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Molecular Varieties of Isozymes[1]

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During the last 20 years great advances have occurred in the extraction, purification and characterization of proteins. These advances have been based upon the development of new techniques for working with these large molecules and upon conceptual clarity concerning the synthesis, structure and function of proteins. Extraction and purification of proteins have always posed difficult problems. Not only must the primary structure, the linear sequence of amino acids, be kept intact, but the overall conformation of the molecule, the secondary, tertiary and quaternary structure, must be maintained if the physiological or catalytic activity of the protein is to be preserved. Denaturation and loss of activity are common consequences of attempts to purify proteins. Many laboratory preparations of proteins are mixtures of native and denatured molecules. Recognition of such heterogeneity has made biochemists reluctant to ascribe the heterogeneity found in laboratory preparations to a corresponding heterogeneity within the cell. Such reluctance is justified because the properties of proteins, as extracted and measured in buffer solution, may very well be quite different from what they were within the cell. Nevertheless many claims for the heterogeneity of single species of proteins are now well substantiated because of the development of modern methods for resolving proteins, particularly chromatographic and electrophoretic methods. Until these methods were developed, most claims for heterogeneity among single enzymes were rejected as artifacts of the processes of preparation.

SMITHIES[2] introduced zone electrophoresis in starch gels as a technique of high resolving power for separating different proteins in homogenates or in blood serum. This method has proved extraordinarily fruitful and is now employed routinely in laboratories throughout the world. When HUNTER and MARKERT[3] combined this technique successfully with histochemical staining procedures for the demonstration of esterases, it became possible to resolve and identify many enzymes in tissue homogenates with ease and precision. For the esterases, this coupling of zone electrophoresis and histochemical staining revealed a vast multiplicity of molecular forms. This demonstration helped explain the different properties of esterase preparations as obtained from different tissues. The differences in properties clearly stemmed from different mixtures of the same esterases and not from uniquely different esterases characteristic of each tissue.

Later this same approach was applied by MARKERT and MØLLER[4] to a number of additional enzymes, particularly to the enzyme lactate dehydrogenase. This enzyme also proved to be heterogeneous and was found to exist in 5 distinct molecular varieties in most mammalian cells. Other techniques that exploit the differences in physical, chemical, immunochemical or kinetic properties of the enzyme molecule have also been used successfully to demonstrate the existence of heterogeneity in enzyme preparations. These various types of analyses have revealed that many enzymes, more than 100 at least, exist in multiple molecular forms in the tissues of single organisms. Such multiple forms of enzymes have been designated *isozymes*, 'the different molecular forms in which proteins may exist with the same enzymatic specificity'[4]. This term (sometimes spelled isoenzyme) served to facilitate communication, simplified and clarified the frame of reference, and most important focused attention on a significant but hitherto neglected biological phenomenon, namely that organisms commonly do synthesize many of their enzymes in several different molecular forms, presumably to fulfill the specialized needs of their different cells or of different metabolic pathways within the cell.

The original definition of isozyme was designed to provide operational utility only and not to define the molecular basis for any particular set of isozymes. The vast literature on isozymes accumulated during the last several years has demonstrated the usefulness of the term, although some new problems of nomenclature

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have arisen. The most useful definition still seems to be the original operational definition. Whatever ambiguities exist stem largely from the definition of an enzyme, but that is an enzymological problem and not a problem in isozyme nomenclature. Once the accepted criteria for defining a collection of molecules as an enzyme have been successfully applied, then if these molecules by any means, electrophoretic, chromatographic, solubility, immunochemical and so forth, can be separated into distinguishable types, then these types represent isozymic forms of the enzyme. Such a broad operational definition embraces several distinct kinds of molecular isozymic systems, and it may be desirable to distinguish these from one another by appropriate adjectives. Thus one might modify isozyme with such terms as allelic, non-allelic, homopolymeric, heteropolymeric, conformational, hybrid, conjugated and so forth. The purpose of this brief review on enzyme heterogeneity is to describe the different molecular bases for the existence of isozymic systems. No comprehensive account of all the different isozymes will be presented, but the molecular basis for each different isozymic system will be described and illustrated with a few specific examples.

Microheterogeneity

Since protein molecules are very large, involving the linking together of up to several hundred amino acid residues, the possibility of errors in synthesis obviously exists. The accumulation of such errors would yield a kind of microheterogeneity[5] so that a purified preparation of a single protein species would, in fact, consist of many slightly different molecules. Some evidence has indeed been presented for errors at the translational level of protein synthesis, as for example in the synthesis of hemoglobin[6,7], but it appears improbable that such mistakes, even if they occur, could account for much of the protein heterogeneity found in cells.

Conformational Isozymes

Folding of the long chains of amino acids leads to the secondary and tertiary structure of proteins, conformations which are essential to functional activity. Such tertiary states have different degrees of stability in different proteins, some being readily denatured into random coils and others being held in relatively rigid conformations by cross links between the chains, such as by disulfide bonds. Because of their great length it may be possible for polypeptide chains to fold into alternate configurations with similar stabilities. Small changes in the tertiary structure undoubtedly occur during catalytic activity and during association with other molecules, particularly with other proteins in the cell. Allosteric transitions are such examples of alterations of the tertiary and/or quaternary structure associated with the activity of the enzyme. Whether or not alternative stable conformational states are probable has been much debated among physical chemists, but considerable evidence does point to their existence. However, these alternate conformations may stem from modifications of primary structure induced by the conjugation of small or large molecules to the protein. Conformational isozymes have been reported for several polymeric enzymes, but these alternate conformations may have arisen from the quaternary state of the aggregate rather than from the tertiary state of the individual subunits – that is, different kinds of subunits may be able to associate in a variety of permutations each with distinct electrophoretic properties. In this paper we shall consider conformational isozymes as those isozymes which differ *only* in their tertiary structure or subunit arrangements.

Isozymes generated solely by conformational differences as opposed to isozymes whose conformational differences are secondary to some other alteration can now be distinguished[8,9]. If, in fact, a set of isozymes differ only in conformation, then unfolding these proteins to a random coil should reduce them to a common state. Renaturation in the same environment should generate the total isozyme pattern from each original isozyme. However, if the initial differences were not due to conformation, then renaturation would not produce identical isozyme patterns. The principal method for detecting conformational isozymes involves electrophoretic resolution in gels, but additional techniques can also be applied, such as thermostability, kinetic, immunochemical and inhibition analyses[10–13]. CANN and GOAD[14] have suggested that multiple banding in gels for single protein species can be generated by the electrophoretic conditions. Their demonstration of such artifactual multiple banding, however, is based upon concentrations of the proteins that are very different from those commonly used during resolution of isozymes from tissue homogenates.

Although many conformational isozymes are clearly not simple artifacts of preparation, their reality within the cell can be questioned. However, several lines of evidence point to their existence within the cell and lead to inferences concerning their physiological utility.

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An esterase of maize, encoded by a single gene, when extracted from different plant tissues exhibits differences in stability to urea denaturation[15–17]. These results suggest that the esterase exists in several different conformations some more sensitive to urea than others. Presumably these conformations are determined by the cellular environment in which the enzyme is synthesized. Another example stems from the sub-bands of lactate dehydrogenase (LDH). These sub-bands are 2 or more isozymes appearing at an electrophoretic position where only 1 would be expected from genetic considerations. Apparently they are generated epigenetically. The electrophoretic resolution of LDH from many mammalian species shows the presence of sub-bands at several LDH positions, particularly in the areas of LDH-2, -3, -4 and -5 (Figure 1). The number of these sub-bands and their relative abundance is characteristic of the tissue from which the LDH was extracted[18,19]. This tissue specificity strongly suggests that the sub-bands are native molecular configurations within the cell. In many cases the LDH sub-bands are very stable. Electrophoretic separation and re-electrophoresis of such sub-bands does not readily generate additional isozymes[18–20].

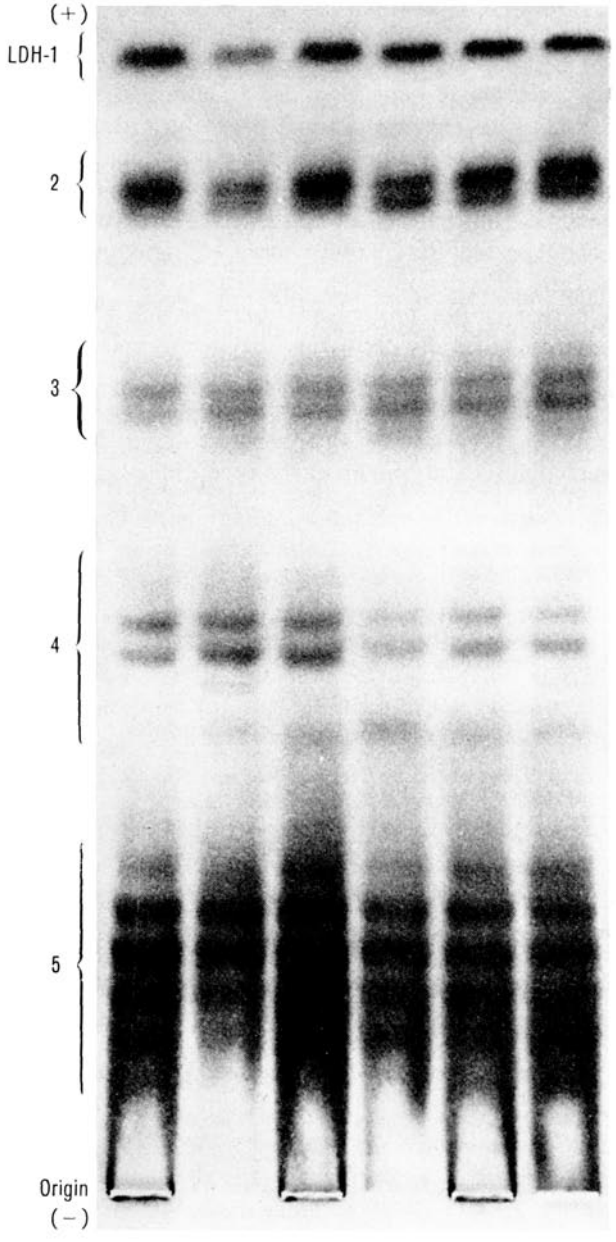

Fig. 1. Subbands of lactate dehydrogenase isozymes in mouse (*Mus musculus*) tissues. Note the multiple banding at all LDH positions except LDH-1.

Perhaps the most studied enzyme, so far as conformational isozymic forms are concerned, is malate dehydrogenase (MDH). This enzyme exists in a variety of isozymic forms and, at least in several organisms, some of these different forms are readily interconvertible by treatment with reducing agents[10], or by reversible acid inactivation[12,13]. The supernatant MDH isozymes of the marine snail *Ilyanassa obsoleta* are all converted to 1 form by treating them with β-mercaptoethanol; this single cathodal isozyme can then regenerate all the original isozymes if the reducing agent is removed by dialysis[10] (Figure 2). The interconversion of these *Ilyanassa* isozymes can also be accomplished without the use of reducing agents. A single MDH isozyme eluted from a starch gel after electrophoresis will, when subjected to re-electrophoresis, generate the other isozymes (Figure 3). This phenomenon has also been observed for the MDH isozymes of *Neurospora crassa*[21,22].

The molecular bases of mitochondrial MDH isozymes of chicken heart have also been investigated[12,13]. When a single isolated enzyme was treated with reversible acid denaturation several isozymes were generated. The 5 isozymes investigated were not significantly different in mol. wt., sp. act., response to inhibition, reactivity with coenzyme analogues or amino acid composition. However, differences were detected among them by semi-microcomplement fixation and by optical rotatory dispersion. All these data suggest that these MDH isozymes are conformational isozymes. More recently, SCHECHTER and EPSTEIN[8], reversibly denatured mitochondrial MDH isozymes from chicken heart with acid and with 7.6 M guanidine-HCl. However, they were unable to generate new isozymes or to shift substantially the isozyme pattern. These results indicate to those authors that the separated MDH isozymes renatured only to their original configuration. However, this issue

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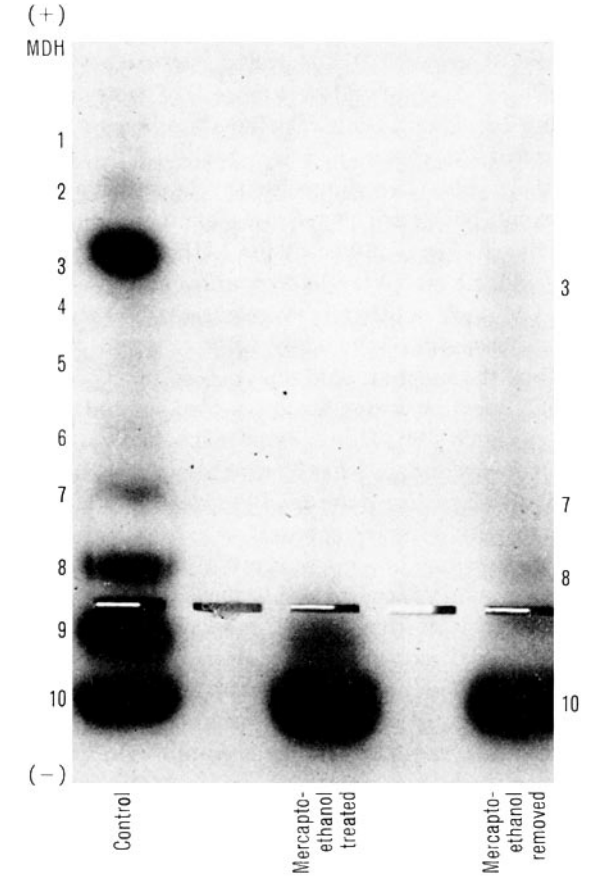

Fig. 2. Interconversion of *Ilyanassa obsoleta* malate dehydrogenase isozymes. Treatment of the MDH isozymes (as represented by the control) with mercaptoethanol converts them mainly to isozymic form number 10. Removal of mercaptoethanol by dialysis results in the regeneration of the original isozymes.

is not yet resolved. 2 of the most important criteria that need to be met before judging whether isozymes differ only in conformation are (1) proof of complete denaturation to a random coil prior to renaturation and (2) exclusion of ligand binding as a cause of the alternate isozymic forms.

Other enzymes reported to exist as conformational isozymes are: glutamate dehydrogenase[23–25], L-amino acid oxidase[26], oxytocinase[27], creatine kinase[28], cholinesterase[29], fructose diphosphatase[30], adenosine deaminase[31] and penicillinase[32].

Cleavage of peptide chain

The polypeptide chain encoded in a gene and initially synthesized is not always the physiologically active form of the molecule. Proteolytic removal of a part of the polypeptide may be essential to confer activity, or the proteolysis may give rise to alternate isozymic forms of an enzyme. Partial proteolytic degradation without loss of enzymatic activity occasionally occurs in vitro during enzyme purification. Examples of such enzymes converted to alternate active forms in vitro by partial proteolysis are phosphoglyceric acid mutase[33,34], lactate dehydrogenase[35], and carboxypeptidase[36,37]. Whether or not proteolysis occurs within the cell to generate naturally occurring isozymic forms of various enzymes is uncertain. However, a few pro-enzymes are converted to the active enzyme by this process. For example, trypsinogen is converted to trypsin through partial autocatalysis. Procarboxypeptidase is similarly

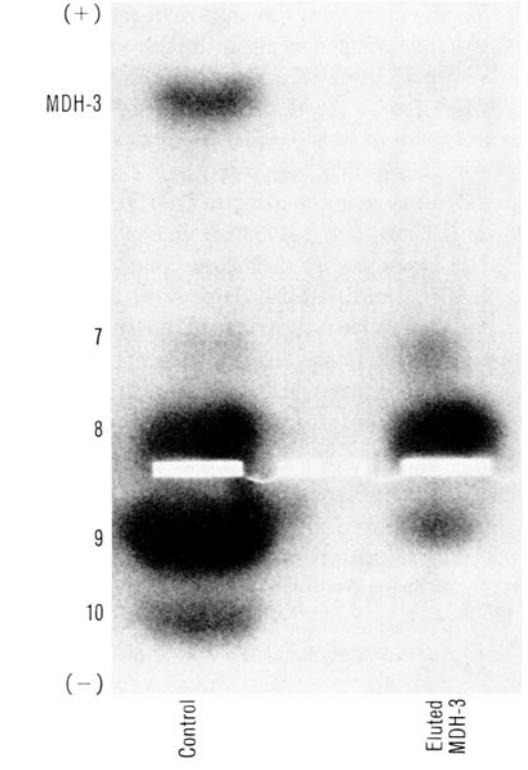

Fig. 3. Electrophoresis of the *I. obsoleta* MDH-3 isozyme after elution from a starch gel. Note that isozymes 7, 8 and 9 are formed from the MDH-3. The control channel contained an aliquot from the original tissue homogenate.

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converted to active carboxypeptidase through proteolytic removal of an inhibiting peptide. Carboxypeptidase A-1 may be converted to carboxypeptidase A-2 through the action of trypsin in removing a single dipeptide. These 2 forms of carboxypeptidase are distinguishable electrophoretically, but appear to have identical chemical properties[36,37]. Hexokinase of yeast is an often quoted example of an enzyme existing in a variety of isozymic forms as a consequence of partial proteolytic degradation of the enzyme. Whether or not these different isozymic forms of hexokinase exist within the yeast cell itself is still uncertain, but the active forms of the enzyme are readily generated in homogenates[38–40].

MASTERS[41] has reported a similar phenomenon for aldolase. He noted that electrophoretic mobility of the isozymes could be altered by prior incubation of tissue extracts with supernatant from a tissue which exhibits the altered isozyme pattern normally. He believes this supports the concept of a modification of aldolases in tissues, presumably by peptidases.

Polymeric series

In recent years we have become aware of the fact that most enzymes are polymers, either homopolymers composed of identical subunits or heteropolymers composed of different kinds of subunits. Many of the isozymes of polymeric enzymes are the same molecular size and do not form a polymer series; i.e., monomer, dimer, tetramer of the same basic units. However, there are several notable examples of a polymeric series of isozymes in which the molecular size varies over a considerable range. Glutamate dehydrogenase is such an enzyme in which the molecular weight may vary from 250,000–1,000,000[42]. The state of aggregation of this particular enzyme is significantly influenced by the presence of steroids, nucleotides, coenzymes and the concentration of the enzyme[23,24,42,43]. All of these substances can be shown to be active in vitro in affecting polymer size, and the presumption is that these same molecules or similar ones act to regulate the degree of aggregation within the cell. The state of aggregation probably plays an important role in the regulation of enzyme activity in specific metabolic pathways.

Deoxythymidine kinase extracted from *Escherichia coli* can exist as either a monomer or as a dimer, the state of aggregation being strongly influenced by various trinucleotides[44]. The monomer is relatively sensitive to temperature, whereas the dimer is much more stable. Thus these 2 forms of the enzyme may serve as a regulatory arrangement for governing the function and stability of the enzyme in the cell. β-Amylase in barley exists in a variety of polymeric states that are readily affected by oxidizing or reducing environments[45–47]. A similar phenomenon has been found to be true for urease[48–50]. The urease subunits appear to be held together by disulfide bridges.

One of the most extensively studied complex enzymes existing in isozymic forms is glycogen phosphorylase. Several conflicting analyses have been reported, but the recent work of DAVIS et al.[51], appears to present a coherent scheme for the interrelationship of the various isozymes of rabbit glycogen phosphorylase. There are 3 distinct *b* forms of the enzyme none of which are phosphorylated, I^b, II^b and III^b. Upon phosphorylation, these 3 *b* forms are converted into their corresponding *a* forms which have slightly different electrophoretic mobilities as compared to their *b* counterparts. The mol. wt. of the I^b form and the I^a form is about 200,000 each. These molecules are dimers, whether phosphorylated or not. The II^b form is also a dimer of the same mol. wt., but when phosphorylated to make the II^a isozyme, it then assumes a configuration that allows it to exist in a state which is in rapid equilibrium with a tetramer. The III^b form is somewhat smaller than the other 2, with a mol. wt. of about 185,000. This molecule is also a dimer, presumably a homopolymer, and when converted to the III^a form by phosphorylation it also undergoes a tetramerization to produce a molecule of about 370,000 mol. wt. In addition, it is presumed that the II^b isozyme is a heteropolymer composed of subunits, one representing the type found in the I^b isozyme and the other the type found in the III^b isozyme. VALENTINE and CHIGNALL[52] also indicate that phosphorylase *b* contains 2 types of subunits. Therefore, the II^b form is probably a heteropolymer, whereas the other 2 unphosphorylated isozymes are probably homopolymers (Figures 4). These various isozymic forms of glycogen phosphorylase do not all have the same sp. act., and the interconversion of one to another regulates the total phosphorylase activity within the cell. Other workers suggest that both phosphorylase *a* and *b* appear to be in a slow equilibrium between dimer and

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tetramer[53]. The equilibrium is displaced in favor of the tetramer by adenosine monophosphate (AMP), which is an allosteric activator. After treatment with *p*-mercuribenzoate, phosphorylase *a* forms a complete progression of successive polymers (monomer, dimer, trimer, tetramer, etc.) as revealed by electrophoretic and sedimentation analysis[53].

Additional enzymes that have been reported to exist as a polymeric series are the following: glucose 6-phosphate dehydrogenase[54], glycollate oxidase[55], xanthine dehydrogenase[56], L-amino acid oxidase[57,58], hexokinase[40,59,60], phosphofructokinase[61], acetylcholinestrase[62], chorismate mutase[63], β-galactosidase[64], apyrase[65,66], fructosediphosphate aldolase[67,68], threonine dehydratase[69], thiosulphate sulphurtransferase[70], β-fructofuranosidase[71,72], DNA polymerase[73], and *o*-Diphenol oxidase[74]. Whether all of these enzymes exist as a polymeric series within the cell is not completely established. Some ambiguity is usually associated with an effort to demonstrate a polymeric series because many proteins tend to aggregate in vitro.

Chemically modified polypeptides

One mechanism for producing enzymatic heterogeneity in cells is to couple the enzyme molecule with other kinds of molecules, either large or small. Such coupling may require enzymatic intervention or it may occur spontaneously. Many proteins are covalently bonded to other molecules, particularly nucleotides and carbohydrates, and some are attached to membranes, possibly through covalent linkage to other proteins. Such attached molecules, if broken loose from the membranes, might well be different in size and charge by virtue of differences in the size of the attached protein. Thus an enzyme might exist in 2 basic states, 1 bound to a protein and 1 in a soluble form, both active and electrophoretically distinct. The penicillinase of *Bacillus licheniformis* exists in 2 forms, 1 covalently bound to the cell membrane through a peptide chain, the other a free soluble form[75]. Both forms are active with similar kinetic and immunochemical properties but are electrophoretically distinct. There is also a bound and a free form of aminopeptidase[76]. The presence of 1 of the glucose-6-phosphate dehydrogenase isozymes is attributed to the complexing of 1 of its subunits to another protein by covalent bonds[54].

One common type of molecule normally bound to many enzymes is the coenzyme, either NAD or NADP. Such binding may occur at the catalytic site, as well as elsewhere on the protein moiety. Whether or not all the varieties of heterogeneity generated by chemical modification of polypeptide chains can be usefully described as isozymic forms of biological significance is a moot question. Alcohol dehydrogenase from *Drosophila*[77,78], and from horse tissues[79] when resolved electrophoretically commonly exist as several discrete bands. Some of

	Non-phosphorylated isozymes require AMP	Phosphorylated isozymes do not require AMP
	I^b Phosphorylase kinase + ATP →	I^a
Molecular weight	200,000	200,000
Polymer	dimer	dimer
Subunit composition	I_2^b	I_2^b
Mobility in electrophoresis	most anodal	slightly anodal to I^b
Tissue location	heart muscle	
	II^b Phosphorylase kinase + ATP →	II^a
Molecular weight	200,000	(dimer in equilibrium
Polymer	dimer	with a tetramer)*
Subunit composition	$I_1^b\ III_1^b$	
Mobility in electrophoresis	intermediate	slightly anodal to II^b
Tissue location	heart muscle	
	III^b Phosphorylase kinase + ATP →	III^a
Molecular weight	185,000	(370,000)*
Polymer	dimer	tetramer
Subunit composition	III_2^b	III_4^b
Mobility in electrophoresis	least anodal	slightly anodal to III^b
Tissue location	heart muscle and skeletal muscle	

* Value obtained by sedimentation analysis; however the behavior of the III^a isozyme during electrophoresis in the gel is that expected for a dimer. Data from DAVIS et al.[51].

Fig. 4. Glycogen phosphorylase *b* and *a* isozymes of rabbit heart and skeletal muscle.

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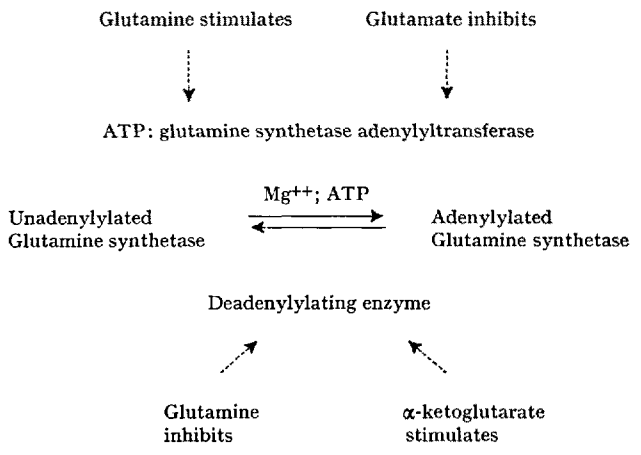
these isozymes are asserted to arise through the binding of varying numbers of coenzyme molecules to a single protein, thus generating the molecular heterogeneity actually observed. Metals also may function as cofactors. At least in 1 case – that of arginase[80], it has been reported that the enzyme exists in human beings in 2 electrophoretically distinct forms. The difference in migration was attributed to differences in the amount of manganese bound to the isozymes. This investigation should be extended but it is not at all certain that differences in the amount of bound manganese would either be characteristic of the enzyme within the cell or have biological significance.

Carbohydrate moieties are among the most common molecular conjugants with proteins, and 1 which frequently appears is sialic acid. Alkaline phosphatase is bound to sialic acid, and removal of the sialic acid by neuraminidase treatment changes electrophoretic mobility[81]. In extracts of kidney several alkaline phosphatase fractions have been found. After treatment with neuraminidase, these can all be reduced to a more slowly migrating form[81]. Thus the initial differences in mobility can be attributed to different amounts of bound sialic acid. A somewhat similar situation has been found by other investigators for acid phosphatase extracted from various human organs[82,83]. At least 4 isozymes of acid phosphatase have been found, and 2 of these can be reduced to the same electrophoretic variety by treatment with neuraminidase[82]. Apparently the distinction between these 2 lies in the amount of sialic acid they contain.

Nucleotides may be covalently bound to enzymes or removed from them by enzymatic processes. An interesting recent case has been presented by HOLZER's group[84,85] and STADTMAN's group[86–88] for the enzyme glutamine synthetase. This enzyme can exist in at least 2 different forms in *E. coli*. These isozymes have the same mol. wt. and essentially the same amino acid composition. The main difference between them is the different amounts of covalently bound AMP. There are 2 main isozymes, 1 with no covalently bound AMP and the other with 12 molecules of AMP bound per mole of enzyme. These isozymes are enzymatically intercovertible as shown in the scheme from SHAPIRO and STADTMAN[88].



Glutamine synthetase presents 1 of the most complex examples of epigenetic generation of isozymes. The 2 main isozymes generated in this manner differ in their sp. act., divalent cation specificity, pH optima, and susceptibility to inhibition by various end products of glutamine metabolism.

Since each of the 12 subunits of the enzyme may bind 1 AMP molecule, the possibility for a large amount of molecular heterogeneity exists. In the growth of *E. coli*, 1 state of nitrogen nutrition favors the almost exclusive production of adenylylated enzyme, whereas a different state of nitrogen nutrition favors production of the form without the AMP. Therefore, the coupling of AMP to the enzyme is almost certainly of physiological significance.

A somewhat less extensive modification of protein occurs through phosphorylation. At least 3 enzymes are characterized by 2 alternate forms generated by phosphorylating the protein. These are glycogen phosphorylase[51], glycogen synthetase[89–91] and phosphoglucomutase[92]. Each of these exists in at least 2 distinct forms, 1 phosphorylated and 1 not. The shift from the phosphorylated to the non-phosphorylated form of each of these enzymes probably plays a significant role in the regulation of enzymatic function.

Many enzymes contain acetyl groups, but whether these are the source of molecular heterogeneity is not generally known. However at least in the case of LDH it has been shown by STEGINK and VESTLING[93] that acetyl groups are present in some of the isozymes and other workers[94,95] have been able to alter the electrophoretic and immunochemical properties of the LDH

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molecule by in vitro acetylation. Nonetheless, acetylation of LDH is not the source of the major isozymic forms of this enzyme but may play a role in the subbanding so commonly seen in LDH preparations from many organisms. There are, of course, many different ways in which the amino acid residues on proteins can be chemically modified. Some of these might involve decarboxylation or removal of amino groups, but there is little direct evidence for enzyme heterogeneity generated by such chemical modifications. However in the preparation of DNAse from spleen, 2 different forms of DNAse were produced[96]. The authors concluded that the major component of DNAse was derived from the minor component by deamidation occurring during the acid extraction of the enzyme from the tissue. Although this is an artifactual generation of enzymatic heterogeneity, it does illustrate the possibility that such conversions might be normally exploited by cells.

The essential role of sulfhydryl groups and disulphide linkages in maintaining normal enzymatic activity is well known. The enzyme citrate synthetase[97] exists in 2 distinct molecular varieties which can be interconverted by treatment with reagents which either break SS-bonds or produce them. Both of these forms of citrate synthetase are active, and therefore one might conclude that such forms are naturally occurring isozymes within the cell. In a recent paper by CANTZ et al.[98], the enzyme catalase in the erythrocytes of humans and horses has been demonstrated to exist as 2 or 3 isozymes by DEAE cellulose chromatography and by electrophoresis. These isozymes are interconvertible by oxidative and reductive processes affecting the –SH and –SS-groups, respectively. These groups could alter conformation at the level of the tertiary protein structure (disulfide linkage within the same chain) or of the quaternary structure (disulfide links between the polypeptide chains).

Gene control of isozymes

It is now well known that individual polypeptides are encoded in individual genes. Thus the old 1 gene-1 enzyme concept has been modified to be 1 gene-1 polypeptide. When enzymes are composed of more than 1 kind of polypeptide then more than 1 gene contributes to the final structure of the enzyme. Thus 2 genes might code for a single enzyme, or if a variety of polymers can be formed, then 2 genes could generate several different isozymic forms. In organisms normally synthesizing homopolymers from subunits encoded in a single gene, the presence of allelic variants of this gene not infrequently gives rise to hybrid heteropolymeric isozymes. Some of these allelic variants leading to allelic heteropolymeric isozymes are glutamate dehydrogenase[99], peptidases[100], lactate dehydrogenase[101,102], ADH[103–107], malate dehydrogenase[108–111], isocitrate dehydrogenase[112–114], phosphogluconate dehydrogenase[115,116], glucose-6-phosphate dehydrogenase[117,118], α-glycerolphosphate dehydrogenase[119], aldehyde oxidase[120], xanthine dehydrogenase[121], catalase[122–124], esterase[125–130], alkaline phosphatase[131,132], acid phosphatase[133], β-glucosidase[134] and leucine aminopeptidase[135].

The fact that heteropolymeric isozymes are often formed in individuals heterozygous at 1 locus demonstrates that such molecules are biologically and thermodynamically possible. In fact, a few heteropolymeric enzymes encoded by 2 or more loci have been reported; e.g., lactate dehydrogenase, aldolase and creatine kinase. These isozymic systems provide a spectrum of molecules of finely graded properties which meet the needs of the cell, and thus have been preserved during evolution.

Moreover, even genes that initially coded for unassociated polypeptides with different functions can also evolve so that their polypeptide products acquire

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the capacity to associate with one another in multienzyme complexes such as tryptophan synthetase, pyruvate dehydrogenase, and fatty acid synthetase[136]. This is particularly true for enzymes that carry out sequential steps in a metabolic pathway such as the pathway for histidine synthesis[137], tryptophan synthesis[138] or aromatic amino acid synthesis[139].

The same evolutionary opportunities (involving gene duplication and divergence through mutation) must have existed for all homopolymeric enzymes. When no heteropolymeric forms of the enzyme are found, one must conclude that, although they probably arose many times, they must have been rejected during the course of evolution.

The existence of isozymes under entirely separate genetic control opens attractive prospects for investigating the genetic regulation of cell metabolism. The independent feedback inhibition of such isozymes each catalyzing the formation of a common precursor leading to several different end products permits a refined discrimination in regulating the synthesis of the products. This isozymic arrangement characterizes the enzyme aspartokinase in which the individual isozymes are differentially inhibited by amino acids[140,141].

A different type of metabolic control through isozymes is provided by 2 dehydroquinases in *N. crassa*[142]. There are 2 isozymes, 1 constitutive and 1 inducible. The constitutive isozyme, which is thermolabile, is part of an enzyme aggregate (mol. wt. 200,000) that functions in the pathway for the synthesis of aromatic compounds. The inducible isozyme, which is thermostable (inducible in wild type or in mutants for the constitutive enzyme) has a mol. wt. of 150,000 and functions in the degradative aromatic pathway. The substrates of these isozymes are kept separate by the utilization of a multienzyme aggregate which effectively isolates the synthetic pathway. A similar example of isozymes synthesized by different loci, 1 form being constitutive the other inducible is that of α-ketoglutaric semialdehyde dehydrogenase[143].

One of the most cogent reasons for the infrequent sharing of subunits among sets of isozymes probably resides in the critical nature of protein-protein interactions within the cell. Protein-protein interactions are commonly essential for the physiological activity of enzymes in cells, and this is particularly true for polymeric enzymes. Any change in a protein that alters its association with other parts of the molecular machinery for a given metabolic pathway would influence its physiological efficiency. Amino acid substitutions that altered the net charge on a protein would be particularly significant. If the change in properties, such as surface charge distribution, were great enough, the normal topographic localization of the molecule within the cell would be prevented. Perhaps the absence of β-glucuronidase from the ergastoplasm of liver cells in certain mutant strains of mice is such an example[144].

Recent work by MUNKRES et al.[145,146] clearly demonstrates the significance of protein structure in regulating the association of proteins to carry out normal metabolic activities. These workers demonstrated that a single amino acid replacement in a mitochondrial structural protein altered the ability of the mitochondria to bind malate dehydrogenase, both in vitro and in vivo. Furthermore, the altered binding led to physiological changes in the function of the mitochondria. In this instance, the structural protein, not the enzyme, was directly altered. However, there are mutants which have a similar effect by directly altering the MDH molecules.

There is also considerable indirect evidence pointing to the importance of net charge in regulating the association of an enzyme with other parts of the cell. This phenomenon is exemplified by LDH. Each species has a fixed repertory of LDH isozymes each with its characteristic net charge; other species have corresponding sets of isozymes with equally characteristic but different net charges. Thus each isozyme of LDH can obviously exist in a variety of functional molecular forms with different net charges. Yet within any 1 species only certain types are found, with the exception of occasional mutants. The selection pressure for maintaining the net charge on an isozyme of LDH in any particular species must therefore be very strong. Since the mutual relationships among macromolecules of the cell are the product of long selection, any change would nearly always prove to be deleterious to the cell and in due course be eliminated. Rarely, a change in net charge would so improve the function of the enzyme that it would persist even in the face of less than optimum interaction with many other proteins in the cell. In such circumstances, selection pressure in favor of new complementary changes in other proteins would exist and mutations changing their charge properties so that they now interacted effectively with the newly mutated enzyme would clearly be favored, even though these same mutations would have been deleterious before the mutated enzyme came into existence. On this view, the

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difference between species in isozyme patterns and the essential uniformity within a species becomes comprehensible, plausible and expected.

Multiple loci, controlling polypeptide subunits that aggregate to form heteropolymeric enzymes, present problems for the mechanisms that identify and regulate the function of these loci. Although such isozymes are fulfilling similar catalytic functions in the cell and are related in other ways, the mechanisms for regulating the corresponding genes must nevertheless be able to distinguish them and to fix independently their relative activities. Such separate control could be achieved by placing the loci on different chromosomes or in different blocks of genes that are controlled independently. The genetic control of isozymes is best illustrated by the enzyme lactate dehydrogenase. This enzyme is present in isozymic forms in nearly all vertebrates and in many invertebrates as well. The molecular bases of the LDH isozymes was first elucidated by APPELLA and MARKERT[147] who succeeded in dissociating LDH into 4 equal sized polypeptide subunits, mol. wt. 35,000, by the use of urea and guanidine hydrochloride treatments. Mixtures of the LDH isozymes after dissociation to subunits contained 2 protein bands as revealed by starch gel electrophoresis. The authors postulated that the 5 LDH isozymes were formed by the random tetrameric association of 2 subunits; i.e., LDH-1 = B_4, LDH-2 = A_1B_3, LDH-3 = A_2B_2, LDH-4 = A_3B_1, and LDH-5 = A_4.

MARKERT[148] suggested that the 2 subunits, if they were different proteins, must be coded by separate loci. The demonstration that LDH-1 and LDH-5 differed in amino acid composition and in their peptide maps[149] established the separate genetic nature of these subunits. Further proof of the structure of LDH was obtained by reversibly dissociating mixtures of LDH-1 and LDH-5 by freezing and thawing in phosphate buffer and NaCl. This procedure generates all 5 isozymes in binomial proportions[150] (Figure 5). Extending this simple but elegant procedure to the LDH isozymes of other species, revealed that in vitro hybridization could occur between LDHs obtained from all vertebrate classes, indicating considerable molecular homology and evolutionary conservatism (Figure 6).

Genetic confirmation of the subunit hypothesis was first brought forth by SHAW and BARTO[102]. They discovered in the field mouse *Peromyscus maniculatus* 2 allelic variants at the ***B*** locus. Heterozygotes produced the predictable 15 isozymes from the 3 subunits, ***A***, ***B***, ***B***^{1}, that were synthesized.

Nearly all vertebrates contain at least 2 genes coding for LDH subunits, but there is good evidence that a third and even a fourth gene may be involved in the synthesis of LDH in certain vertebrates[151]. If all these polypeptide subunits were freely accessible to one another and combined at random to make tetrameric molecules, then for 2 genes, 5 isozymes would be formed, for 3 genes, 15 isozymes, and for 4 genes, 35 isozymes as shown by Figure 7. The LDH isozyme composition of vertebrates ranges from 1 to as many as 18 or more. Thus the maximum theoretical number of tetramers is seldom found. One possible explanation is that all of the LDH genes do not function simultaneously in each cell. Another possibility is that the LDH subunits, though produced, will not form all of the expected combinations. In mammals and birds and in many other vertebrates at least 2 of the genes for LDH synthesis are active in each cell; thus 5 isozymes, tetrameric combinations of the 2 different kinds of subunits, are formed. However, in many fish and amphibia, the 2 LDH subunits do not combine at random with one another to make the 5 expected tetramers. Some fish

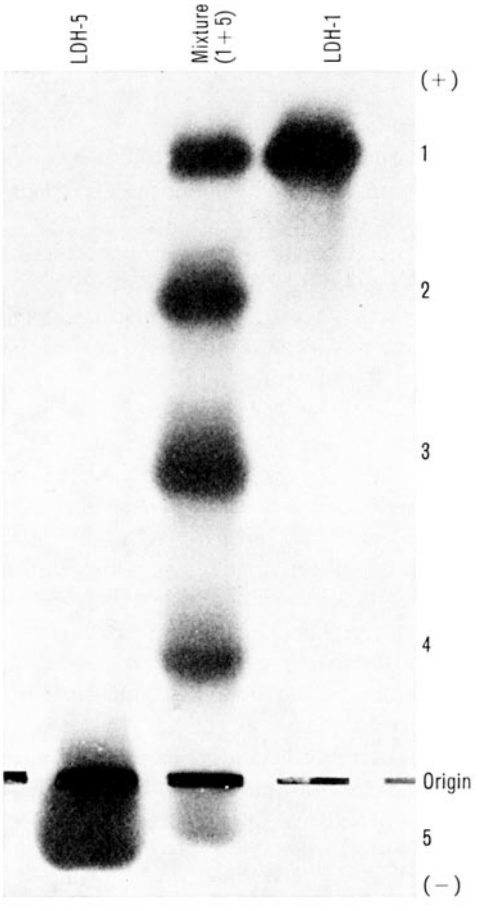

Fig. 5. In vitro hybridization of *Bos taurus* lactate dehydrogenase isozymes. Freezing and thawing mixtures of LDH-1 and LDH-5 generates all 5 isozymes (the 2 homotetramers and the 3 heterotetramers) in a binomial distribution.

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produce only 2 isozymes of LDH, presumably the 2 homopolymers; and other fish produce 3 isozymes, the 2 homopolymers and the symmetrical heteropolymer. These isozymes are tetramers that may be dissociated to subunits that will then combine at random with unrelated LDH subunits to produce 5 tetrameric molecules. Thus the failure to form the expected 5 isozymes in vivo is due to the genetically imposed structure of the polypeptide chain, a structure which specifically rejects combination with alternate types of subunits present in the same species. Where 3 genes are active, as in most mammals, the third gene does not contribute a subunit that is freely available for combination with the ***A*** and ***B*** subunits, because of the restriction of the activity of this gene to primary spermatocytes where the other 2 genes are non-functional. A corresponding case exists in the eyes of many fish in which an additional LDH subunit is synthesized. These subunits form tetra-

$$I = \frac{(S + N - 1)!}{N!\,(S - 1)!}$$

N \ S	1	2	3	4
2	1	3	6	10
3	1	4	10	20
4	1	5	15	35

Fig. 7. Formula and chart for the determination of the number of isozymes expected from random assemblage of subunits into all possible polymers. (I) No. of isozymes; (N) No. of subunits/polymer; (S) No. of different subunits.

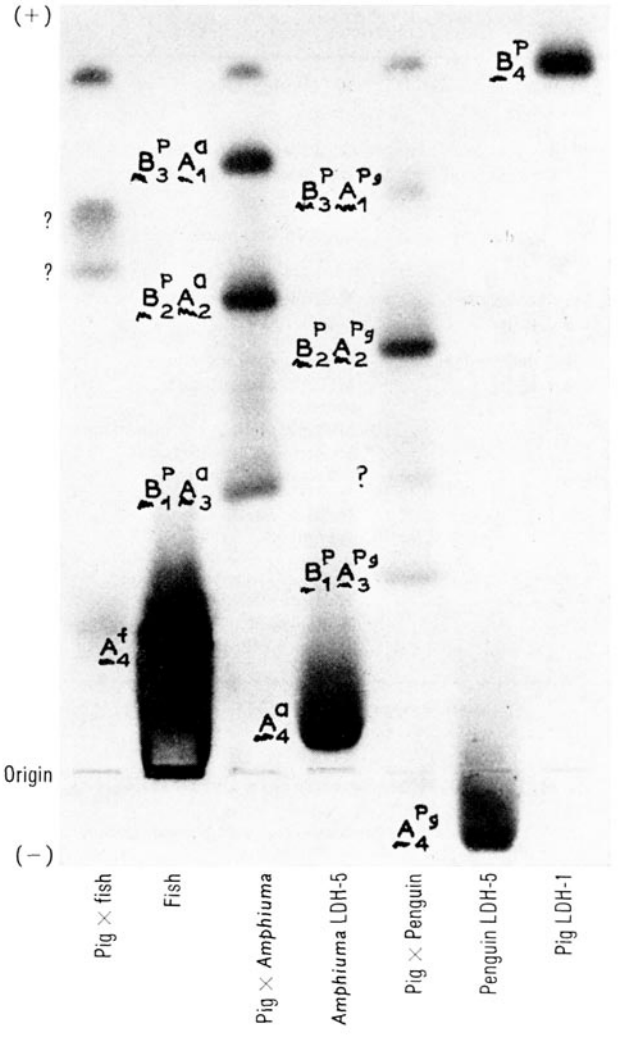

Fig. 6. Hybridization of lactate dehydrogenase isozymes between vertebrate classes. The LDH-1 isozyme of pig (*Sus scrofa*) was hybridized against LDH-5 isozymes from penguin (*Pygoscelis adeliae*), *Amphiuma sp.* and fish (*Paralichthys dentatus*). The subunit composition of the principal isozymes is indicated on the Figure.

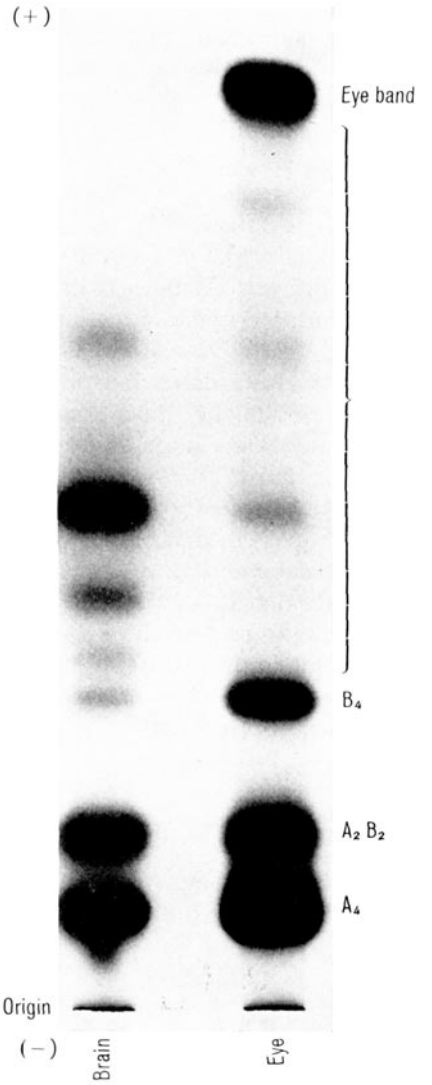

Fig. 8. Lactate dehydrogenase isozyme patterns of the eye and brain of mackerel (*Scomber scombrus*). The mackerel is a three-isozyme fish in which only LDH-5 (A_4), LDH-3 (A_2B_2) and LDH-1 (B_4) are present in most tissues. However in the eye and brain additional isozymes appear anodal to the main three. These are probably under the control of a separate genetic system. The bracketed region indicates heteropolymeric isozymes presumably formed from the subunits of the eye band and *A* or *B* subunits.

meric molecules but in some fish do not have free access to ***A*** and ***B*** subunits in vivo so that fewer than the expected 15 isozymes are found on analysis of fish eyes (Figure 8).

Epigenetic control over isozyme patterns is indicated by the failure to form the expected tetramers in the expected ratios. This occurs in certain fish such as the alewife (*Alosa pseudoharengus*) in which 5 isozymes are formed but in non-binomial proportions. The asymmetrical heteropolymers are in greatly reduced amounts and this appears to be due to a preferential assembly of the remaining 3 isozymes from the ***A*** and ***B*** subunits. When such a non-binomial distribution is dissociated and the subunits allowed to recombine in vitro, then a binomial distribution is generated (Figure 9). These results suggest a metabolic regulation of subunit combination in vivo. Although the isozyme patterns found on analysis of various tissues probably reflect differential synthetic activity, it must be recognized that the final pattern could also be modified by differential lability of isozymes. In vitro studies show that the isozymes are not equally stable. These differences in stability might be exploited by the cell to alter the isozymic repertory catabolically in response to changing physiological needs of the cell. No other enzyme has been so well studied with reference to its genetic control as LDH, but 2 other enzymes appear to be similar to LDH. These are creatine kinase and aldolase. Creatine kinase appears to be a dimer existing in 3 distinct isozymic forms, presumably the 2 homopolymers and the heteropolymer. Dissociation and recombination of this enzyme in vitro also indicate that it is a dimer[28,152–156]. Until recently aldolase was believed to be a trimer. However, evidence based on in vitro dissociation and recombination of subunits as well as other data now indicate that it is at least a tetramer, which like LDH, appears to be assembled randomly from at least 2 different kinds of subunits[157–160].

Many enzymes in which the molecular basis for the isozymic forms has been adequately described are listed in the Table.

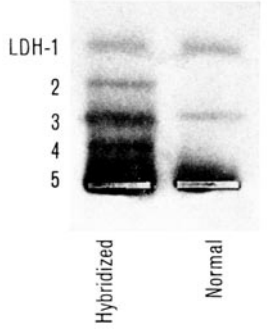

Fig. 9. Lactate dehydrogenase isozyme pattern of the alewife (*Alosa pseudoharengus*) after in vivo hybridization (normal) and after in vitro hybridization of the normal pattern. The failure to exhibit a binomial distribution of isozymes in vivo indicates an epigenetic control over subunit assembly.

Discussion and perspectives for the future

Isozymes are a relatively new and general aspect of enzymology. The very large amount of information now available demonstrates that many enzymes exist in multiple molecular forms. This multiplicity apparently extends to all organisms in which a thorough search has been made. In plants alone[161] more than 50 enzymes have been recorded in isozymic forms. Isozymes, as shown in this review, stem from a variety of different molecular bases; further research is likely to reveal additional types of molecular diversity which have been used by cells to generate sets of isozymes. The principal kinds of molecular multiplicity now known to generate isozymic sets are: different polypeptides encoded by allelic and also by non-allelic genes; polymers of various

Molecular varieties of isozymes[a]

Enzyme	Molecular basis for isozymes	References
Acetylcholinesterase (EC 3.1.1.7)	Polymeric series	62
Acid phosphatase (EC 3.1.3.2)	Conjugation with sialic acid	82,83
	Multiple alleles-shared subunits	133
Adenosine deaminase (EC 3.5.4.4)	Conformational isozymes	31
Adenylate kinase (EC 2.7.4.3)	Multiple alleles-subunits not shared	163
Alcohol dehydrogenase (EC 1.1.1.1)	Binding with coenzyme	77–79
	Multiple loci-subunits not shared	103,105
	Multiple alleles-subunits shared	103–107,164
	Multiple alleles-subunits not shared	103
Aldehyde oxidase (EC 1.2.3.1)	Multiple alleles-shared subunits	120
Alkaline phosphatase (EC 3.1.3.1)	Conjugation with sialic acid	81,166
	Multiple alleles-shared subunits	131,132
	Multiple alleles-subunits not shared	165

[a] Explanation on page 989.

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Molecular varieties of isozymes[a]

Enzyme	Molecular basis for isozymes	References
L-Amino acid oxidase (EC 1.4.3.2)	Conformational isozyme	26
	Polymeric series	57,58
α-Amylase (EC 3.2.1.1)	Multiple loci-subunits not shared	167–169
	Multiple alleles-subunits not shared	169
β-amylase (EC 3.2.1.2)	Polymeric series	45–47
Apyrase (EC 3.6.1.5)	Polymeric series	65,66
Arginase (EC 3.5.3.1)	Binding with manganese	80
Aspartokinase (EC 2.7.2.4)	Multiple loci	140,141
Carbonic anhydrase (EC 4.2.1.1)	Multiple loci-subunits not shared	170–174
Carboxypeptidase (EC 3.4.2.1)	Cleavage of peptide chain	36,37
Catalase (EC 1.11.1.6)	Disulfide-sulfhydryl interconversion	98
	Multiple alleles-shared subunits	122–124
Chorismate mutase	Polymeric series	63
Citrate synthase (EC 4.1.3.7)	Disulfide-sulfhydryl interconversion	97
Creatine kinase (EC 2.7.3.2)	Conformational isozymes	28
	Multiple loci-shared subunits	28,152–156
5-Dehydroquinate dehydratase (EC 4.2.1.10)	Multiple loci-subunits not shared	142
Deoxythymidine kinase	Polymeric series	44,175
o-Diphenol oxidase (EC 1.10.3.1)	Polymeric series	74
DNA polymerase (EC 2.7.7.7)	Polymeric series	73
Esterases (EC 3.1.1.–)	Conformational isozymes	15–17,29,127
	Multiple alleles-shared subunits	110,125,126 128–130,176
	Multiple alleles-subunits not shared	177–180,199
β-Fructofuranosidase (EC 3.2.1.26)	Polymeric series	71,72
Fructosediphosphate aldolase (EC 4.1.2.13)	Polymeric series	67,68
	Cleavage of peptide chain	41
	Multiple loci-shared subunits	157–160
β-Galactosidase (EC 3.2.1.23)	Polymeric series	64,181
Glucose-6-phosphate dehydrogenase (EC 1.1.1.49)	Polymeric series	54
	Conjugation with protein	54
	Multiple alleles-shared subunits	117,118
	Multiple alleles-subunits not shared	182,183
β-Glucosidase (EC 3.2.1.21)	Multiple alleles-shared subunits	134
Glutamate dehydrogenase (EC 1.4.1.2 and 1.4.1.4)	Conformational isozymes	24,25
	Polymeric series	23,24,42,43
	Multiple alleles	99
Glutamine synthetase (EC 6.3.1.2)	Conjugation with AMP	85–88

Molecular varieties of isozymes[a]

Enzyme	Molecular basis for isozymes	References
Glycogen phosphorylase (EC 2.4.1.1)	Phosphorylation	51,53
	Polymeric series	51,53 184–186
Glycogen synthetase (EC 2.4.1.11)	Phosphorylation	89–91
Glycerolphosphate dehydrogenase (EC 1.1.99.5)	Multiple alleles-shared subunits	119
Glycollate oxidase (EC 1.1.3.1)	Polymeric series	55
Hexokinase (EC 2.7.1.1)	Polymeric series	40,59,60
	Cleavage of peptide chain	38–40
Hexosediphosphatase (EC 3.1.3.11)	Conformation isozymes	30
Isocitrate dehydrogenase (EC 1.1.1.42)	Multiple alleles-shared subunits	112–114
Lactate dehydrogenase (EC 1.1.1.27)	Conformational isozymes	18–20, 187–189
	Polymeric series	190–192
	Multiple loci-shared subunits	147–150
	Multiple alleles-shared subunits	101,102,151
Leucine aminopeptidase (EC 3.4.1.1)	Multiple loci-subunits not shared	135,194
	Multiple alleles-shared subunits	135
	Multiple alleles-subunits not shared	135, 193–195,199
Lysosyme (EC 3.2.1.17)	Polymeric series	196
	Cleavage of peptide chain	200
Malate dehydrogenase (EC 1.1.1.37) and (EC 1.1.1.40)	Conformational isozymes	10–13,21,22
	Multiple alleles-shared subunits	108–111,113
Oxytocinase	Conformational isozymes	27
Penicillinase (EC 3.5.2.6)	Conformational isozymes	32
	Conjugation to protein	75
Phosphofructokinase (EC 2.7.1.11)	Polymeric series	61
Phosphoglucomutase (EC 2.7.5.1)	Phosphorylation	92
Phosphogluconate dehydrogenase (EC 1.1.1.43)	Multiple alleles-shared subunits	115,116
Phosphoglyceromutase (EC 2.7.5.3)	Cleavage of peptide chain	33,34
Pyruvate kinase (EC 2.7.1.40)	Multiple loci-subunits not shared	198
Thiosulphate sulphur-transferase (EC 2.8.1.1)	Polymeric series	70
Threonine dehydratase (EC 4.2.1.16)	Multiple loci	197
	Polymeric series	69
Urease (EC 3.5.1.5)	Polymeric series	48–50
Xanthine dehydrogenase (EC 1.2.3.2)	Polymeric series	56
	Multiple alleles-shared subunits	121

[a] This table lists most of the enzymes in which the molecular basis for the isozymic forms has been adequately described. All pertinent references have not been listed but those given should provide an adequate introduction to the literature.

sizes; homopolymers and heteropolymers; polypeptides secondarily modified in various ways; and various conformations produced by permutations of polymer subunits or alternate tertiary and quaternary configurations of proteins. Such molecular diversity indicates that organisms have exploited both genetic and epigenetic mechanisms for tailoring molecular properties of enzymes to fit special metabolic requirements. Just what these special metabolic requirements might be, however, is scarcely known in a single case. Some data exist to indicate that isozymes of various enzymes, including LDH and malate dehydrogenase, are located in different parts of the cell. Differential centrifugation and cytochemical and immunochemical procedures have all been used in an effort to determine the precise intracellular location of these isozymes. Although some useful information has been obtained, the results are still not definitive. New techniques seem to be required. Aside from specific location in various regions of the cell, the physiological significance of isozymes may well lie in their specific kinetic properties. The isozymic forms of any enzyme, though catalyzing the same reaction, do generally have distinct catalytic properties that presumably enables each of them to function somewhat differently in fulfilling specialized physiological roles in different metabolic sequences. It should be noted in this connection that many measurements of enzyme properties in the past have been made on what we now know to be mixtures of isozymes in different proportions. Many of these measurements should be repeated on separated individual isozymes or at least on preparations in which the isozymic composition is known.

One of the most fruitful areas for investigation provided by isozymes involves the physical chemistry of polypeptide association and the relationship between the primary, secondary, tertiary and quaternary structures of proteins. For small proteins, such as ribonuclease, the genetic control of the final conformation through specifying amino acid sequences may represent all of the information required to make the completed molecule. However, for large complicated proteins, the evidence clearly suggests an epigenetic direction of conformational folding or subunit association to make polymers each with its characteristic properties. The sub-bands of the many isozymes of LDH may illustrate the capacity of a given polymeric protein to exist in alternate conformations of similar stability. In any event, the significance of epigenetic direction of conformational folding is an area of investigation in which the isozymes of LDH and other enzymes provide exceedingly rich material for investigation.

The origin of many isozymic systems based upon the activity of multiple genes has probably occurred through gene duplication and then subsequent divergence through mutation. Such evolutionary relationships among genes can be investigated by comparing the amino acid sequences of their proteins. For lactate dehydrogenase, and possibly other proteins, enough information is now available to make further study of the evolutionary relationship among the different isozymes quite profitable.

Another area in which isozymes provide useful experimental material is in the study of the molecular mechanisms that regulate gene function. Since the several genes controlling the subunits of LDH show great cell specificity in function, there must be mechanisms within such cells for selectively identifying these genes and for regulating the degree to which each of them functions. To produce the observed highly specific isozyme patterns of a cell requires the coordinated function of 2 or more genes in precise but progressively changing rela-

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tive amounts as the cell differentiates. Whatever may be the mechanisms of gene regulation, the genes for LDH synthesis clearly offer rich material for studying them. The isozymic expression of gene function can also be studied in hybrid cells between very unrelated organisms such as the mouse-rat somatic cell hybrids of WEISS and EPHRUSSI[162], or in hybrids between closely related cells of different genotype as in the mouse cells used by MINTZ and BAKER[114]. These hybrid cells form hybrid molecules, thus demonstrating the activity of genes of both parental genotypes. Although terminal adult patterns of enzymes in cells, and this includes isozymes, can generally be accounted for by the differential function of the corresponding genes, such explanations are apparently not entirely adequate for cells during rapid differentiation. Under such conditions the change in isozyme pattern is so rapid as to require differential degradation of previously existing isozymes to make way for the new patterns that characterize the differentiating cell. The mechanisms underlying the specificity of this catabolic process need investigation.

The most important biological questions posed by protein heterogeneity, specifically by the existence of isozymes, concern their role in cellular metabolism. They must clearly confer great advantages on organisms, else they would not be so prevalent, but just what each isozyme does that distinguishes it from the others is still unknown. Many investigators have already found that isozymic systems are useful and important in studies of various problems of protein chemistry, enzymology, physiology, genetics, evolution, and developmental biology. With the continued and increased recognition of the problems of the physiological significance of isozymes and of their utility in many kinds of investigations, we may confidently hope that great progress in analyzing the multiplicity of molecular forms for many enzymes will be made in the near future.

Conclusion

The main events that led to the burgeoning study of isozymes were the development of zone electrophoresis and the application of histochemical staining techniques to the supporting media after electrophoresis. Isozymes exist in a variety of molecular forms, some of which have more physiological significance than others. The principal kinds of molecular heterogeneity now known to generate isozymes are: different polypeptides encoded by allelic or non-allelic genes; these polypeptides may assemble at random to form both homopolymers and heteropolymers or they may exhibit restricted assembly; polymers of different sizes; polypeptides secondarily modified in various ways such as by cleavage of the peptide chain or by conjugation to acetyl groups, sialic acid, or nucleotides or by binding to coenzymes and cofactors, or by phosphorylation, or by disulphide-sulfhydryl interconversion; conformational changes generated by permutations of polymer subunits or by alternate tertiary conformations.

Many of these isozymes have distinct kinetic properties that enable them to function somewhat differently in fulfilling specialized physiological roles. They may also be located in different parts of the cell or be integrated into distinct metabolic pathways. They permit a more refined and specific control of cellular metabolism. Several areas of investigation can be effectively exploited by the use of isozymes. These involve the genetic and epigenetic regulation of enzyme synthesis, the evolution of protein structure following gene duplication, the mechanisms responsible for selective degradation of proteins, and the formation of alternative conformational states through changes in quaternary or tertiary structure.

Zusammenfassung. Die wichtigsten Faktoren, welche zu den Fortschritten auf dem Gebiet der Isozyme führten, waren die Entwicklung der Zonen-Elektrophorese und die Anwendung histochemischer Färbungen der Elektrophorese-Medien. Isozyme existieren in verschiedenen molekularen Formen, von denen einige mehr physiologische Bedeutung haben als andere. Die folgenden Ursachen dieser molekularen Heterogenität sind bis heute bekannt geworden: Verschiedene Polypeptide, die von allelen oder nichtallelen Genen kodiert werden; diese Polypeptide können sich entweder zufällig zu Homo- oder Heteropolymeren vereinigen, oder es können nur bestimmte Polymere gebildet werden. Es können auch Polymere verschiedener Grösse auftreten. Die Polypeptide können auf verschiedene Weise sekundär modifiziert werden, z.B. durch Spalten der Peptidkette, Konjugation durch Acetylgruppen, Neuraminsäure oder Nukleotide, Bindung zu Koenzymen und Kofaktoren, durch Phosphorylierung oder durch Bildung bzw. Spaltung von Disulfidbrücken. Schliesslich können auch Konformationsänderungen durch Permutation der Polymer-Untereinheiten oder alternative Tertiärstrukturen erzeugt werden.

Viele dieser Isozyme haben bestimmte kinetische Eigenschaften, die ihnen eine spezielle physiologische Funktion ermöglichen. Sie können auch in bestimmten Regionen der Zelle lokalisiert oder in bestimmte Reaktionsketten des Stoffwechsels eingeschaltet sein. Sie erlauben eine verfeinerte und spezifische Kontrolle des Zellstoffwechsels; verschiedene Fragenkomplexe können mit Hilfe der Isozyme angegangen werden. Diese umfassen die genetische und epigenetische Regulation der Enzymsynthese, die Evolution der Proteinstruktur nach Genduplikation, den Mechanismus der selektiven Degradation der Proteine und die Bildung alternativer Konformationszustände durch Änderungen in der Quartär- und Tertiärstruktur.