16
Nucleoside diphosphate kinase	17, 17.3, 17.7, 18
Purine deoxynucleoside kinase	19
GMP kinase	(18.4), (19), (20), (20.5), (22), (24)
AMP kinase I	22, 23, 23.6
AMP kinase III	23.4
AMP kinase II	30
dTMP kinase	25
UMP kinase	(17), (26), (26), (28), (28)
d·cytidine kinase	28, 30
d·guanosine kinase	29, 30
Uridine kinase	21, 25, 26.5, 28, 30, 30, 32
d·thymidine kinase	28, 35, 42, 42, 44, 48, 68
PRPP synthase	31, 33.2, 40
Nucleoside phosphotransferase	38
d·adenosine kinase	(40), (40.7)
Adenosine kinase	(40), (40), 40.7, 51, (56)
d·guo/d·ado kinase	56
3. Hydrolases	
A. Nucleosidases and nucleotidases	
Uridine nucleosidase	16.5

Table A5 (continued)
SUBUNIT MOLECULAR WEIGHTS OF ENZYMES ARRANGED BY ENZYME CLASS

	Subunit M_r ($\cdot 10^{-3}$)
Dinucleoside tetraphosphatase	(19.8)
dUTPase	16, 22.5, 35, 46
dTTPase	24
Pyrimidine 5′-nucleotidase	28
AMP nucleosidase	54, 54
Nucleoside diphosphate hydrolase	65
5′-Nucleotidase	74, 75, 76
Acid nucleotidase	79.5
B. Deaminases	
dCMP deaminase	20, 20, 21
Cytidine deaminase	33, 35
Cytosine deaminase	(34)
Adenosine deaminase	(29), 36, (38), (38), 40, 41.7
Methenyltetrahydrofolate cyclohydrolase	(30)
Dihydroorotase	38.4, 55
Dihydropyrimidinase	56.5
dCMP deaminase	53
Guanine deaminase	56
AMP deaminase	60, (70), 74
4. Lyases	
OMP-decarboxylase	26, 27, 27.5
Adenylosuccinate lyase	27.6
5. Isomerases	
Phosphopentomutase	32.5
6. Ligases (synthetases)	
Phosphoribosylformylglycinamidine cyclo-ligase	38.5
Phosphoribosylamine-glycine ligase	(38), (45)
Adenylosuccinate synthase	45, 53
CTP synthase	52, 66
Formyltetrahydrofolate synthase	60
GMP synthase	63, (83), (85)
Carbamoylphosphate synthase, α subunit	110, 130
Phosphoribosylformylglycinamidine synthase	133, 133, 135
7. Multifunctional proteins	
UMP synthase (*pyr 5,6*)	51.5
Phosphoribosylaminoimidazole succinocarboxamide synthase (*pur 6,7*)	52
d·guo/d·ado kinase	56
dTMP synthase/dihydrofolate reductase	56, 56, 56, 56.7, 70, 70, 100
IMP synthase (*pur 9,10*)	67, 71
Tetrahydrofolate synthase	95, 100, 100, 104, 108.5
Phosphoribosylaminoimidazole synthase (*pur 2,3,5*)	102, 110, 150
Dihydroorotate synthase (CAD, *pyr 1-3*)	220, 235, 240

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