Dependence of the Aggregation and Conformation States of Uridine 5′-Phosphate Synthase on Pyrimidine Nucleotides. Evidence for a Regulatory Site†

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ABSTRACT: Uridine 5′-phosphate (UMP) synthase is a multifunctional protein that contains the last two enzyme activities of the de novo pathway for UMP biosynthesis, orotate phosphoribosyltransferase (EC 2.4.2.10) and orotidine-5′-phosphate (OMP) decarboxylase (EC 4.1.1.23). We have previously reported that UMP synthase from mouse Ehrlich ascites cells can exist in at least three distinct aggregation and/or conformation states, as measured by changes in sedimentation through sucrose gradients [Traut, T. W., & Jones, M. E. (1979) *J. Biol. Chem. 254*, 1143–1150]. The major sedimenting species were a 3.6S monomer, a 5.1S dimer, and a 5.6S species. The formation of the 5.1S dimer from the 3.6S monomer occurs in the presence of ligands that are competitive inhibitors at the OMP decarboxylase catalytic site. This paper presents evidence for a regulatory site, distinct from either of the two catalytic sites, which appears to mediate the conversion of the 5.1S dimer to the 5.6S form upon binding certain pyrimidine nucleotides (OMP, UMP, and 6-azaUMP). Since UMP synthase sediments predominantly as a dimer in the presence of substrates, regulation of the aggregation/conformation state of this multifunctional protein may be physiologically significant.

The two enzyme activities of UMP synthase,[1] orotate phosphoribosyltransferase (EC 2.4.2.10) and orotidine-5′-phosphate decarboxylase (EC 4.1.1.23), catalyze the last two steps of de novo UMP biosynthesis.[2] These two enzyme activities are on a single multifunctional protein, since a purified and homogeneous polypeptide contains both activities (McClard et al., 1980). A variety of aggregation states have been reported for UMP synthase: a monomer, dimer, and tetramer in human erythrocytes (Brown et al., 1975; Brown & O'Sullivan, 1977a); monomers with different conformational states in a murine leukemia cell line (Reyes & Guganig, 1975) and in mouse brain and liver (Reyes & Intress, 1978); multiple molecular forms in mouse liver (Reyes et al., 1978); three different forms in human erythrocytes (Grobner & Kelley, 1975); a monomer and dimer in Ehrlich ascites cells (Shoaf & Jones, 1973). Recently, we presented studies on UMP synthase from Ehrlich ascites cells that demonstrated three major different species with $s_{20,w}$ values of 3.6, 5.1, and 5.6 (Traut & Jones, 1979). Sedimentation of UMP synthase in the presence of substrates, products, or their analogues always produces the larger 5.1S or 5.6S forms.

In earlier work, we presented evidence that the formation of the 5.1S species was produced by effectors binding to the OMP[3] decarboxylase catalytic site (Traut & Jones, 1979). We now present evidence that the formation of the 5.6S species is promoted by effectors binding to a noncatalytic, regulatory site.

Experimental Procedures

Materials

Tetrasodium pyrophosphate, tetrasodium P-Rib-PP, dithiothreitol, NAD, NADH, alcohol dehydrogenase (horse liver), nucleotides, and Tris were obtained from Sigma. Lactate dehydrogenase (beef muscle) was from Boehringer/Mannheim, and bovine hemoglobin was a product of Miles Laboratories. [7-^{14}C]OMP and [6-^{14}C]orotate were purchased from New England Nuclear. Poly(ethylenimine)–cellulose plates were obtained from Brinkmann, and ultrapure sucrose was purchased from Schwarz/Mann. PEI–cellulose powder was from Machery-Nagel and Co.

Methods

Enzyme Preparation. The two enzyme activities of UMP synthase were prepared from mouse Ehrlich ascites cells as described previously (Jones et al., 1978). In the present work, the protein fraction precipitating between 1.7 and 2.6 M ammonium sulfate was used. This fraction is purified 8–10-fold.

Enzyme Assays. Orotate phosphoribosyltransferase in the biosynthetic reaction was measured by the production of [6-^{14}C]OMP and [6-^{14}C]UMP from [6-^{14}C]orotate as described previously (Traut & Jones, 1977a), using thin-layer chromatography on PEI–cellulose sheets to separate substrate and products. This procedure was modified for K_m determinations in the degradative reaction as follows. A final reaction volume of 750 μL contained 20 mM Tris-HCl (pH 7.4 at 37 °C), 2 mM dithiothreitol, 5 mM $MgCl_2$, 0.1 mM EDTA, 5 mM NaF,

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[1] Previous publications on UMP synthase have used the designation "complex U" for the protein which possesses these two enzyme activities.[2] Recent purification (McClard et al., 1980) of this protein to homogeneity has shown that the protein is composed of one rather than two polypeptide chains, so the term "complex U" has been replaced by "UMP synthase". A more precise term has also been suggested, namely, "multienzyme *pyr 5,6*" (Jones, 1980; Christopherson et al., 1980).

[2] The two reactions are

$$\text{orotate} + \text{P-Rib-PP} \xrightleftharpoons[\text{orotate phosphoribosyltransferase}]{Mg^{2+}} \text{OMP} + PP_i$$

$$\text{OMP} \xrightarrow{\text{OMP decarboxylase}} \text{UMP} + CO_2$$

[3] Abbreviations used: P-Rib-PP, 5-phosphoribosyl 1-pyrophosphate; OMP, orotidine 5′-phosphate; azaUMP, 6-azaUMP; PEI, poly(ethylenimine).

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and 0.1 mM azaUMP. AzaUMP was added to block the OMP decarboxylase activity, and NaF was added to block an inorganic pyrophosphatase activity that is present in this enzyme preparation (Traut & Jones, 1977b). For determination of the K_m of OMP, the reactions also included 0.3 mM sodium PP_i and [7-^{14}C]OMP (35.7 Ci/mol) at concentrations indicated. For determination of the K_m of PP_i, the reactions included 10 μM [7-^{14}C]OMP (35.7 Ci/mol) and sodium PP_i as indicated. Reactions were started by the addition of UMP synthase (6 μg of protein), and incubations were for 14 min at 37 °C. Reactions were stopped by the addition of 75 μL of ice-cold 0.1 M EDTA (pH 7.0) after which the mixture was held on ice for 20 min. Normally, the conversion of substrate to product was not greater than 10%.

Because of the low concentrations and limiting specific radioactivity of the [7-^{14}C]OMP, the reverse reaction for the phosphoribosyltransferase cannot be measured by thin-layer chromatography. Instead, PEI–cellulose columns (5 × 40 mm) were prepared by using Pasteur pipets. Of the EDTA-quenched reaction mixture, 0.6 mL was loaded on a PEI–cellulose column and then washed with 0.5 mL of water. [7-^{14}C]Orotate was then eluted with 2.5 mL of 0.2 N KCl, and [7-^{14}C]OMP was eluted with 2.5 mL of 1 N KCl. Total radioactivity recovered in the fractions collected was normally 95–104% of input.

OMP decarboxylase activity was measured as the production of $^{14}CO_2$ from [7-^{14}C]OMP (2 Ci/mol) by methods described previously (Jones et al., 1978).

For studies of inhibitors, the two UMP synthase activities were measured at two or three different inhibitor concentrations selected to be reasonably near their K_i values as determined in earlier studies (Traut & Jones, 1977a).

Sucrose Gradient Ultracentrifugation. Linear sucrose density gradients, 10–40% (w/v), were prepared, centrifuged, and fractionated as described elsewhere (Traut & Jones, 1979). Gradients of 12 mL were centrifuged at 41 000 rpm with an SW 41 rotor for 65 h at 3 °C. Compositions of sucrose solutions are described in the legends to figures and tables. The recovery of enzyme activity from sucrose gradients varied in accordance with the effectors included in the sucrose solution: the amount of OMP decarboxylase activity recovered was at least 60% when only buffer and dithiothreitol were present, and as high as 90% when high concentrations of OMP, azaUMP, or other nucleotides were present.

Samples of 200 μL containing 4 mg of enzyme protein plus three marker proteins (1.6 mg of hemoglobin, 1.6 mg of alcohol dehydrogenase, 30 μg of lactate dehydrogenase) were layered on top of the gradients. The sedimentation coefficient of UMP synthase in the various solvent systems listed was determined from a calibration curve of the standard proteins by using values from the literature for hemoglobin (4.3 S), alcohol dehydrogenase (5.1 S), and lactate dehydrogenase (7.3 S). Only the OMP decarboxylase activity was normally monitored, since both enzyme activities cosediment exactly, as previously shown (Traut & Jones, 1979). In the measurement of OMP decarboxylase activity of sucrose gradient fractions, the effect of inhibitors present in the sucrose gradient was diminished in the enzyme assay by diluting the sample 20–50-fold, and by using OMP at saturating concentrations.

Results

Kinetic Studies with UMP Synthase. Except for orotate, all the substrates and products of the two enzymes activities promote the aggregation of UMP synthase. It was therefore important to be able to compare affinity constants of these effectors at the two different catalytic sites. For the transferase activity from mammalian cells, no kinetic data were available for this enzyme in the degradative reaction,[2] and we therefore determined the apparent K_m values for both OMP and PP_i. Proper separation and resolution of substrates and products required chromatography on PEI–cellulose columns.

Table I: Kinetic Constants for the Orotate Phosphoribosyltransferase Reaction

substrate	K_m (μM)	enzyme velocity (nmol min^{-1} mg^{-1})
P-Rib-PP	9.6	4.21 (V_f)
orotate	2	
PP_i	15.4	3.69 (V_r)
OMP	0.07	

Table II: Apparent Michaelis or Inhibition Constants for Substrates or Competitive Inhibitors of Orotate Phosphoribosyltransferase (OPRTase) and Orotidine-5′-phosphate Decarboxylase (OMP Dcase)

inhibitor	K for OPRTase[a]	K for OMP Dcase[b]
P_i	5.6 mM	17 mM
Cl^-		95 mM
Ac^-		135 mM
P-Rib-PP	(9.6 μM)[c]	950 μM
PP_i	(15.4 μM)	520 μM
OMP	(0.07 μM)	(0.23 μM)
azaUMP	1.8 mM	0.003 μM
UMP	2.1 mM	24 μM
AMP	2.6 mM	100 μM
GMP	0.5 mM	244 μM
XMP		0.05 μM

[a] Inhibition constants are vs. the substrate P-Rib-PP. [b] Inhibition constants are vs. the substrate OMP. [c] Values in parentheses are K_m values; all others are K_i values.

All the kinetic constants for the transferase reaction in either direction[2] are shown in Table I. The K_m value for P-Rib-PP is lower than the value of 16 μM determined previously (Kavipurapu & Jones, 1976). This is probably because we have analyzed our commercial P-Rib-PP more precisely, as described previously (Traut & Jones, 1977b). Using the values in Table I in the Haldane equation[4] produces a ratio of 0.073, which is in excellent agreement with the K_{eq} for this reaction that we have previously determined as 0.068 (Traut & Jones, 1977b).

In addition, we have determined inhibition constants for the various effectors at the two catalytic sites, as shown in Table II. All the compounds listed in Table II promote aggregation of the 3.6S monomer to the 5.1S dimer. The K_i of P-Rib-PP vs. OMP is essentially the same whether Mg^{2+} is absent or present in the reaction mixture. Both azaUMP and xanthosine 5′-phosphate (XMP) are excellent inhibitors of OMP decarboxylase activity, consistent with other studies on azaUMP (Brown & O'Sullivan, 1977b; Handschumacher, 1960) and XMP (Brown & O'Sullivan, 1977b). Two compounds stand out in that they have fairly similar affinities for both catalytic sites. The first of these is OMP. Repetitive measurements have produced a K_m of 0.23 μM at the decarboxylase site, which is in good agreement with the value of 0.29 μM published earlier (Kavipurapu & Jones, 1976). OMP has an even lower K_m of 0.07 μM at the phosphoribosyltransferase site. This value is significantly lower than the K_m of 8 μM determined with yeast orotate phosphoribosyltransferase (Victor et al., 1979). The second compound is inorganic phosphate, which is a moderate inhibitor of both catalytic activities.[5]

[4] The Haldane equation is $K_{eq} = (V_f^2 K_{mP} K_{mQ})/(V_r^2 K_{mA} K_{mB})$, where A is P-Rib-PP, B is orotate, P is PP_i, and Q is OMP.

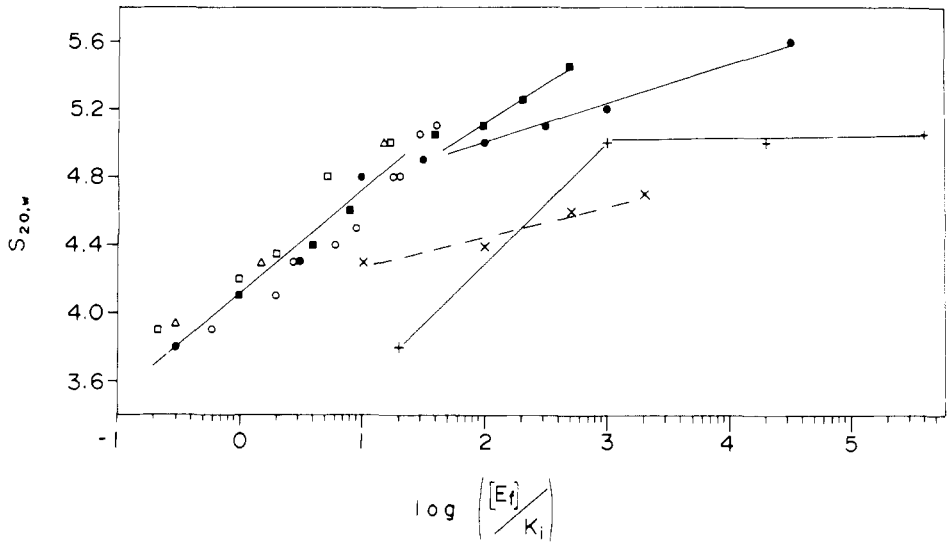

FIGURE 1: Sedimentation of UMP synthase in the presence of a variety of effectors as a function of the effector's binding affinity at the OMP decarboxylase catalytic site. The sedimentation of UMP synthase was measured on 10–40% sucrose gradients containing the standard buffer (20 mM Tris-HCl, 2 mM dithiothreitol) and effectors. Separate experiments were done with sucrose gradients containing each of the following effectors: (●) azaUMP; (■) UMP; (○) P_i; (□) P-Rib-PP; (△) Cl^-; (×) AMP; (+) XMP. The abscissa shows effector concentrations relative to their K_i vs. OMP in the decarboxylase reaction (Table II).

Sedimentation of UMP Synthase. We had previously shown that a variety of effectors (which were competitive inhibitors vs. OMP) was able to convert the 3.6S monomer to the 5.1S dimer (Traut & Jones, 1979). Of all the compounds that we have tested, only pyrimidine nucleotides produce the 5.6S form. These studies are summarized in Figure 1. The abscissa in Figure 1 represents the relative concentration of an effector in terms of the effector's K_i at the OMP decarboxylase site (Table II). The absolute concentrations varied enormously: the effective conversion of all (or almost all) of the 3.6S monomer to the 5.1S dimer requires 0.5 M P_i, 1 mM UMP, or 0.1 μM azaUMP. Except for the purine nucleotides, the amount of any effector that is necessary to convert the 3.6S monomer to the 5.1S dimer is inversely proportional to the effector's ability to bind at the OMP decarboxylase site. The apparently anomalous results obtained with purine nucleotides are probably related to steric hindrance resulting from the extra bulk of the purine base, and this will be considered in more detail later.

We feel that the results in Figure 1, demonstrating that a wide variety of effectors falls on a common line when we plot their ability to produce the 5.1S dimer of UMP synthase as a function of their K_i vs. OMP decarboxylase, provide compelling evidence that the binding of ligands to the OMP decarboxylase site produces a change in the monomer that leads to dimerization. The fact that the concentrations of UMP or azaUMP required for promoting the 5.6S species of UMP synthase were (1) in great excess of the concentrations required to produce the 5.1S dimer and (2) not identically related to their K_i at the OMP decarboxylase site (Figure 1) suggested that a different binding site is involved for converting the 5.1S dimer to the 5.6S dimer of UMP synthase. An obvious other site is the phosphoribosyltransferase site.

The most suitable ligand for the phosphoribosyltransferase catalytic site is the substrate Mg(P-Rib-PP). In fact, sedimentation studies showed that the addition of 20 mM $MgCl_2$ significantly enhanced the ability of P-Rib-PP to promote the

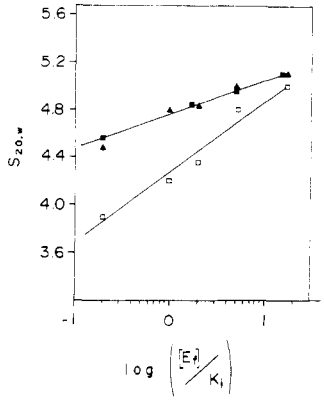

FIGURE 2: Sedimentation of UMP synthase in the presence of P-Rib-PP, with or without $MgCl_2$. Sedimentation was measured as described in the legend to Figure 2. Observed sedimentation for gradients containing P-Rib-PP (□) or P-Rib-PP + 20 mM $MgCl_2$ (■). Calculated sedimentation for gradients containing P-Rib-PP + 40 mM Cl^- (▲). The abscissa is the same as in Figure 1.

5.1S dimer (Figure 2). Since inhibition studies with the decarboxylase activity showed that the K_i values for Mg(P-Rib-PP) and P-Rib-PP vs. OMP were essentially identical, these results might suggest that the observed increase in sedimentation was the result of Mg(P-Rib-PP) binding to the phosphoribosyltransferase site, suggesting this site to have at least a secondary role in promoting dimerization. This increased sedimentation is not, however, produced by Mg^{2+}. Rather, it is the counterion Cl^- which we have shown will by itself promote dimerization (Figure 1). By addition of the sedimentation attributable to 40 mM Cl^- to the sedimentation curve for P-Rib-PP, a curve is produced that is identical with the observed curve for Mg(P-Rib-PP), as shown in Figure 2. Thus, we have no data to support the concept that the phosphoribosyltransferase site is involved in promoting the monomer → dimer transition, or the 5.1S → 5.6S transition.

Competition Studies. We have previously shown that various combinations of some effectors are additive in promoting the conversion of the 3.6S monomer to the 5.1S dimer (Traut & Jones, 1979). This was also observed when combinations of the nucleotide effectors were tested (Figures 3 and 4). Thus, if the nucleotides OMP, UMP, and azaUMP are

[5] The competitive inhibition of both the phosphoribosyltransferase and the decarboxylase enzyme activities by P_i was first observed by Dr. P. R. Kavipurapu.

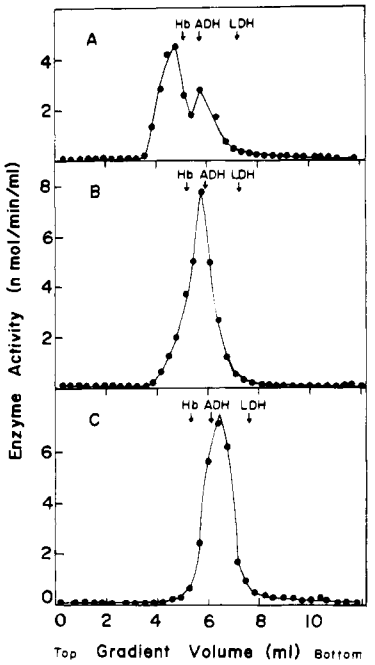

FIGURE 3: Sedimentation of UMP synthase in the presence of the nucleotide effectors OMP and azaUMP. Only the OMP decarboxylase activity was assayed, and sedimentation was measured as described in the legend to Figure 1. Sucrose gradients contained 30 μM OMP (A), where the two peaks sedimented at 3.6S and 5.6S; 0.1 μM azaUMP (B), where the peak is at 5.0S; and 30 μM OMP plus 0.1 μM azaUMP (C), where the peak is at 5.6S. Hb, hemoglobin; ADH, alcohol dehydrogenase; LDH, lactate dehydrogenase.

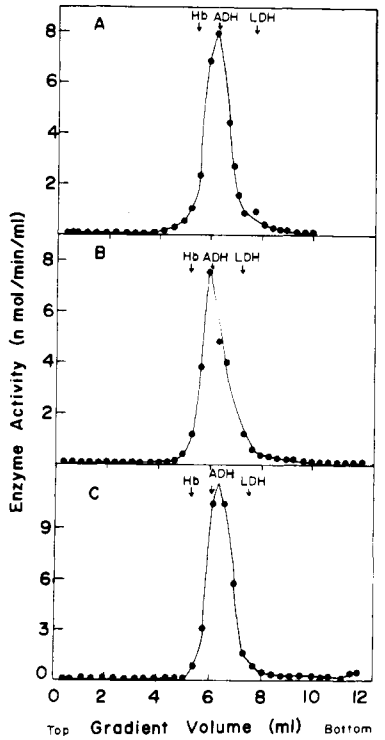

FIGURE 4: Sedimentation of UMP synthase in the presence of the nucleotide effectors UMP and azaUMP. Sedimentation was measured as described in the legend to Figure 1. Sucrose gradients contained 1 mM UMP (A), where the peak is at 5.1S; 1 μM azaUMP (B), where the peak is at 5.0S; and 1 mM UMP plus 1 μM azaUMP (C), where the peak is at 5.5S. Hb, hemoglobin; ADH, alcohol dehydrogenase; LDH, lactate dehydrogenase.

combined at concentrations where individually they would result only in mixtures of the 3.6S and 5.6S forms (Figure 3A) or in the 5.1S form (Figures 3B, 4A,B) the combined effectors

Table III: Sedimentation of UMP Synthase in the Presence of Competing Effectors[a]

solvent[b]	obsd s value (S)
OMP (0.2)	5.6
OMP (0.2) + P_i (5)	5.5
OMP (0.2) + P_i (50)	5.0
OMP (0.2) + P_i (200)	5.1
azaUMP (0.1)	5.6
azaUMP (0.1) + P_i (50)	4.9
UMP (12)	5.5
UMP (12) + P_i (50)	5.1

[a] Conditions for centrifugation are described under Experimental Procedures. [b] Values in parentheses are concentrations in mM. All solvents contained standard buffer: 20 mM Tris-HCl (pH 7.4) and 2 mM dithiothreitol.

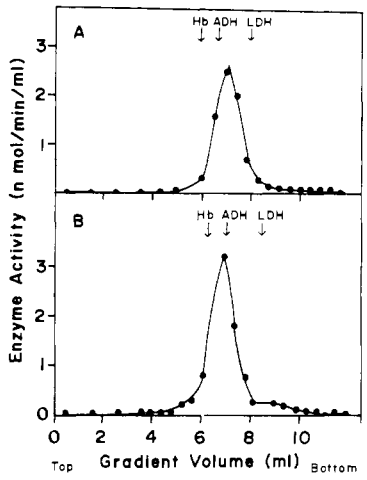

FIGURE 5: Sedimentation of UMP synthase in the presence of OMP and P_i. Sedimentation was measured as described in the legend to Figure 1. Sucrose gradients contained 200 μM OMP (A), where the peak is at 5.6S; and 200 μM OMP plus 50 mM P_i (B), where the peak is at 5.0S. Hb, hemoglobin; ADH, alcohol dehydrogenase; LDH, lactate dehydrogenase.

will convert all the enzyme to the 5.6S form (Figures 3C, 4C).

A clear exception to this is provided by inorganic phosphate. P_i by itself will convert the 3.6S monomer to the 5.1S dimer (Traut & Jones, 1979), but P_i when combined with OMP, for example, will prevent the formation of the 5.6S species that OMP normally produces (Figure 5). As shown in Table III, P_i is competitive vs. all three nucleotide effectors that normally promote the 5.6S species and clearly prevents the conversion of the 5.1S dimer to the 5.6S species. P_i, even at high concentrations, does not prevent the formation of the 5.1S dimer by the nucleotides. At the concentrations listed in Table III, P_i by itself would result in UMP synthase sedimenting at 3.8S (5 mM P_i), 4.3S (50 mM P_i), or 4.5S (200 mM P_i) (Traut & Jones, 1979).

Discussion

Studies on the reaction mechanism of the phosphoribosyltransferase activity have only been done with the enzyme from yeast (Goitein et al., 1978; Victor et al., 1979), and the data reported support a ping-pong mechanism. While we have not conducted sufficient studies to unambiguously define the reaction mechanism for the mammalian phosphoribosyltransferase activity, the kinetic values that we have determined (Table I) are consistent with a ping-pong mechanism.

The K_i values reported in Table II show that nucleotides in general are much better inhibitors of the OMP decarboxylase than of the orotate phosphoribosyltransferase, in

agreement with earlier studies with UMP synthase from Ehrlich ascites cells (Traut & Jones, 1977a). In studies with UMP synthase from human erythrocytes, a different interpretation was presented since nucleotides were reported as being better inhibitors of the phosphoribosyltransferase activity (Tax et al., 1976; Tax & Veerkamp, 1979). Tax and colleagues have studied the inhibition of the phosphoribosyltransferase activity in crude hemolysates by using a coupled assay that depends on the OMP decarboxylase activity to produce $^{14}CO_2$ when [7-^{14}C]orotate is the original substrate. We feel that such an assay system with crude hemolysates is not very reliable because the same hemolysate contains high levels of a nucleotidase activity that readily dephosphorylates OMP (Tax et al., 1979). Thus, we suggest that in the inhibition studies by Tax et al., nucleotide inhibitors will be most effective at the decarboxylase site, leading to accumulation of OMP, which in turn can be degraded to orotidine. This nucleoside cannot be decarboxylated and would no longer produce the expected product, CO_2; such results would be interpreted as inhibition of the phosphoribosyltransferase, when actually the inhibition occurs at the OMP decarboxylase site. We anticipate that kinetic studies with a more purified preparation of erythrocyte UMP synthase will yield results similar to those with the enzyme from mouse Ehrlich ascites cells. Certainly, any assay which merely measures CO_2 production and does not follow the fate of the pyrimidine base is insufficient to clarify this issue.

We have previously reported (Traut & Jones, 1979) that in sedimentation studies UMP synthase appeared capable of existing as four different species: the native monomer which contains both catalytic sites and sediments at 3.6S; a dimer of the 3.6S form that sediments at 5.1S; a putative hybrid species of 4.7S produced only by AMP at concentrations of 40 mM or greater; and a 5.6S species which is formed reversibly from the 5.1S dimer by certain nucleotide effectors (OMP and its analogues, UMP or azaUMP). We concluded that the 3.6S, 5.1S, and 5.6S forms should all exist in vivo, and we suggest that the regulation of the aggregation state may be significant since the OMP decarboxylase activity is uniquely, or at least predominantly, associated with the 5.6S dimer of UMP synthase (Traut & Payne, 1980). UMP synthase has at least two sites, corresponding to the two catalytic centers for (1) orotate phosphoribosyltransferase and (2) OMP decarboxylase. We have no data to suggest that binding of substrates or other ligands to site 1 produces any significant effect on the aggregation or conformation state of UMP synthase. Effectors for which vastly different concentrations are required to produce the 5.1S dimer (e.g., P_i, UMP, or azaUMP) have essentially quite similar K_i values at site 1, but appropriately different K_i values at site 2 (Table II). We are reasonably certain that it is the binding of effectors to site 2 that produces a conformational change in the 3.6S monomer such that monomers more readily bind to one another to form 5.1S dimers (Figure 1).

How is the 5.1S dimer → 5.6S dimer transition achieved? To interpret our data, we have considered both a two-site and a three-site model for UMP synthase.

Two-Site Model for UMP Synthase. This model has the virtue of economy but requires that all changes in aggregation or conformation are the result of effectors binding to site 2. Thus, the 5.6S form would then be produced as the enzyme undergoes a conformational change in the act of catalysis. Since the substrate OMP is most effective in producing the 5.6S form, this interpretation is attractive. However, it is difficult to completely account for the data of Figure 1,

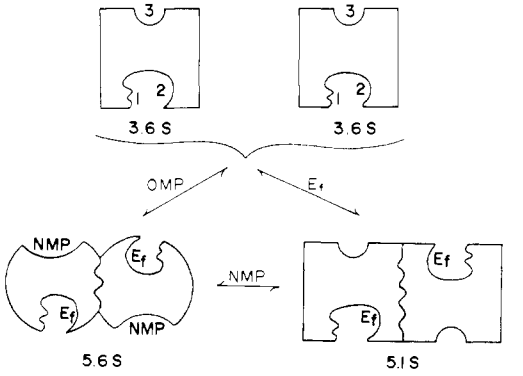

FIGURE 6: Schematic representation of changes in the aggregation and conformation of UMP synthase. The subunit is the 3.6S monomer, containing a catalytic domain for the orotate phosphoribosyltransferase activity (site 1) and for the OMP decarboxylase activity (site 2). The third site is a regulatory site distinct from sites 1 and 2. E_f, effectors (listed in Table II); NMP, pyrimidine nucleoside monophosphate (OMP, UMP, or azaUMP). NMP is a subset of E_f. The number of sites which must be filled is unknown.

showing that greater concentrations of effectors are required to produce the 5.6S species and, further, that these concentrations are not identically related to the effector's K_i for site 2 (compare the curves for UMP and azaUMP in Figure 1). Thus, to convert all of UMP synthase to the 5.1S dimer, presumably all of the monomer units have one ligand bound at site 2. With azaUMP, for example, this can be achieved at a concentration of about 0.1 μM. But 100 μM azaUMP is required to convert all of UMP synthase to the 5.6S dimer. Thus, if 0.1 μM azaUMP saturates site 2, it is hard to explain why vastly greater concentrations are required to have an additional effect at the same site, and why there should be a change in the affinity for site 2 as suggested by the break in the azaUMP curve (Figure 1).

Three-Site Model for UMP Synthase. The scheme for aggregation and conformational changes depicted in Figure 6 is consistent with all the data presented here and in an earlier publication (Traut & Jones, 1979). Sites 1 and 2 are the catalytic sites, and site 3 is a regulatory site distinct from sites 1 and 2. Binding at site 2 merely promotes the 3.6S → 5.1S dimerization. Any anion effector that can imitate the phosphate group of OMP could be expected to interact with a positively charged residue in the catalytic pocket (arginine would be a good candidate) and thereby initiate a conformational change that favors dimerization. The better an effector fits the complete catalytic site (i.e., the lower the K_i), the more efficiently it promotes dimerization.

While purine nucleotides also promote dimerization, they are relatively poor effectors[6] (Figure 1). It may be that purine nucleotides, because of the extra bulk in the purine base, do not totally fit into the catalytic pocket. Thus, they may protrude somewhat and offer some steric hindrance to the conformation change necessary for dimerization.

Only the binding of certain nucleotide effectors, principally OMP, to site 3 produces a conformational change in the dimer such that it sediments with a value of 5.6S. In postulating a regulatory site, it is implicit that such a site has a definite function in promoting the 5.6S dimer. Kinetic studies with

[6] We have run control experiments to measure decomposition of purine nucleotides. When [^{14}C]AMP was incubated for 65 h in a sample mixture equivalent to those layered on gradients, less than 5% of the AMP had been degraded to adenosine and adenine, as measured by thin-layer chromatography and autoradiography. Therefore, decomposition is not significant.

UMP synthase suggest that the 5.6S conformation is necessary for OMP decarboxylase activity (Traut & Payne, 1980).

A three-site model can more easily account for the data of Figure 1. Thus, when site 2 is saturated, all of UMP synthase exists as the 5.1S dimer. Higher concentrations of effectors are required to produce the 5.6S dimer because these effectors bind poorly to site 3. This also explains the break observed in the curves for UMP and azaUMP (Figure 1). Using the amount of each pyrimidine nucleotide required to produce the 5.6S species (i.e., to saturate site 3) as an estimate for binding to site 3, we predict that approximate values for a dissociation constant at site 3 would be azaUMP ≤ 10 μM and UMP $\leq$ 5 mM. For OMP, we estimate a value of about 1 nM on the basis of kinetic studies at low OMP concentrations, the interpretation that only the 5.6S dimer is active, and the observation that there are no breaks in a plot of V^{-1} vs. $[OMP]^{-1}$ for OMP concentrations in the low nM range (Traut & Payne, 1980).

We do not have definitive data that the 5.1S dimer is an obligatory intermediate in the conversion of 3.6S monomer $\rightarrow$ 5.6S dimer. With OMP, which appears to bind better to site 3 than to site 2, we have never observed UMP synthase sedimenting at 5.1S. It should be noted that interpretation of such studies with OMP is complicated by the fact that OMP is the only effector tested that is significantly degraded (by the OMP decarboxylase) during the experiment. Thus, if sedimentation studies are done with low concentrations of OMP, the OMP is completely decarboxylated, and UMP synthase sediments essentially as a monomer. If sufficient concentrations are used so that the OMP is not completely decarboxylated, UMP synthase sediments as the 5.6S dimer. However, for effectors such as UMP and azaUMP which bind better to site 2, the data of Figure 1 suggest the sequence 3.6S $\rightarrow$ 5.1S $\rightarrow$ 5.6S as first site 2, and then site 3 is filled.

For either model, the failure of purine nucleotides to produce the 5.6S dimer is most likely a function of their extra bulk which would make them fit improperly, or not at all, and thereby ineffective in inducing the 5.1S $\rightarrow$ 5.6S conformational transition. Also, P_i appears to compete with the pyrimidine nucleotides that produce the 5.6S dimer (Figure 5, Table III). This is probably due to P_i binding at that part of the site which binds the phosphate group of the nucleotide. If the pyrimidine base or nucleoside structure of OMP, UMP, and azaUMP is necessary to produce the conformational change that results in the 5.6S dimer, then P_i does not have sufficient structure to accomplish this. The binding of P_i does, however, block OMP and other nucleotides from this regulatory site, and thereby inhibits them from promoting the normal 5.1S $\rightarrow$ 5.6S transition.

Although not tested in these studies, certain artificial nucleotide analogues that have been shown to be potent inhibitors of the OMP decarboxylase activity should promote the 5.1S form of UMP synthase: the 5′-nucleotides of allopurinol (4-hydroxypyrazolo[3,4-*d*]pyrimidine) and oxipurinol (4,6-dihydroxypyrazolo[3,4-*d*]pyrimidine) (Fyfe et al., 1973; Tax et al., 1976); the 5′-nucleotide of pyrazofurin (3-β-D-ribofuranosyl-4-hydroxypyrazolo-5-carboxamide) (Ohnuma et al., 1977); and the 5′-nucleotide of barbituric acid (Komura et al., 1980). Using UMP synthase prepared from erythrocytes, Grobner & Kelley (1975) demonstrated that it sedimented in the range 5.1–5.3S in the presence of allopurinol ribonucleotide or oxipurinol ribonucleotide.

The only purine nucleotides that might promote formation of the 5.6S dimer are 3-XMP and 7-oxipurinol nucleoside 5′-phosphate. Both of these nucleotides have the glycosidic linkage to the nitrogen on the 6-member ring of the purine base (N3 or N7) that corresponds to the N1 position of the pyrimidine base. Since the purine base is normally in the anti conformation in respect to the ribose (Sundaralingam, 1969), it is less likely that steric hindrance would result. By the same reasoning, these two compounds would be expected to be similar to the majority of effectors in promoting the 5.1S dimer (Figure 1).

Previously, we had suggested that the 5.6S species of UMP synthase could be either an altered conformer of the 5.1S dimer or a hybrid trimer, containing the 5.1S dimer plus some small but unidentified macromolecule, and we favored the latter interpretation (Traut & Jones, 1979) because the calculated M_r difference between the 5.6S and 5.1S forms was equivalent to the calculated M_r difference between the 3.6S and 4.7S species. Our current results make it more likely that the 5.6S species is also a dimer, but with a different conformation than the 5.1S dimer. In addition, preliminary sedimentation studies by McClard and Jones have also shown that completely purified UMP synthase has a sedimentation value of 5.6S in the presence of OMP.[7] This observation quite strongly supports the interpretation that the 5.6S form of UMP synthase is a dimer.

The recent purification of UMP synthase made it possible to calculate $\bar{v}$, the partial specific volume of this protein, from its amino acid composition.[7] With this newly determined value for $\bar{v}$ of 0.743 cm^3/g, our earlier M_r estimates (based on an assumed $\bar{v}$ = 0.725 cm^3/g) can now be revised. Using the Stokes radii determined previously (Traut & Jones, 1979) and the sedimentation constants for the different forms of UMP synthase, we find for the 3.6S monomer M_r = 50 100 and for the 5.1S dimer M_r = 102 700. While it has a different S value, the 5.6S dimer should have the same M_r as the 5.1S dimer.

A similar interpretation is possible for the fourth form of UMP synthase previously proposed—the 4.7S species observed only with AMP (Traut & Jones, 1979). More recent studies suggest that AMP has not been tested at sufficient concentrations. Extrapolating the AMP curve (Figure 1) suggests that AMP at an unattainable concentration of 20 M should completely convert the 3.6S monomer to the 5.1S dimer. XMP, which is a much more potent inhibitor of the OMP decarboxylase (Table II), can effectively promote the 5.1S dimer of UMP synthase. Because of our inability to distinguish, by means of sedimentation studies, between a profile (of the type shown in Figures 3–5) that represents an equilibrium mixture of monomers and dimers sedimenting with an average value of 4.7S and a profile of a hydrid species sedimenting at 4.7S, a definitive interpretation is difficult. However, the simplest interpretation that conforms to both our previous and current data is that the 4.7S species observed in the presence of AMP is not a unique hybrid dimer, as suggested earlier (Traut & Jones, 1979), but simply a freely equilibrating mixture of monomers and dimers.

References

Brown, G. K., & O'Sullivan, W. J. (1977a) *Biochemistry 16*, 3235–3242.

Brown, G. K., & O'Sullivan, W. J. (1977b) *Biochem. Pharmacol. 26*, 1947–1950.

Brown, G. K., Fox, R. M., & O'Sullivan, W. J. (1975) *J. Biol. Chem. 250*, 7352–7358.

Christopherson, R. I., Traut, T. W., & Jones, M. E. (1980) *Curr. Top. Cell. Regul.* (in press).

[7] R. W. McClard and M. E. Jones, unpublished experiments.

Fyfe, J. A., Miller, R. L., & Krenitsky, T. A. (1973) *J. Biol. Chem. 248*, 3801–3809.
Goitein, R. K., Chelsky, O., & Parsons, S. M. (1978) *J. Biol. Chem. 253*, 2963–2971.
Grobner, W., & Kelley, W. N. (1975) *Biochem. Pharmacol. 24*, 379–384.
Handschumacher, R. E. (1960) *J. Biol. Chem. 235*, 2917–2919.
Jones, M. E. (1980) *Annu. Rev. Biochem. 49*, 253–279.
Jones, M. E., Kavipurapu, P. R., & Traut, T. W. (1978) *Methods Enzymol. 51*, 155–167.
Kavipurapu, P. R., & Jones, M. E. (1976) *J. Biol. Chem. 251*, 5589–5599.
Komura, H., Nakanishi, K., Potvin, B. W., Stern, H. J., & Krooth, R. S. (1980) *J. Am. Chem. Soc. 102*, 1208–1210.
McClard, R. W., Black, M. J., Livingstone, L. R., & Jones, M. E. (1980) *Biochemistry 19*, 4699–4706.
Ohnuma, T., Roboz, J., Shapiro, M. L., & Holland, J. F. (1977) *Cancer Res. 37*, 2043–2049.
Reyes, P., & Guganig, M. E. (1975) *J. Biol. Chem. 250*, 5097–5108.
Reyes, P., & Intress, C. (1978) *Life Sci. 22*, 577–582.
Reyes, P., Intress, C., & Sandquist, R. B. (1978) *Fed. Proc., Fed. Am. Soc. Exp. Biol. 37*, 1342.
Shoaf, W. T., & Jones, M. E. (1973) *Biochemistry 12*, 4039–4051.
Sundaralingam, M. (1969) *Biopolymers 7*, 821–860.
Tax, W. J. M., & Veerkamp, J. H. (1979) *Biochem. Pharmacol. 28*, 829–831.
Tax, W. J. M., Veerkamp, J. H., Trijbels, F. J. M., & Schretlen, E. D. A. M. (1976) *Biochem. Pharmacol. 25*, 2025–2032.
Tax, W. J. M., Veerkamp, J. H., & Schretlen, E. D. A. M. (1979) *Biol. Neonate 35*, 121–125.
Traut, T. W., & Jones, M. E. (1977a) *Biochem. Pharmacol. 26*, 2291–2296.
Traut, T. W., & Jones, M. E. (1977b) *J. Biol. Chem. 252*, 8374–8381.
Traut, T. W., & Jones, M. E. (1979) *J. Biol. Chem. 254*, 1143–1150.
Traut, T. W., & Payne, R. C. (1980) *Biochemistry* (following paper in this issue).
Victor, J., Greenberg, L. B., & Sloan, D. L. (1979) *J. Biol. Chem. 254*, 2647–2655.

Dependence of the Catalytic Activities on the Aggregation and Conformation States of Uridine 5′-Phosphate Synthase†

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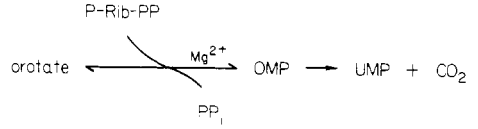
ABSTRACT: Uridine 5′-phosphate (UMP) synthase is a multifunctional protein that contains the last two enzyme activities for the de novo biosynthesis of UMP, orotate phosphoribosyltransferase (EC 2.4.2.10) and orotidine-5′-phosphate (OMP) decarboxylase (EC 4.1.1.23). The native enzyme from mouse Ehrlich ascites cells exists in at least three distinct aggregation/conformation states as measured by sedimentation in sucrose gradients: a 3.6S monomer, a 5.1S dimer, and a conformationally altered 5.6S dimer. It has previously been reported that a variety of ligands (of which the most effective is OMP) mediate the conversion of the 3.6S monomer to the two types of dimers. Initial velocity studies with the enzyme in the different native states show that all three forms of UMP synthase have phosphoribosyltransferase activity but that the OMP decarboxylase is either uniquely or at least predominantly associated with the 5.6S form. Activation of this enzyme activity by the substrate appears to be the result of both a dimerization and a conformation step.

The two enzyme activities of UMP synthase,[1] orotate phosphoribosyltransferase (EC 2.4.2.10) and orotidine-5′-phosphate (OMP) decarboxylase, catalyze the last two enzymatic steps of de novo UMP biosynthesis.



Previous studies have shown that native UMP synthase exists in at least three different physical states, with sedimentation constants of 3.6 for the monomer and 5.1 or 5.6 for the dimer (Traut & Jones, 1979; Traut et al., 1980). The aggregation of the 3.6S monomer to the 5.1S dimer is mediated by a variety of effectors [nucleoside monophosphates, 5-phosphoribosyl 1-pyrophosphate (P-Rib-PP), P_i, etc.] binding to the OMP decarboxylase catalytic site (Traut & Jones, 1979). A conformational alteration of the 5.1S dimer to the 5.6S dimer is produced only by pyrimidine nucleotides acting on a regulatory site that appears to be distinct from either of the two catalytic sites (Traut et al., 1980). The formation of the 5.6S species of UMP synthase is promoted most effectively by OMP, the product of one catalytic center, and the substrate for the second catalytic site of UMP synthase. Kinetic studies suggest that the aggregation/conformation state produced by the substrate OMP increases the catalytic activity of the OMP decarboxylase.

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[1] Previous publications on UMP synthase have used the designation "complex U" for the single polypeptide chain which possesses these two enzyme activities. Because of the recent purification of a single homogeneous polypeptide chain with both enzyme activities, the term "complex U" has been replaced by "UMP synthase". A more precise term has also been suggested, "multienzyme *pyr 5,6*" (Jones, 1980; Christopherson et al., 1980).

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