Subunit Interactions in Aspartate Transcarbamylase

A MODEL FOR THE ALLOSTERIC MECHANISM* (Received for publication, July 9, 1974)

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SUMMARY

The conformational changes in aspartate transcarbamylase upon binding of substrates or regulatory ligands and the effects of alterations in the subunit structure on the allosteric interactions are reviewed. The available information including recent results from studies of the c<r6 complex (c denotes the catalytic polypeptide and r, the regulatory polypeptide) is considered in terms of the existing models for the allosteric mechanism of this enzyme. It is found that many of the discrepancies between experimental observations and the present models could be resolved by postulating an important role for r:r interactions in the allosteric mechanism.

A new model is presented in which an obligatory conformational change upon binding of substrates results in an alteration in the relative orientation of c versus r. As a consequence of symmetry conservation, the r:r domain is shifted to a position of higher potential energy. By favoring one or the other alternative r:r domains, CTP and ATP can respectively enhance and reduce the sigmoidal character of substrate saturation. The model is shown to be consistent with all of the important known properties of the enzyme. Because the heterotropic effects of CTP or ATP are postulated to operate via a mechanism separate from that for the homotropic effects of the substrates, this model accounts satisfactorily for the observation by Kerbiriou and Hervé (Kerbiriou, D., and Hervé, G. (1973) J. Mol. Biol. 78, 687-702) that homotropic effects can be abolished whereas heterotropic effects are retained in the altered enzyme from Escherichia coli grown in the presence of 2-thiouracil.

RESULTS AND DISCUSSION

In the following presentation, the existing evidence relevant to the allosteric mechanism is first reviewed briefly in order to provide the background for discussion of the models. The next section summarizes the new evidence on subunit interactions from studies of the c<r6 complex. Then, previous models are discussed in the light of the recent information. Finally, the details of the new model are given together with explanations of the known properties of the enzyme in terms of the model.

Submit Structure of Aspartate Transcarbamylase and Allosteric Phenomenon—The number of subunits and their arrangement within the quaternary structure of aspartate transcarbamylase have been discussed at length elsewhere (8, 9). Only those binding of substrates or the regulatory ligands (CTP and ATP); and (b) observations of changes in the homotropic and heterotropic interactions of aspartate transcarbamylase as a result of alterations in the structure, especially the subunit structure.

In the preceding two papers (3, 4), we described the properties of the c<r6 complex which is missing 1 of the catalytic subunits (c) of the native structure (c<r6). In these studies, it was possible to determine the effects (or the lack of them) of certain ligands on particular types of subunit interactions. The new information obtained in this work is difficult to reconcile with the existing models* of allosteric mechanism proposed for this enzyme (1, 5, 6). We have therefore formulated a new model to account for the allosteric properties of aspartate transcarbamylase in terms of subunit interactions. The model which is presented here is based on the evidence from our work on the c<r6 complex as well as on the large body of existing knowledge concerning the structure and function of the enzyme. A special feature of this model is the distinction between two types of subunit contacts (c:r and r:r) in terms of their participation in homotropic and heterotropic ligand interactions. The model appears to be consistent with all of the important known properties of the enzyme. It can also explain in a simple manner certain unusual results such as the properties of the altered enzyme obtained by growth of E. coli in the presence of 2-thiouracil (7).

*The abbreviations used are: c, catalytic polypeptide; r, regulatory polypeptide.

1 In the present discussion, we shall consider only models which offer an explanation of the allosteric mechanism of aspartate transcarbamylase on a molecular basis and exclude schemes which represent the enzyme in a number of interconvertible states whose structural relationships are not specified.

There is now considerable information available which is relevant to the molecular mechanism of allosteric behavior in aspartate transcarbamylase of Escherichia coli (for reviews, see Refs. 1 and 2). The following two types of studies have been particularly important in providing this information: (a) investigations of the changes in molecular parameters upon the...
features which are essential for an understanding of the allosteric mechanism will be summarized here. The native enzyme consists of six catalytic polypeptides (c) containing active sites and six regulatory polypeptides (r) containing regulator (CTP or ATP) binding sites (10). The catalytic polypeptides are organized as trimeric (c₃) subunits whereas the regulatory polypeptides are grouped into dimeric (r₂) subunits. The two c₂ subunits are apparently connected to each other by the three r₃ subunits in such a way that each catalytic polypeptide in one c₃ subunit is linked to a corresponding catalytic polypeptide located on the other c₃ subunit. Treatment of the native enzyme with p-hydroxymercuribenzoate results in the dissociation into c₃ and r₂ particles which can be separated (11). When the isolated subunits are mixed under appropriate conditions, reconstitution of the native structure can take place (12).

The allosteric phenomenon which we seek to explain in terms of the above structure can be briefly stated as follows. The sigmoidal saturation curve for the substrate aspartate (and similar binding curves for the substrate analog succinate) indicates cooperative interaction between the active sites (13). CTP inhibits by shifting the aspartate saturation curve toward higher aspartate concentrations and enhances the sigmoidal character whereas ATP activates by shifting this curve in the opposite direction and reducing sigmoidicity. The question of interest here is what combination of subunit interactions is responsible for these properties.

Conformational Changes Induced by Succinate and Carbamyl Phosphate—The changes in physical and chemical properties of aspartate transcarbamylase brought about by the substrate analog succinate in the presence of carbamyl phosphate are numerous and have been extensively reviewed (1, 2, 5). Many of these changes clearly reflect changes in the conformation of the protein upon binding of these ligands. It is now evident from several studies that more than one type of conformational change can occur (14, 15). For our present discussion, it is useful to distinguish between “local” and “gross” changes (to use the terms of Kirschner and Schachman). The former occurs regardless of whether the ligands bind to the native enzyme (c₃+r₂) or to the isolated catalytic subunit (c₂), whereas the latter are found only with binding to the native enzyme. The local alterations in conformation have been strongly implicated in the catalytic mechanism (2) but can only be of secondary importance in the allosteric mechanism. On the other hand, the gross conformational changes must be correlated with the well known homotropic and heterotropic interactions which are present in the native enzyme but absent in the isolated catalytic subunit. The most clear-cut example of a distinction between the two types of conformational changes is provided by measurements of the sedimentation coefficient (16, 17). Thus, succinate in the presence of carbamyl phosphate increases the S value of the isolated catalytic subunit but decreases that of the native enzyme. This observation indicates that conformational changes of opposite nature are occurring in the two cases. Most probably a contraction of the catalytic subunit upon binding of these ligands is accompanied in the native enzyme by an over-all expansion of the structure (18). The same structural alterations are apparently responsible for other changes in properties (e.g., optical rotatory dispersion spectra, Ref. 19 and sensitivity toward sodium dodecyl sulfate, Ref. 20) which also occur in opposite directions for the catalytic subunit and for the native enzyme. Similarly, circular dichroism spectra (21) can distinguish between the local and gross conformational changes discussed here. On the other hand, studies of -SH group reactivity (16) and of sensitivity toward proteolysis (22) probably only characterized the gross effects of succinate and carbamyl phosphate because reactivity in the regulatory subunit is monitored whereas binding occurs at the catalytic subunit. The coupling of local and gross conformational changes has been reviewed by Schachman (18) and it is clear from several studies that intermediate or hybrid conformations must exist (14, 15, 21). Obviously, any model of the allosteric mechanism must be able to accommodate the structural changes discussed above.

Conformational Changes Induced by CTP and by ATP—Jacobson and Stark (2) have pointed out that it is not clear in many instances whether the effects of CTP and of ATP reflect conformational changes in the regulatory subunit or whether gross changes also occur. It is probably safe to assume that these nucleotide ligands by themselves do not cause large alterations in the quaternary structure of the type induced by succinate together with carbamyl phosphate. Thus, no specific change in the sedimentation coefficient occurs in the presence of CTP (19). The only clear-cut case where the binding of CTP or ATP to the regulatory subunit induces a change in the catalytic subunit is the spin-label study by Buckman (23). The results of this study can be interpreted to mean that a group localized in the catalytic subunit in the neighborhood of the c:r domain becomes less mobile upon the binding of CTP or ATP to the regulatory subunit. The fact that dissociation of the enzyme into subunits substantially increases the mobility of the label is consistent with this interpretation. However, the label could well be located away from the c:r domain and still be affected in the above manner through conformational changes. The point emphasized here is that there is no absolute need to postulate a substantial conformational change in the catalytic subunit as a result of CTP or ATP binding. It seems almost certain that the spin-label was not located at or near the active site because succinate and carbamyl phosphate had little effect on it and the labeled derivative was highly active. The study by Colman and Markus (20) regarding the effects of CTP and ATP on the stability of aspartate transcarbamylase toward sodium dodecyl sulfate can also be interpreted by postulating a tightening of the c:r domain because both nucleotides protect aspartate transcarbamylase against dissociation.

Although it is thus uncertain whether CTP or ATP induce gross conformational changes in the absence of substrates (or analog), there is much evidence for the effects of these ligands on the conformational changes induced by succinate and carbamyl phosphate discussed earlier. For example, the decrease in the sedimentation coefficient of aspartate transcarbamylase due to 0.5 mM succinate in the presence of 1.8 mM carbamyl phosphate is reduced 70% by 1 mM CTP (16). Similarly, the enhancement in the reactivity of -SH groups caused by succinate and carbamyl phosphate is also diminished by BrCTP (an analog of CTP). The antagonistic effect of CTP toward succinate and carbamyl phosphate also extends to the local conformational change induced by the latter ligands. This effect was seen in the change in spectrum of nitrotyrosyl groups in the catalytic subunit (15). As in the case of steady state kinetics (13), these effects of CTP are overcome by saturating concentrations of substrates (or their analogs). The results of relaxation rate studies also show synergistic effects between substrate analogs and CTP (14). These data are consistent with a scheme involving two conformations for the enzyme nucleotide complex, one of which is stabilized by CTP and the other by ATP (24, 25). In this scheme, the presence of carbamyl phosphate and succinate favors the conformation stabilized by ATP.
Effects of Structural Alterations on Homotropic and Heterotropic Interactions—The observation that homotropic and heterotropic interactions are both absent in the isolated catalytic subunit (11) indicates the importance of the regulatory subunit in mediating these interactions. The parallel loss of both types of interactions as a result of proteolytic digestion of the regulatory subunit in the native enzyme (22) is also consistent with the assignment of a key role to the regulatory subunit in the allosteric mechanism. Similarly, high pH (pH 10.2) or the presence of moderate concentrations of urea abolishes both the sigmoidal saturation curve for aspartate and the sensitivity to CTP (26). However, the work of Kerbiriou and Hervé (7) shows that homotropic and heterotropic interactions can be affected differentially. In this case, the modified enzyme obtained by growing E. coli in the presence of 2-thiouracil has a hyperbolic saturation curve for aspartate but is still sensitive toward CTP and ATP. Taken together, the above observations indicate that the homotropic and heterotropic interactions are closely linked to each other but do not operate via the same mechanism.

Studies of hybrid aspartate transcarbamylase containing succinylated and unmodified catalytic subunits have provided important insight into the allosteric mechanism (18). Because the succinylated subunits do not bind the substrate aspartate, it is possible to test for cooperativity between unmodified catalytic chains in various relative positions. Thus, it is evident that active sites on unmodified polypeptides located in the same half of the molecule (on the same trimeric catalytic subunit) can influence each other if the complete quaternary structure is present, even though the other catalytic subunit is succinylated. In this case, the cooperativity must be mediated through the regulatory subunits and the succinylated catalytic subunit. Interactions can also occur between active sites on unmodified polypeptides located on different catalytic subunits presumably again via the regulatory subunits. In both of these derivatives, CTP inhibition still occurs. Although the homotropic and heterotropic interactions are reduced in the hybrid molecules, it is not clear whether this is due to the relative position occupied by the unmodified polypeptide in the quaternary structure or to the effect of succinylation in the other polypeptides. In the case of the derivative containing only one unmodified polypeptide in each catalytic subunit, three isomers can theoretically exist and the degree of cooperativity might depend on whether the unmodified polypeptides are linked directly via a single regulatory dimer.

Recently, a derivative of aspartate transcarbamylase lacking 1 regulatory subunit has been reported by several laboratories (27-29). The c:r structure in this derivative still allows homotropic and heterotropic interactions to occur. Furthermore, the reduction of these effects by approximately one-third suggests that the primary allosteric unit consists of two catalytic polypeptides situated on different catalytic subunits but linked directly via a regulatory dimer (i.e. a c:r:c system). This mode of interaction had been suggested previously by Endrenyi et al. (30) from their analysis of succinate binding data and independently by Markus et al. (5) as part of their functional model for aspartate transcarbamylase.

Evidence from Work on c:r Complex—The hyperbolic saturation curve for aspartate shown by the c:r complex (3,31) is consistent with the idea of a primary allosteric unit discussed above. In this complex, each of the regulatory dimers is presumably attached to a catalytic polypeptide but does not form a link between two catalytic polypeptides. Therefore the primary allosteric unit is incomplete and no homotropic interaction should be expected.

Another significant property of the c:r complex is that its $K_m$ for aspartate (3), which is much lower than that of the isolated catalytic subunit (c), is close to that expected for the relaxed state of the native enzyme (2,32). Furthermore, the substrate inhibition at pH 8.5 and the pH profile of the c:r complex at high aspartate concentration also resemble the corresponding properties of the native enzyme. Thus, the c:r complex can be considered a stabilized relaxed state of aspartate transcarbamylase. This observation should be of considerable significance for an understanding of the allosteric mechanism.

The insensitivity of c:r toward CTP and ATP (3) shows that the establishment of c:r contact is not sufficient for the expression of heterotropic interactions. Furthermore, CTP and ATP were shown to have no effect on the apparent association constant for the formation of c:r from c and r (4) suggesting that these nucleotides have no influence on c:r interactions in this complex. The same ligands however inhibit the conversion of c:r to the native enzyme. These results imply that although the c:r interaction remains the same, the structure of c:r is somehow altered by these ligands possibly through changes in the r:r contact. Some of these observations are difficult to reconcile with the existing models of allosteric mechanism proposed for aspartate transcarbamylase (1,5,6).

Previous Models of Allosteric Mechanism in Aspartate Transcarbamylase—The rotational model of Gerhart (1) postulates rigid bonding for both the c:r and the r:r domains and therefore specifies that the allosteric transition between the “tight” and the “relaxed” forms occurs with a change only in the c:r domain. An integral part of this model is the highly concerted nature of the transition between the two states making hybrid conformations very unstable. Although the conformational change accompanying substrate binding postulated by this model is qualitatively consistent with changes in properties such as sedimentation coefficient and -SH group reactivity, it seems incompatible with a number of more recent findings. Thus, the existence of intermediate states between the completely tight and the completely relaxed conformation is indicated by studies of optical rotatory dispersion (19), circular dichroism difference spectra (21), and relaxation rate (14). These results do not favor a two-state highly concerted mechanism. It is also difficult to understand with the rotation model how the absence of a regulatory subunit in the c:r derivative would reduce the cooperativity by one-third. Similarly, the parallel decrease (but not complete disappearance) of cooperativity and the substrate-analog-induced conformational change in hybrid molecules containing succinylated and unmodified subunits argues against a completely concerted transition.

If as postulated in the rotation mechanism, the c:r domain is the only one involved in the allosteric transition, then it is difficult to explain how homotropic interactions can be abolished whereas heterotropic interactions are retained in the modified enzyme from E. coli grown in the presence of 2-thiouracil (7). Although Gerhart did not specify in his mechanism how CTP and ATP exerted their effects on aspartate transcarbamylase, the c:r domain was also implied in these interactions because the other domains were assumed to be rigid. The results from our study of c:r which indicate no effect by CTP and ATP on the strength of c:r interactions are difficult to reconcile with this implied role for the c:r domain.

The model of Markus et al. (5), unlike the rotational model, is able to account satisfactorily for the existence of hybrid con-
formations. In this model, the basic allosteric unit consists of two c catalytic chains linked by a regulatory dimer and is postulated to operate in a manner more or less independent of the other identical systems in the same molecule. The reduction in cooperativity as a result of omission of a single regulatory subunit or of introduction of succinylated catalytic chains follows naturally from the proposed mechanism. However, the postulated role of the regulatory subunit, directly hindering the binding of substrates to the active site, is not consistent with our finding that the association of the catalytic subunit with the regulatory subunit to form cT r4 is accompanied by an increase in affinity for aspartate (3). Furthermore, the postulated mechanism of the action by CTP and ATP in this model operates directly at the c:r domain and is therefore in disagreement with the observation that these ligands have no effect on c:r interactions in the cT r4 complex (4). As in the rotational model, the hypothesis that the homotropic and heterotropic interactions involve the same mechanism and only one type of domain (c:r) is difficult to reconcile with the selective abolishment of homotropic interactions in a modified form of aspartate transcarbamylase (7).

Finally, there is the suggestion by Warren et al. (6) that the allosteric mechanism involves control of substrate diffusion through access channels to the central cavity (where the active sites are apparently located). This novel idea however does not seem to agree with the properties of the cT r4 derivative studied by several groups recently (27-29). The observation that the K", for aspartate actually decreased when regulatory subunits associate with a catalytic subunit to form cT r4 also argues against steric hindrance as a major factor. In any case, it is difficult to see how cooperative binding of succinate (33) could be explained by restricted diffusion since the process studied is at an equilibrium.

New Model for Allosteric Mechanism of Aspartate Transcarbamylase—The results from studies of the cT r4 complex pose several questions which are pertinent to the development of an allosteric model for native aspartate transcarbamylase. For example, because the cT r4 complex resembles the relaxed state (with high affinity for aspartate) of the native enzyme, it is natural to ask what is the structural basis for the tight state. It is also relevant to consider what mechanism would allow the action of CTP and ATP in the native enzyme but not in the cT r4 complex. One can also wonder how CTP and ATP can exert their effects without directly affecting the c:r domain. A model which provides satisfactory answers to these questions is presented below.

In this model, the primary allosteric unit consists of two catalytic chains located on different catalytic subunits and linked noncovalently via an r2 bridge. A schematic diagram of 2 such allosteric units is shown in Fig. 1a. The complete molecule contains 3 such units with the 3rd unit located behind the 2 units shown here and arranged in such a way that a 3-fold symmetry axis runs horizontally across the diagram. The 3rd allosteric unit has been omitted here for the sake of a clearer presentation. The coupling of conformational change between catalytic chains in each primary allosteric unit is postulated to be strong though not necessarily concerted whereas the coupling between adjacent units will be considered somewhat weaker and probably pH dependent. The idea of an allosteric unit and its arrangement in the molecule have been derived from the model of Markus et al. (5).

It is postulated that a conformational change in the catalytic chain is obligatory for the proper binding of aspartate (with carbamyl phosphate already present in the active site concerned). This local conformational change is assumed to be a tightening of the tertiary structure of the c chain as reflected in the increase in sedimentation coefficient of the isolated c4 in the presence of succinate and carbamyl phosphate (16). When the catalytic chain is making contacts with a regulatory chain (as in the native enzyme or in the cT r4 complex), a significant change in the relative orientation of the two chains is postulated to accompany the above conformational change (Fig. 1b). In the native enzyme, this relative movement between the subunits is opposed by quaternary constraints2 and results in the shifting of the r:r contact to a new position. As a consequence of the tendency to conserve symmetry, the other catalytic chain in the primary allosteric unit is induced to take up a conformation much more favorable for substrate binding.4

Fig. 1. Postulated conformational changes accompanying the binding of substrates to native aspartate transcarbamylase. For reasons of clarity, only two-thirds of the molecule is shown. cT represents the "tight" or T conformation of the catalytic subunit whereas cR represents the "relaxed" or R conformation. The shaded bullet-shaped object represents aspartate and carbamyl phosphate. The active sites are shown as being located inside the central aqueous cavity according to x-ray diffraction studies (6, 9).

\[4\] It should be pointed out that the shape of the subunits given in Figs. 1 to 4 and the movements between them shown there are simply hypothetical examples to illustrate the type of conformational changes that can give rise to the desired effects. When the structure of the enzyme is eventually revealed at atomic resolution, many detailed aspects of these changes would likely be found to differ from those shown here. These details should not affect the central postulates of the model concerning the basis of the tight state and the role of r:r interactions.

\[2\] Quaternary constraint is defined here as the restriction of local conformational change imposed by the quaternary structure.
second substrate to bind to the other active site in the allosteric unit little further expenditure of energy is required. A central feature of this model is that CTP and ATP exert their effects on native aspartate transcarbamylase by favoring different r:r contacts. Fig. 2 shows diagrammatically how these effects might occur. A regulatory dimer is shown here with its r:r domain capable of existing in three positions. (It is not intended to exclude the possibility that the domain can vary in an almost continuous manner between the extreme positions, 1 and 3.) In the absence of the nucleotide ligands, the r:r contact is most stable in Position 2. Binding of ATP is postulated to shift the contact to a new position of stability whereas CTP causes a shift in the opposite direction. When the regulatory subunits are incorporated into the structure of the native enzyme, these changes in the r:r domain are subject to quaternary constraint and cannot occur without the participation of the substrate-induced conformational change discussed earlier. The difference in free energy at the r:r domain between the normal position and those stabilized by CTP or ATP is considered to be significantly smaller than the change in free energy accompanying the substrate-induced conformational change in the catalytic subunit. As a consequence, the binding of CTP or ATP to the regulatory subunits in the native enzyme does not lead to extensive conformational changes at the catalytic subunit. Instead, the conformational changes postulated in the above model are consistent with the sedimentation coefficient changes (16), the changes in -SH reactivity (16), the sensitivity towards proteolysis (22), and various optical studies (19, 21). The primary allosteric unit similar to that proposed by Markus et al. (5) accounts satisfactorily for the properties of the c6r4 derivative (27–29) and the reduced cooperativity in various succinylated derivatives (18).

Experimental Observations Accounted For in Terms of New Model—The conformational changes postulated in the above model are consistent with the sedimentation coefficient changes (16), the changes in -SH reactivity (16), the sensitivity towards proteolysis (22), and various optical studies (19, 21). The primary allosteric unit similar to that proposed by Markus et al. (5) accounts satisfactorily for the properties of the c6r4 derivative (27–29) and the reduced cooperativity in various succinylated derivatives (18).

This model is also able to explain in a natural way the results obtained with the c6r4 complex (3, 4, 31). Thus, it follows from the structure of the postulated allosteric unit that no homotropic interactions can occur in c6r4 because one of the catalytic chains in each unit is missing (Fig. 3). The similarity of c6r4 to the R state of the native enzyme (3) is also predicted by the model because the conformational change accompanying substrate binding can now take place without shifting the r:r domain to an energetically unfavorable position (Fig. 3b). The insensitivity of c6r4 to CTP and ATP (4) is also easily understood in terms of this model because the r:r domain can now shift freely without affecting substrate binding. This model is also consistent with the observation that CTP or ATP has no effect on the strength of c:r interactions (4) as measured by the equilibrium: $c_5 + 3r_z \rightleftharpoons c_{6r}$. Finally, the relative orientation of c and r in c6r4 (in the absence of any ligands) is postulated to be close to that in the native enzyme (c6r4) under the same conditions (compare Fig. 3a with Fig 1a). This similarity in orientation may be expected to facilitate the conversion of c6r4 to c6r4. Therefore, by shifting the r:r domain and consequently altering the orientation, both CTP and ATP should reduce the rate of conversion of c6r4 to c6r4. This was indeed found to be the
for incorporation into the native enzyme (compare Fig. 3b with version of care to csrB. This observation would also be consistent by the above model in a simple manner. This modified cnzymc, Fig. 1b).

Table 1

<table>
<thead>
<tr>
<th>Form of enzyme</th>
<th>Ligand added</th>
<th>$K_m$ for aspartate</th>
<th>$V_{max}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-thiouracil-aspartate</td>
<td>CTP (0.5 mM)</td>
<td>28.8</td>
<td>100</td>
</tr>
<tr>
<td>transcarbamylase</td>
<td>None</td>
<td>14.4</td>
<td>82</td>
</tr>
<tr>
<td></td>
<td>ATP (1 mM)</td>
<td>7.4</td>
<td>68</td>
</tr>
<tr>
<td>$c_r$</td>
<td>None</td>
<td>24</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>None</td>
<td>45</td>
<td>100</td>
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<td>None</td>
<td>20</td>
<td>100</td>
</tr>
<tr>
<td>$c_r$s</td>
<td>None</td>
<td>10</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>None</td>
<td>7.9</td>
<td>63</td>
</tr>
</tbody>
</table>

* Results taken from the work of Kerbiriou and Hervé (7), assays were done at pH 7.0 in 50 mM cacodylate buffer.
* CTP and ATP have no significant effects on $c_r$ and $c_r$s.
* Radioactive assays in Tris-acetate (0.2 M, pH 8.5).
* Titrator assays at pH 8.5 in potassium acetate (0.1 M).
* Radioactive assays at pH 7.0, 8.0, and 8.7 from the work of Porter et al. (35).

The unusual properties of the aspartate transcarbamylase prepared by Kerbiriou and Hervé (7, 34) can also be explained by the above model in a simple manner. This modified enzyme, obtained by growth of E. coli in the presence of 2-thiouracil shows Michaelis-Menten type kinetics but is still inhibited by CTP and activated by ATP. The effect of CTP is to increase both the $K_m$ for aspartate and the $V_{max}$ (Table 1) whereas ATP leads to a decrease in both these parameters. The clue to the understanding of this 2-thiouracil-aspartate transcarbamylase comes from comparing these changes with similar changes which occur when $c_r$ associates with $r$ to form $c_r$s. This process is also accompanied by reduction in $K_m$ and $V_{max}$ of comparable magnitude (3). Because the data on $c_r$ and $c_r$s were obtained at pH 8.5 (radioactive assay in Tris-acetate buffer or titration assay in potassium acetate solution) whereas the data on the modified aspartate transcarbamylase were taken at pH 7 (in cacodylate buffer), direct comparison of these results is not possible. However, Porter et al. (35) have shown that the $K_m$ for $c_r$ (20 mM) is unchanged over the pH range 7.0 to 8.7. It can be seen from Table 1 that the difference between CTP in inhibited 2-thiouracil-aspartate transcarbamylase and ATP-activated 2-thiouracil-aspartate transcarbamylase in a 4-fold reduction in $K_m$ and a decrease in $V_{max}$ to 68%. The corresponding change when $c_r$ is converted to $c_r$s is also a 4-fold decrease in $K_m$ (radioactive assay) and a decrease in $V_{max}$ to 56%. The results with the titrator assay are of similar magnitude. Thus, the effects of CTP and ATP on 2-thiouracil-aspartate transcarbamylase operate by respectively converting the active sites to a state corresponding to that in the isolated catalytic subunit ($c_r$) and to a state resembling that in the $c_r$s complex (which itself is similar to the R state of the native enzyme).

As has been pointed out (2), the $K_m$ of $c_r$ for aspartate (20 mM) is much higher than that calculated for the R state of the enzyme (5.8 mM). In our model, the isolated catalytic subunit is considered to exist in the I state which binds the substrates somewhat imperfectly (Fig. 4a). It is postulated that when the c:r domain is formed, a portion of the domain induces a change at the active site which results in a transition to the R state with good binding for substrates.

In the case of the 2-thiouracil-aspartate transcarbamylase, it is known from hybrid experiments that modification in the regulatory subunit is responsible for its unusual properties (7). Our model explains these properties by postulating that the c:r domain is weakened. In the case of normal aspartate transcarbamylase, the binding of substrates at one active site is postulated to cause a shift at the r:r domain. In the case of 2-thiouracil-aspartate transcarbamylase, the weakened c:r domain is unable to do so and is postulated to "slip" (Fig. 4c). The portion of the c:r domain which normally induces the transition from the I state to the R state is only partially functioning. This situation leads to binding properties partially resembling that of the isolated catalytic subunit. As a consequence of the inability to shift the r:r domain, the conformational change is not transmitted to the other active site in the allosteric unit, resulting in the disappearance of the homotropic effects. Because CTP induces the r:r domain to take up a position opposite to that required for allosteric transition, the c:r domain is considered to slip further (Fig. 4b) resulting in a state identical in substrate binding with the isolated $c_r$. Again, homotropic interactions between pairs of active sites are not possible. In the presence of ATP, the r:r domain favorable to the substrate-induced conformational change is stabilized and therefore there is no strain to cause the c:r domain to slip as the
binding of substrate occurs. Therefore, the active site simply exists in the R state induced by the complete formation of the c:r domain. The weakening of the c:r contact is also consistent with the observation that 2-thiouracil-aspartate transcarbamylase is more heat-sensitive than normal aspartate transcarbamylase since heat treatment is known to dissociate the native enzyme into c2 and r2 (20).

Discussion of Model in Relation to Other Allosteric Models—The model presented here does not conform strictly to either of the two generalized models (i.e., the concerted model of Monod et al. (36) or the sequential model of Koshland et al. (37)) but contains certain features derived from them. Thus, the postulated change in the tertiary structure occurs as a response to substrate binding as specified in the Koshland model but the result of this change is not necessarily the storage of energy in two springs in the tertiary structure and diminishes the work required to change the tertiary structure and diminishes the work required to change the tertiary structure.

In this regard, it should be noted that Kirschner and Schachman (15) have observed that 50% of the maximal change in the sedimentation coefficient occurs at a succinate concentration at which only 7% of the maximal change in nitrotyrosyl spectrum at the catalytic subunit occurs. Similarly, the results of circular dichroism studies (21) indicate that the half-maximal change at the metal site of the regulatory subunit in native aspartate transcarbamylase occurs at a lower (0.2 mm) concentration of succinate than the concentration (0.4 mm) required to cause half-maximal change at the tryptophanyl residues of the catalytic subunit. We therefore envisage that most of the quaternary structural change over the entire molecule (excluding the shift at the r:r domain) occurs as the first active site binds the substrates. This condition does not necessarily imply that most of the potential energy change has occurred as the small residual structural change may require a disproportionately high amount of free energy. We consider it likely that a pH-dependent variation in the coupling between local and gross conformational changes or in the accompanying changes in potential energy may account for the change in the Hill coefficient of the enzyme from about 1.7 to 2 at pH 7 to about 4 at pH 8.5 (38).

The model is similar in some respects to the earlier model of Perutz for hemoglobin in which "... the step by step release of the constraints on the unreactive quaternary structure... changes the equilibrium in favor of the reactive quaternary structure and diminishes the work required to change the tertiary structure of each subunit from the unreactive to the reactive form" (39). The countereffecting effects of the c:r and r:r domains have similarities with the storage of energy in two springs in the Hopfield model of hemoglobin (40) and it is possible that in aspartate transcarbamylase the free energy is also distributed as small strains in a number of bonds as postulated for hemoglobin.

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