Interconversion of Different Molecular Weight Forms of the Orotate Phosphoribosyltransferase·Orotidine-5'-'phosphate Decarboxylase

Enzyme Complex from Mouse Ehrlich Ascites Cells*

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Complex U consists of orotate phosphoribosyltrans-ferase (EC 2.4.2.10) and orotidine-5'-phosphate decarboxylase (EC 4.1.1.23), the last two enzymes of the de novo pathway for UMP biosynthesis. Earlier studies had shown that orotate and magnesium 5-phosphoribosyl-1-pprophosphate, substrates for the transferase, and orotidine 5'-monophosphate (OMP), the product of the transferase and also the substrate for the decarboxylase, could alter the conformation of Complex U as measured by changes in sedimentation on sucrose gradients (Traut, T. W., and Jones, M. E. (1977) J. Biol. Chem. 252, 8374-8381). More extensive studies using both density gradient sedimentation and gel filtration chromatography reveal that Complex U has four different aggregation states: a 3.6 S species (Mr = 45,400) which is probably a monomer, a 4.7 S species (Mr = 78,000), a 5.1 S dimer (Mr = 92,800), and a 5.6 S species (Mr = 118,000). The 4.7 S and 5.6 S species are interpreted as hybrids formed with either the monomer or the dimer of Complex U and an as yet unidentified molecule, whose Mr is about 29,000.

A wide variety of compounds (Pi, 5-phosphoribosyl-1-pyrophosphate, chloride, OMP, UMP, azaUMP) promote the formation of the dimer. Of these effectors, OMP is a substrate for the decarboxylase, and the rest are all competitive inhibitors (versus OMP) of the decarboxylase reaction. The data suggest that formation of the dimer is achieved by the binding of these effector molecules to the decarboxylase catalytic site since each of these inhibitors, at a concentration 10 times its Ki, versus OMP, will completely convert the 3.6 S monomer to the 5.1 S dimer. Only the nucleotides OMP, UMP, and azaUMP can convert Complex U entirely to the 5.6 S species.

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EXPERIMENTAL PROCEDURES

Materials

Tetrasodium pyrophosphate, tetrasodium P-Rib-PP, diithiothreitol, NAD, NADH, alcohol dehydrogenase (horse liver), catalase (beef liver), ferritin (horse spleen), 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-'C]OMP and [6-'C]orotate were purchased from New England Nuclear. Polyethyleneimine cellulose plates were obtained from Brinkmann and ultrapure sucrose was purchased from Schwarz/Mann.

Methods

Enzyme Preparation—The two enzyme activities of Complex U were prepared from mouse Ehrlich ascites cells as described previously (2). In the present work, the protein fraction precipitating between 1.7 and 2.6 M ammonium sulfate was used. This fraction is purified 8 to 10-fold. For the studies in Fig. 4, diithiothreitol was omitted during the preparation of the enzyme fraction and from the storage buffer.

Enzyme Assays—Orotate phosphoribosyltransferase was measured either by the release of 14CO2 from [7-'C]orotate, when the reaction was coupled with orotidine-5'-phosphate decarboxylase, or by measuring the production of [6-'C]OMP and [6-'C]UMP from [6-'C]orotate using thin layer chromatography. Orotidine-5'-phosphate decarboxylase was measured by the production of 14CO2 from [7-'C]OMP. Unless stated otherwise, the composition of the reaction mixtures and procedures for measuring the products were exact as described before (14).

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* The abbreviations used are: P-Rib-PP, 5-phosphoribosyl-1-pyrophosphate; OMP, orotidine 5'-phosphate;azaUMP, 6-azaUMP.
Interconversion of Different M. Forms of Complex U

Sucrose Gradient Ultracentrifugation—Linear sucrose density gradients, 10 to 40% (w/v), were prepared, centrifuged, and fractionated as described elsewhere (11). Some gradients were 5 ml and were centrifuged at 64,500 rpm with a Beckman SW 65 rotor for 16 to 24 h. Most gradients were 12 ml and were centrifuged at 41,000 rpm with an SW 41 rotor for 65 h. Compositions of sucrose solutions are described in the legends to figures and tables.

Samples of 100 ml containing 1 mg of enzyme protein plus four marker proteins (750 µg of hemoglobin, 1.5 µg of alcohol dehydrogenase, 150 µg of lactate dehydrogenase, and 100 µg of catalase) were layered on top of the 5-ml gradients. For the 12-ml gradients, samples were 200 ml with the enzyme and marker protein at the same concentrations.

The sedimentation coefficient of the enzyme complex in the various solvent systems listed was determined from a calibration curve of the four standard proteins using values from the literature (15) for hemoglobin (4.3 S), alcohol dehydrogenase (6.1 S), lactate dehydrogenase (7.0 S), and catalase (11.3 S). For some gradients, only the three smaller markers were used to estimate the Sedimentation coefficient of Complex U.

Gel Filtration—Ascending chromatography of Complex U was done on a Sephadex G-200 column (1.6 x 92 cm) with solvents as described in figure legends. A 0.3-ml sample containing 20 to 200 µg of enzyme protein plus five marker proteins (8 mg of hemoglobin, 8 mg of alcohol dehydrogenase, 0.4 mg of lactate dehydrogenase, 0.3 mg of catalase and 2 mg of ferritin) was applied to the column and eluted at flow rates of 2 to 4 ml/h. Ferritin was monitored by its absorbance at 407 nm, and the other protein markers were assayed as described previously (11).

Calculation of Mₐ—The following equations, whose derivation is described by Siegel and Monty (16), were used to calculate the molecular weight and frictional coefficient of different forms of Complex U:

\[ Mₐ = \frac{6\eta N \alpha}{(1 - \phi) f/f₀} \]

\[ \frac{f}{f₀} = \frac{3Mₐ}{4\eta N} \]

where \( \eta \) is the viscosity of the medium, N is Avogadro’s number, \( \alpha \) is the Stokes radius in centimeters, \( f/f₀ \) is the partial specific volume of Complex U (assumed to be 0.725, as discussed in Ref. 17), and \( \phi \) is the density of the solvent. The variables \( \alpha \) and \( f/f₀ \) are determined by gel filtration chromatography and by ultracentrifugation, respectively, as described above.

Determination of Monomer-Dimer Ratios—As detailed under “Results,” the monomer and dimer forms of Complex U are interpreted as being in a rapid equilbrium with one another, so that mixtures of the two forms sediment as single peaks on ultracentrifugation with sedimentation coefficients intermediate between those for pure monomer and pure dimer. Using molecular weight values described in Table II, one can calculate an “average” molecular weight for any mixture with a given proportion of dimer to monomer. This calculated value can then be correlated with a sedimentation coefficient from a calibration curve of our marker proteins.

As an example, a mixture that contains 50% of the Complex U enzyme activities in the dimer form would contain one dimer with a molecular weight of 105,000 and two monomers of about 45,000. The average molecular weight would be 60,000, which corresponds to an s value of 4.0 on our standard curve. This procedure was used to calculate the “per cent of Complex U as dimer” scale in Fig. 1.

RESULTS

Sedimentation of Complex U—An extensive survey of the sedimentation of Complex U was done with a variety of substrates, products, or their analogs (Table I). Some of these data have been reported in a preliminary paper (12). The sedimentation profile of the enzyme complex normally had the form of a sharp and symmetric peak, with S values that increased gradually as effector concentrations were increased in the solvent. An increase in any single effector always produces an increase in the S value. This is shown more clearly in Fig. 1, where the sedimentation of Complex U increases from 3.6 S° to 5.1 S as the concentration of ortho-phosphate increases from 0 to 10 mM. In the 12-ml gradients, for subsequent studies increased the resolution in measuring sedimentation and produced a lower limit of 3.6 S for the sedimentation of Complex U in sucrose gradients containing only Tris-HCl/dithiothreitol buffer.

Table I: Sedimentation of Complex U in presence of different effectors

<table>
<thead>
<tr>
<th>Solvent*</th>
<th>Observed S values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tris (20)</td>
<td>3.70</td>
</tr>
<tr>
<td>Tris (20), KCl (100)</td>
<td>3.70</td>
</tr>
<tr>
<td>Tris (20), Pi (1), MgCl₂ (5), EF (10)</td>
<td>3.75</td>
</tr>
<tr>
<td>Tris (20), Pi (1), MgCl₂ (5), P-Pi (0.2), Pi (1)</td>
<td>3.75</td>
</tr>
<tr>
<td>Tris (20), Pi (1), MgCl₂ (5), P-Pi (0.2), Pi (1), P-Pi (0.2)</td>
<td>3.80</td>
</tr>
<tr>
<td>Tris (20), Pi (1), MgCl₂ (5), P-Pi (0.2), Pi (1), P-Pi (0.2), Pi (1)</td>
<td>3.90</td>
</tr>
<tr>
<td>Tris (20), Pi (1), MgCl₂ (5), P-Pi (0.2), Pi (1), P-Pi (0.2), Pi (1), P-Pi (0.2)</td>
<td>4.00</td>
</tr>
</tbody>
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* All solvents contained 2 mM dithiothreitol; values in parentheses are concentrations in mM.

Figure 1. Dependence of Complex U sedimentation on the concentration of Pi. The sedimentation of Complex U was measured on 5-ml (A) or on 12-ml (B) to 40% sucrose gradients containing the standard buffer (20 mM Tris-HCl, 2 mM dithiothreitol) and Pi as indicated. The location of the Complex U peak was determined by measuring the activity of orotidine-5′-phosphate decarboxylase in gradient fractions, and the S value was obtained from comparison with the marker proteins hemoglobin, alcohol dehydrogenase, lactate dehydrogenase, and catalase, which were measured in the same gradients as Complex U. The scale along the right ordinate shows the per cent of the enzyme complex that would be in the dimer form for any peak-sedimenting intermediate between the monomer and dimer species.
phosphate (P_i) is increased from 0 to 500 mM. Higher concentrations of P_i (up to 1 m) did not increase the sedimentation beyond 5.1 S. A sedimentation coefficient of 5.1 is the appropriate value for a dimer of a molecule with a sedimentation coefficient of 3.6, and intermediate sedimentation values represent mixtures of monomer and dimer that are in rapid equilibrium, and therefore sediment as a single sharp peak. Such sedimentation behavior of monomer-dimer systems has been described in theory (18, 19) and in experimental studies (20, 21). P-Rib-PP and chloride also promote aggregation of the monomer of Complex U to the 5.1 S species.

In the presence of certain nucleotides, the aggregation of Complex U increased further to a species that has an upper S value of 5.6. Fig. 2 shows such an increase in sedimentation in the presence of increasing concentrations of azaUMPA, an analog of OMP and potent inhibitor of the decarboxylase activity (22). As azaUMPA is increased, the enzyme complex increases its sedimentation from 3.6 S in buffer alone (Fig. 2A) through 5.1 S (Fig. 2C) and finally to 5.6 S at very high concentrations of azaUMPA (100 μM, Fig. 2D). Similar profiles showing a single peak are obtained with UMP, the product of the decarboxylase.

OMP also produces the 5.6 S species of Complex U, but this compound, which is the product of the first enzyme activity and the substrate for the second enzyme activity of Complex U, is unusual in that it alone can simultaneously produce two different species of Complex U (when the enzyme activities are stabilized by diithiothreitol) that are separable on centrifugation. As shown in Fig. 3, both enzyme activities sediment in the 3.6 S form (Fig. 3A) and continue to co-sediment in both a 3.6 S and a 5.6 S form (Fig. 3B and C) as OMP is increased to 30 and 100 μM, respectively. When OMP is at 300 μM, all of the enzyme activities are in the 5.6 S form. The fact that the 3.6 S and 5.6 S species sediment as distinct peaks indicates that these two forms of Complex U are not in rapid equilibrium, as is true of the 3.6 S and 5.1 S forms of the enzyme complex. In the presence of OMP, below 200 μM, we always observed two distinct peaks, and we never observed peaks that have values intermediate between 3.6 S and 5.1 S, or between 3.6 S and 5.6 S, as is true with azaUMPA and UMP.

The reason for the unique sedimentation behavior of Complex U in the presence of OMP is, at least partly, due to the fact that OMP is the only effector whose concentration changes with time during sedimentation experiments (i.e. OMP is decarboxylated to UMP by Complex U as the enzyme complex sediments through the sucrose gradient). We have done experiments similar to those of Fig. 3 with [7-3H]OMP in the sucrose gradient. After centrifugation, the remaining OMP concentration was measured, and ranged from 0.9 to 1.2 μM in the upper region of the gradients through which Complex U had sedimented; in the denser part of the gradient, where the enzyme complex had not yet migrated, the original OMP concentration was unchanged. Thus, the amount of OMP necessary to cause aggregation of Complex U to the 5.6 S form is much smaller than the initial concentration used, and the starting concentration needed is, in turn, a function of the time needed for sedimentation. With the SW 41 rotor, it takes 65 h at top speed to clearly resolve the 3.6 S and 5.6 S peaks from one another. In such an experiment, it requires about 200 μM OMP at the beginning of the centrifugation to maintain all of Complex U in the 5.6 S form. With the higher speed SW 65 rotor, a similar experiment lasts 16 h and requires only 40 μM OMP initially to maintain all of Complex U in the 5.6 S form. With the Sorvall TV-865B vertical rotor, this can
be repeated in 7 h with 15 μM OMP (data not shown). We cannot do shorter time experiments without sacrificing resolution of the two peaks, but the above three experimental values can be extrapolated to less than 1 μM OMP at zero time. That is, the instantaneous concentration of OMP required to maintain all of Complex U in the 5.6 S form is about 1 μM or less; this is in very good agreement with the actual determination of OMP remaining in the gradient after centrifugation, as described above.

The aggregation state of Complex U is completely dependent upon the environment in which the enzyme complex is studied. As shown in Figs. 1 to 3, an increase in the concentration of any effector causes conversion of the monomer form toward the 5.1 S or 5.6 S species. However, these higher molecular weight forms can also be shifted back to the 3.6 S monomer. We have pooled the high molecular weight peaks from gradients, concentrated them, and resedimented them through gradients containing only buffer (data not shown). In such studies only the 3.6 S peak is observed.

Other investigators have reported that Complex U prepared from human erythrocytes (10) and liver (8), or from mouse liver (23), is unstable on storage, and is converted to both larger (8, 10, 23) and smaller (8, 10) sedimenting species after storage for 2 weeks or more at −20°C. In all our studies, we routinely maintain Complex U in the presence of 2 mM dithiothreitol, beginning with the cell homogenate in the preparation of the enzyme and throughout storage and assay. This compound stabilizes the two enzyme activities of Complex U (2), so that not more than 15% of either activity is lost over 6 months of storage at −85°C, and the sedimentation behavior of such stored preparations remains unchanged. Since our data are at variance with studies reporting on the stability and aggregation states of Complex U from other tissues where dithiothreitol was not used (7, 8, 10, 13), we have repeated our studies in the absence of dithiothreitol in an attempt to detect species of Complex U different from those shown in Figs. 2 and 3.

When the enzyme fraction is prepared in the absence of dithiothreitol, the specific activities that can be measured are greatly reduced, even when assayed in the presence of dithiothreitol; after 3 days of storage at 4°C, only about 10% of the decarboxylase activity remains, and the transferase activity is undetectable. However, if such enzyme preparations are incubated for 4 h at 4°C with 2 mM dithiothreitol prior to assay, the two enzyme activities are completely recovered in 3-day-old preparations, and 80 to 90% recovered in 1-week-old preparations.

The sedimentation profiles of Complex U prepared without dithiothreitol are shown in Fig. 4. A freshly prepared enzyme fraction, Fig. 4A, and similarly prepared fractions stored at −20°C for 1 week (Fig. 4B) or for 4 weeks (Fig. 4C) were sedimented in sucrose gradients containing only 20 mM Tris buffer, without dithiothreitol. In all three experiments, both a 3.6 S and a 5.6 S species are clearly evident. By contrast, in the presence of dithiothreitol, only the 3.6 S peak would be observed (see Figs. 2A and 3A). In addition, all three experiments of Fig. 4 show enzyme activity associated with broad range of sedimentation larger than the 5.6 S species. Such larger aggregations of complex U have never been observed in any significant quantity with enzyme samples that were stabilized by dithiothreitol. If an experiment similar to that of Fig. 4B is repeated with 30 μM OMP in the gradient, then the 5.6 S peak becomes larger than the 3.6 S peak (i.e. part of the 3.6 S is converted to the 5.6 S species, similar to the observation in Fig. 3B).

A significant result from the experiments of Fig. 4 is that no species of Complex U smaller than 3.6 S can be detected in an enzyme preparation that has been made unstable by storage in the absence of dithiothreitol. Although the 4-week-old preparation (Fig. 4C) has lost more than 90% of both enzyme activities, the shapes of the sedimentation profiles for both the transferase and decarboxylase are the same as those of fresher preparations (Fig. 4A and B). Thus, whether Complex U from Ehrlich ascites cells is prepared with, or without, dithiothreitol, whether such preparations are stable or unstable during storage, there is no evidence in our studies that storage per se leads to aggregation of the native enzyme complex, or to its dissociation to subunits smaller than 3.6 S. While the sedimentation profiles in Fig. 4 show some enzyme activity associated with species larger than 5.6 S, these larger aggregation species are already present in the enzyme sample that was freshly prepared without dithiothreitol (Fig. 4A) and, therefore, cannot be attributed to storage.

OMP is the substrate for the decarboxylase activity of Complex U, and all other effectors used produce competitive inhibition versus OMP. This suggested that the site on the enzyme complex at which these compounds bind, in causing aggregation to the 5.1 S or 5.6 S species, could be the decarboxylase catalytic site. To test this hypothesis, sedimentation data for six effectors have been replotted as a function of their relative binding strength at the decarboxylase catalytic site. As shown in Fig. 5, OMP is the most effective in producing the 5.6 S form. Of the other five effectors, all but AMP are
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almost identical in the relative increment of their concentrations above their individual \(K_i\) values (versus OMP) required to shift Complex U from the 3.6 S to the 5.1 S form. An additional effector (not shown) that would also plot on the curve between 3.6 S and 5.1 S is chloride ion. We interpret these results as supporting the hypothesis that the formation of the 5.1 S dimer from the 3.6 S monomer is caused by effector molecules binding at the decarboxylase catalytic site. The formation of the 5.6 S species probably results from binding of effector molecules to some secondary site on Complex U since the curves diverge between 5.1 S and 5.6 S in Fig. 5.

The curve for AMP in Fig. 5 is quite distinct from the others since AMP, even at concentrations of 200 mM, produces an aggregate of Complex U with an upper size of 4.7 S. The significance of this 4.7 S species will be discussed below.

**Gel Filtration Chromatography of Complex U**—Comparison of \(M_t\) values and sedimentation coefficients for Complex U indicate that this is an asymmetric molecule (12). Therefore, we determined the Stokes radius of each of the three major species by gel filtration chromatography on Sephadex G-200 as described under “Experimental Procedures.” Columns were first equilibrated with buffer plus an effector known to produce the 3.6 S, 5.1 S, or 5.6 S forms on sedimentation. As can be seen in Fig. 6A, the 3.6 S form of Complex U elutes faster than hemoglobin, while on sucrose gradients it sediments more slowly than hemoglobin (Figs. 2A and 3A). In similar fashion, the 5.1 S form, which co-sediments with alcohol dehydrogenase, elutes from the molecular sieve well ahead of this marker (Fig. 6B), and the 5.6 S form elutes near catalase (Fig. 6C). Clearly, Complex U in its different forms has a more asymmetric shape than the more spherical marker proteins. As shown in Table II, the Stokes radii for these three forms of Complex U are 3.05, 4.45, and 5.10 nm. The 3.6 S form has a \(f/f_0\) of 1.30, while the 5.1 and 5.6 S forms are more elongated and have similar \(f/f_0\) values of 1.47 and 1.51, respectively. With these values and the sedimentation coefficients determined above, we have calculated the molecular weights for the three species as described under “Experimental Procedures.” The monomer of Complex U has a \(M_t\) of 45,000 ± 800

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**Fig. 5.** The sedimentation of Complex U in the presence of a variety of effectors, as a function of their binding affinity at the decarboxylase catalytic site. The sedimentation of Complex U was measured, separately for each effector, in the presence of the four nucleotides identified in Fig. 4, as well as Pi ( ), P-Rib-PP ( ), and AMP ( ).

**Fig. 6.** Elution profiles of Complex U on Sephadex G-200. The elution of Complex U, measured by following the activity of orotidine-5'-phosphate decarboxylase, was done as described under “Experimental Procedures.” Columns were equilibrated and eluted with standard buffer (A), with buffer plus 0.5 M Pi (B), or with buffer plus 100 \(\mu\)M azaUMP (C). The inset in B shows the calibration curve obtained for the marker proteins: ferritin ( ), catalase ( ), lactate dehydrogenase (LDH) ( ), alcohol dehydrogenase (ADH) ( ), and hemoglobin (Hb) ( ).
in a number (indicated in parentheses) of different gradients. The size of the gradient fractions collected. For any single gradient, that is evident in Fig. 4 is clearly missing in Figs. 2A and 3A. Ehrlich ascites Complex U (Figs. 2A and 3A). The 5.6 S peak ultracentrifugation. values determined by a combination of gel filtration and S) are always in rapid equilibrium and cannot be separated with the latter complex the monomer (3.6 S) and dimer (5.1 S). The 5.6 S and 5.1 S are clearly evident, and a small fraction of the nucleotide analogs of OMP converted all the enzyme to a form of Complex U has a mass of 118,000 ± 1,500 daltons (n = 10). This latter value is clearly a noninteger multiple of the monomer value, and its significance will be considered below.

**DISCUSSION**

The degree of aggregation exhibited by Complex U from Ehrlich ascites cells is dependent on the presence or absence of dithiothreitol. When Complex U is prepared and centrifuged in the absence of dithiothreitol, two major species at 3.6 S and 5.6 S are clearly evident, and a small fraction of the enzyme activities is associated with aggregation species larger than 5.6 S (Fig 4). Studies of Complex U from other tissues that were done without dithiothreitol (7, 10, 13) have also reported multiple aggregation states. Brown et al. (7) have studied Complex U in human erythrocytes and reported three distinct elution peaks on gel chromatography with M, of 62,000, 115,000, and 250,000 (although the two larger peaks cannot be consistently resolved from each other). With a similar preparation of Complex U (i.e. human erythrocytes; no dithiothreitol), Grobner and Kelley have reported that ultracentrifugation studies show two major forms (sometimes three) of this enzyme complex, one at 3.6 S and the other at 5.0 S (13). We feel that the enzyme complex from Ehrlich ascites cells is quite similar to the complex from erythrocytes. The M, values that we would obtain by gel filtration chromatography alone are 75,000, 120,000, and 215,000 for the 3.6 S, 5.1 S, and 5.6 S forms, respectively, and these values are in reasonable agreement with those of Brown et al. (7). The S values obtained by Grobner and Kelley (13) of 3.6 and 5.0 are almost identical to the values we have obtained for the monomer and dimer. However, we feel that the sedimenting peak assigned a value of 5.0 S by Grobner and Kelley is most likely equivalent to the 5.6 S species of the ascites complex since the larger complex observed by Grobner and Kelley (13) are a little lower than ours, and those of Reyes and Guganig (9) are higher than ours. Reyes and Guganig, however, concluded that the larger form produced by nucleotides were not dimers but more likely conformers of the monomer (9).

While dithiothreitol causes higher aggregates to dissociate, the dissociated monomers can be reaggregated by a variety of effectors, as shown in Table I and Figs. 1 to 3. Similar aggregation of the monomer has been observed in earlier reports on the ascites Complex U (11, 12) and has also been reported by Grobner and Kelley with erythrocyte Complex U (13) and by Reyes and Guganig with Complex U from murine leukemia (9). In the former study (13), it was reported that nucleotide analogs of OMP converted all the enzyme to a species sedimenting at 5.2 to 5.3 S, similar to the 5.6 S form we see. In the latter study (9), the authors found that 5 mM UMP produces a species with an S value of 6.0, while 100 µM OMP produces two species, the native monomer plus an aggregate of about 6.1 S. The degree of aggregation of the native monomer in the presence of effectors is the same in the two studies above as it is with enzyme complex from Ehrlich ascites cells (22), although the S values determined for the aggregated species by Grobner and Kelley (13) are a little lower than ours, and those of Reyes and Guganig (9) are higher than ours. Reyes and Guganig, however, concluded that the larger forms produced by nucleotides were not dimers but more likely conformers of the monomer (9).

We feel that the combination of gel filtration studies and extensive sedimentation studies we have done makes it possible to give a more definitive interpretation of this enzyme complex, as illustrated in Fig. 7. The 3.6 S form is the monomer, containing both the transferase and decarboxylase activities, and the 5.1 S form is the dimer. The formation of the dimer is enhanced by a variety of effectors which probably produce dimerization by binding at the decarboxylase catalytic site since all are competitive inhibitors versus OMP and, at concentrations 10 times their individual K, versus OMP, they all produce the 5.1 S dimer.

The formation of the 5.6 S species, which, in the presence of dithiothreitol, is only promoted by nucleotides, has three possible interpretations. The 5.6 S aggregate could represent an altered conformer of the 5.1 S dimer, a trimer of the 3.6 S monomer, or a hybrid complex between the dimer and some other moiety. The first interpretation is not favored by our data, since the 5.6 S species has essentially the same f/fo as

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**Table II**

**Physical Parameters for Complex U**

<table>
<thead>
<tr>
<th>Effector*</th>
<th>Stokes radius <em>nm</em></th>
<th>ηweighted <em>S</em></th>
<th>M, <em>values</em></th>
<th>f/f°</th>
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<tbody>
<tr>
<td>None</td>
<td>3.05</td>
<td>3.6</td>
<td>45,400</td>
<td>1.30</td>
</tr>
<tr>
<td>500 µM P</td>
<td>4.45</td>
<td>5.05</td>
<td>92,800</td>
<td>1.47</td>
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<tr>
<td>100 µM azaUMP</td>
<td>5.10</td>
<td>5.6</td>
<td>118,000</td>
<td>1.51</td>
</tr>
</tbody>
</table>

* Each condition included 20 mM Tris-HCl, pH 7.4, and 2 mM dithiothreitol.

(n = 12),4 the dimer is 92,800 ± 1,000 (n = 8), with a commensurate increase in its frictional coefficient. The 5.6 S form of Complex U has a mass of 118,000 ± 1,500 daltons (n = 10). The 5.6 S peak ultracentrifugation. values determined by a combination of gel filtration and S) are always in rapid equilibrium and cannot be separated with the latter complex the monomer (3.6 S) and dimer (5.1 S), Grobner and Kelley have reported that ultracentrifugation studies show two major forms (sometimes three) of this enzyme complex, one at 3.6 S and the other at 5.0 S (13). We feel that the enzyme complex from Ehrlich ascites cells is quite similar to the complex from erythrocytes. The M, values that we would obtain by gel filtration chromatography with M, of 62,000, 115,000, and 250,000 (although the two larger peaks cannot be consistently resolved from each other). With a similar preparation of Complex U (i.e. human erythrocytes; no dithiothreitol), Grobner and Kelley have reported that ultracentrifugation studies show two major forms (sometimes three) of this enzyme complex, one at 3.6 S and the other at 5.0 S (13). We feel that the enzyme complex from Ehrlich ascites cells is quite similar to the complex from erythrocytes. The M, values that we would obtain by gel filtration chromatography alone are 75,000, 120,000, and 215,000 for the 3.6 S, 5.1 S, and 5.6 S forms, respectively, and these values are in reasonable agreement with those of Brown et al. (7). The S values obtained by Grobner and Kelley (13) of 3.6 and 5.0 are almost identical to the values we have obtained for the monomer and dimer. However, we feel that the sedimenting peak assigned a value of 5.0 S by Grobner and Kelley is most likely equivalent to the 5.6 S species of the ascites complex since the larger complex observed by Grobner and Kelley (13) are a little lower than ours, and those of Reyes and Guganig (9) are higher than ours. Reyes and Guganig, however, concluded that the larger form produced by nucleotides were not dimers but more likely conformers of the monomer (9).

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* The limiting measurement in the calculation of M, is the observed ηweighted. The lower limit, for the resolution of this value is determined by the size of the gradient fractions collected. For any single gradient, the limiting resolution is equivalent to ±4,000 for the 3.6 S species, ±5,300 for the 5.1 S species, and ±6,100 for the 5.6 S species. The values in the text are standard deviations for repeated measurements in a number (indicated in parentheses) of different gradients.
the 5.1 S dimer, yet the 5.6 S form behaves as a molecule that is larger than the 5.1 S species, both in gel filtration and on sedimentation (Table II), suggesting a real increment in mass above the 5.1 S form. However, the 5.6 S species is not large enough to be a trimer, because a trimer of the 3.6 S monomer would have a \( M_r \) of 136,000 and an expected \( s_{20, w} \) > 6.2, and our resolution is good enough to distinguish between such a trimer and the 5.6 S species, which, therefore, probably represents a hybrid complex between a 5.1 S dimer and some other molecule. We feel that the 4.7 S species observed with AMP represents a similar complex between the monomer of Complex U and a foreign molecule.

It is our interpretation (see "Results") that almost all of the enzyme complex could be maintained in the 5.6 S form by OMP concentrations of 1 \( \mu \)M or less. Since physiological concentrations of OMP are about 0.07 \( \mu \)M (25), it is likely that in \( \text{in vivo} \) a fraction of Complex U may exist in the 5.6 S form. Additionally, the 5.6 S form is found in enzyme preparations that do not contain dithiothreitol (Fig. 4). The significance of this postulated 5.6 S hybrid complex will depend on the demonstration of the unknown moiety associated with Complex U after further purification of the enzyme complex. An example of such a hybrid complex was reported by Wild and Belcr (26, 27). In studying these two enzyme activities in \( \text{Serratia marcescens} \), they found that a complex including dihydroorotase (EC 3.5.2.3) could be formed; ion exchange chromatography separated the dihydroorotase from the transferase and decarboxylase.  

One interpretation of the 4.7 S species produced by AMP is that AMP is unable to promote the complete dimerization of Complex U and that the observed 4.7 S peak is simply a mixture of monomer and dimer. However, a 4.7 S peak produced by AMP is distinctly narrower than a 4.7 S peak produced by Pi, which represents a mixture of monomer and dimer. Alternatively, the 4.7 S species might also represent a hybrid complex between a monomer of Complex U and some other molecule. In the latter hypothesis, we can calculate from the sedimentation data that this foreign molecule would have a mass of about 33,000 daltons, which is not too different from the calculated mass increment of 25,000 daltons in the formation of the 5.6 S species from the 5.1 S dimer. It is therefore conceivable that the same unknown molecule (with a \( M_r \) of 29,000 ± 4,000) could associate with the monomer of Complex U to form the 4.7 S species and with the dimer to form the 5.6 S species. However, we have no evidence so far that the 4.7 S species can be converted to a 5.6 S form.

The molecular weight of 45,400 that we have assigned to the monomer (Table II) is lower than previously reported for Complex U from Ehrlich ascites cells (1, 2). In the earlier study, sedimentation was measured in 10 mM Pi buffer, and as shown in Fig. 1, this small amount of Pi is sufficient to shift the sedimentation to a slightly higher value. In similar fashion, the value of 65,000 assigned by Reyes and Guganig to the monomer of Complex U from murine leukemia cells was determined in the presence of 50 mM Pi buffer (9) and is similar to the apparent molecular weight that we would calculate for the same conditions. In human lymphoblasts, this complex has been reported to have \( M_r \) of 41,000 for the smallest form observed (29).

We feel that the variety of studies that we have done, in the absence or presence of dithiothreitol, with a wide variety of effectors, and using both density gradient centrifugation and gel filtration chromatography, allows us to have confidence in our \( M_r \) assessments, and the model postulated in Fig. 7 is consistent with the data presented here, as well as with most of the data from other laboratories.

However, other investigators have reported that Complex U dissociates to subunits of \( M_r \approx 35,000 \) (8, 10, 24) after storage, and to even smaller subunits when the erythrocyte enzyme complex is denatured with guanidine hydrochloride (10). While we have not attempted to denature the enzyme complex from ascites cells, we have examined preparations stored without dithiothreitol at \(-20^\circ C\) for 1 month, or with dithiothreitol for up to 2 months at \(-20^\circ C\), and for over 6 months at \(85^\circ C\), and observed no form of Complex U smaller than 3.6 S. In preparations of Complex U from Ehrlich ascites cells that were stored without dithiothreitol, there is a considerable loss of both enzyme activities. However, in these preparations, there is no discernible shift in the sedimentation profiles after storage. If dissociation to subunits occurred, they had no enzymatic activity and, thus, were not detectable. The fact that stored preparations containing dithiothreitol have been very stable, retaining 90 to 100% of original activity for both the transferase and decarboxylase, makes it unlikely that the 3.6 S monomer could have dissociated in part to a smaller, but inactive, subunit in these preparations of Complex U.

While it is possible that the human enzyme complex is different from the mouse Ehrlich ascites cell Complex U in that it has dissociable subunits with \( M_r \approx 35,000 \) and smaller, it is less likely that the mouse liver enzyme should be so different from the ascites cell complex. Since the enzyme preparations used in these studies (9, 10, 24) that reported subunits were much cruder than ours (i.e., they have specific activities much lower than the value of 12 nmoles/min/mg for the decarboxylase in our studies), it is also quite possible that subunits containing only one of the two enzyme activities of Complex U were produced by one or more protease activities in these crude preparations. Protease activity would also explain the loss of enzyme activities after storage in the above studies. In fact, Reyes and Guganig have separated the two activities of Complex U by digestion with elastase (9). Since our enzyme preparation is very stable and since we cannot demonstrate dissociation of Complex U to separable subunits with only one enzyme activity, we feel that the model illustrated in Fig. 7 best represents the different physical states of Complex U.
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Interconversion of different molecular weight forms of the orotate phosphoribosyltransferase orotidine-5'-phosphate decarboxylase enzyme complex from mouse Ehrlich ascites cells.

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