Wintersberger, E., Neurath, H., Coombs, T. L., and Vallee, B. L. (1965), *Biochemistry 4*, 1526.

Wolff, E. C., Shirmer, E. W., and Folk, J. E. (1962), *J. Biol. Chem. 237*, 3094.

Subunit Interactions in Aspartate Transcarbamylase†

Robert A. Cook

ABSTRACT: Binding studies of cytidine triphosphate (CTP) to native *Escherichia coli* asparate transcarbamylase reveal both positive and negative cooperative-like behavior. Equilibrium dialysis studies at 4 and 23° are consistent with six binding sites for CTP on the transcarbamylase. Complete saturation of the CTP binding sites is not attained at 23°. Complex Hill plots are obtained with at least two interaction coefficients values (η_H), with one break in the Hill plot occurring at approximately 35% saturation. The results are explained by a ligand-induced sequential model with subunit interactions which lead to a mixture of positive and negative cooperativity. A model involving dissimilar binding sites is also discussed.

Aspartate transcarbamylase of *Escherichia coli* has been the subject of numerous physicochemical and kinetic studies due to the availability of large quantities of homogeneous enzyme and the central role this enzyme has played in the development of allosteric theory. The enzyme is now generally accepted to be hexameric in structure, consisting of six regulatory subunits (mol wt 17,000) and six catalytic subunits (mol wt 33,500) (Weber, 1968; Meighen *et al.*, 1970; Rosenbusch and Weber, 1971). The enzyme can be dissociated by a variety of physical techniques to yield two types of subunits, a catalytic trimer (mol wt 100,000) and a regulatory dimer (mol wt 33,000) (Gerhart and Schachman, 1965). Based on the unusual association pattern of subunits following dissociation of the native molecule and the crystallographic data of Wiley and Lipscomb (1968), several models for the transcarbamylase molecule have been proposed (Gerhart, 1970; Rosenbusch and Weber, 1971; Markus *et al.*, 1971).

Support for the hexameric structure has come primarily from binding studies on native aspartate transcarbamylase and the isolated subunits. By the method of continuous variation, Hammes *et al.* (1970) have determined a total of six binding sites for BrCTP[1] on the native enzyme and three binding sites for carbamyl phosphate on the isolated catalytic trimer. Three binding sites for succinate, an aspartate analog, have also been indicated on the isolated catalytic trimer (Rosenbusch and Weber, 1971).

The determination of the allosteric mechanism involved in the regulation of the transcarbamylase requires more detailed binding and kinetic studies with the native, untreated enzyme. The binding of CTP to native aspartate transcarbamylase has been examined by the use of spin-label probes (Buckman, 1970) and by equilibrium dialysis (Changeux *et al.*, 1968; Winlund and Chamberlin, 1970). The results of these authors have not been in agreement. The original binding experiments of Changeux *et al.* (1968) revealed four independent binding sites for CTP, in agreement with the original tetrameric model for the enzyme. The binding experiments of Winlund and Chamberlin (1970) revealed six binding sites total, and these authors have suggested two different classes of CTP binding sites, including three independent "tight" sites and three independent "weak" binding sites. The CTP binding results of Buckman (1970) have been explained in terms of three independent "tight" binding sites but six independent "weak" binding sites. The binding of succinate to native transcarbamylase has been examined by the technique of equilibrium dialysis (Changeux *et al.*, 1968). A total of four binding sites for succinate were obtained, a value well below the expected value of six based on the number of catalytic subunits.

The discrepancy between the binding data for ligands and the proposed hexameric structure of the transcarbamylase prompted a detailed examination of the binding characteristics of the inhibitor, CTP, and the substrate analog, succinate. In this paper, the binding of CTP has been examined by equilibrium dialysis utilizing [^{14}C]CTP in an effort to understand the mechanism of enzyme inhibition.

The similarity of the present results to the results obtained in an examination of yeast glyceraldehyde-3-phosphate dehydrogenase (Cook and Koshland, 1970) has prompted an explanation of the results on the basis of "sequential" changes (Conway and Koshland, 1968; Koshland *et al.*, 1966; Kirtley and Koshland, 1967) of subunit conformation induced by the binding of CTP. A combination of both positive and negative cooperative effects is concluded.

Experimental Section

Materials. Aspartate transcarbamylase was purified from a strain of *Escherichia coli* kindly provided by Dr. J. Gerhart. The enzyme was purified essentially as described by Gerhart and Holoubek (1967). The only modification of the original procedure was an additional DEAE-cellulose column step added at the end of the purification to remove a minor contaminant band visible in cellogel electrophoresis. The enzyme then appeared to be homogeneous when tested by cellogel electrophoresis in Tris–borate buffer (pH 8.6) (Boyer *et al.*,

† From the Department of Biochemistry, University of Western Ontario, London, Ontario, Canada. *Received September 8, 1971.* This work was supported by a research grant from the Medical Research Council (MA-3848).

[1] Abbreviations used are: BrCTP, 5-bromocytidine triphosphate; CTP, cytidine triphosphate; n_H, Hill coefficient; n_M, maximum number of binding sites; S_f, free substrate; S_b, bound substrate; E_t, total enzyme; K_i', binding constant.

1963). The enzyme was routinely stored at 0–4° as a solution of 20 mg of protein/ml in 0.04 M potassium phosphate buffer (pH 7.0)–2 × 10^{-3} M 2-mercaptoethanol–2 × 10^{-4} M Na-EDTA. No loss of enzyme activity was noted during the course of this investigation. In all experiments reported here, an enzyme preparation with a specific activity of 7500–7800 units/mg of protein was used. Protein concentration was estimated by the method of Lowry *et al.* (1951) with bovine serum albumin as reference standard. Estimation of the protein concentration from the extinction coefficient (0.70 cm^2/mg) gave essentially identical results.

Equilibrium Dialysis. Equilibrium dialysis was routinely carried out in 0.15-ml cells at 4 and 23°, using Visking 20–32 dialysis tubing. The dialysis tubing was washed several times in hot EDTA and then phosphate buffer (pH 7.0), but was not otherwise treated to increase porosity. Controls of CTP *vs.* buffer indicated that equilibrium was reached in 24 hr at 23° and 48 hr at 4°. The presence of ATCase did not affect the time for equilibration. The enzyme was checked periodically for any denaturation during experiments by using a standard reaction mixture which contained 20 μmoles of potassium phosphate (pH 7.0), 7.5 μmoles of potassium aspartate (pH 7.0), and 2 μmoles of carbamyl phosphate in an 0.5-ml total volume. The sensitivity of the enzyme activity to CTP inhibition was unaltered by the equilibration time. After equilibrium was reached, ligand concentration was determined on aliquots from each cell compartment. Samples of 0.075 ml were counted in 10 ml of Bray's (1960) solution in a scintillation counter and monitored for possible quenching. Four different stock CTP solutions were made to cover the wide range of CTP concentrations used in this study. The counts were therefore not constant but varied from 1350 to 200,000 cpm. All samples were therefore counted to constant efficiency.

2-[^{14}C]CTP was purchased from Schwarz with a specific activity of 21.8 Ci/mole. A routine check of purity indicated 0.5% contamination, resulting in a maximum error of 6% in the estimation of CTP free at the lowest concentration of CTP employed in these studies. CTP and carbamyl phosphate were purchased from Sigma.

Results

Binding Studies of CTP to E. coli Aspartate Transcarbamylase. Equilibrium dialysis with the transcarbamylase from *E. coli* was carried out as previously described in the Experimental Section with the CTP concentrations varied over the range of 0.025 × 10^{-5} M to 600 × 10^{-5} M. The enzyme concentration in all cells was 6.45 × 10^{-5} M or 20 mg of protein per ml, assuming a molecular weight for the transcarbamylase of 310,000. A typical binding curve for CTP at 23° is presented in Figure 1A,B. The binding curve exhibits obvious sigmoidicity at low CTP concentration and does not reach a maximum at high CTP concentrations. When the data were replotted in the Scatchard plot, a curve characteristic of both positive and negative cooperative-like behavior was observed (Figure 2). There was no loss in enzyme activity after the dialysis procedure was completed. Plotting the data of Figure 1 in a Hill plot gives three η_H values of 2.5 below 5% saturation, 0.80 below 35% saturation, and 0.32 above 35% saturation[2] (Figure 3).

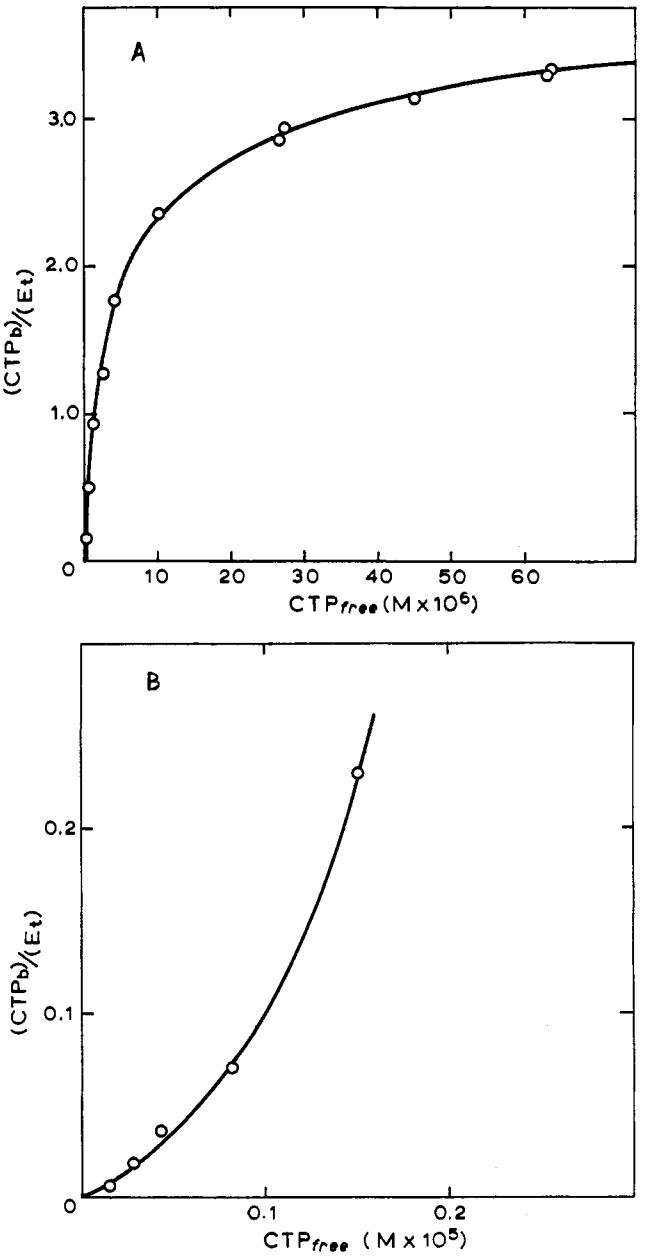

FIGURE 1: Equilibrium dialysis study of binding of CTP to the aspartate transcarbamylase of *E. coli* at 23°. Conditions: 0.04 M potassium phosphate buffer–2 mM 2-mercaptoethanol–0.2 mM EDTA (pH 7.0), 23° 6.45 × 10^{-5} M aspartate transcarbamylase.[3] (A) $\bar{Y}$ *vs.* [S] plot at high CTP concentrations; (B) $\bar{Y}$ *vs.* [S] plot at low CTP concentrations.

A typical binding curve for CTP at 4° is shown in Figure 4A,B. The shape of the curves are similar to those obtained at 23° but differ in the degree of positive cooperativity at low CTP concentrations. A greater degree of binding is obvious at 4° as can be seen from the values of $[CTP]_b/[E]_t$ on the vertical axis. When the data were replotted in the Scatchard plot, a mixture of both positive and negative cooperative-like behavior was observed (Figure 5). Plotting the data in a

[2] Assuming an n_M value of 4.8 for CTP instead of the theoretical value of 6.0, a similar Hill plot was obtained with a break at 35% saturation but with values of n_H of 1.06 and 0.48, respectively.

[3] No correction has been made for Donnan effects in these experiments. Controls in which equilibrium dialysis was carried out in the presence of 0.1 M KCl gave essentially the same results as were obtained in the absence of added salt. Such controls were attempted at extremely low CTP concentration, moderate concentration, and extremely high concentration.

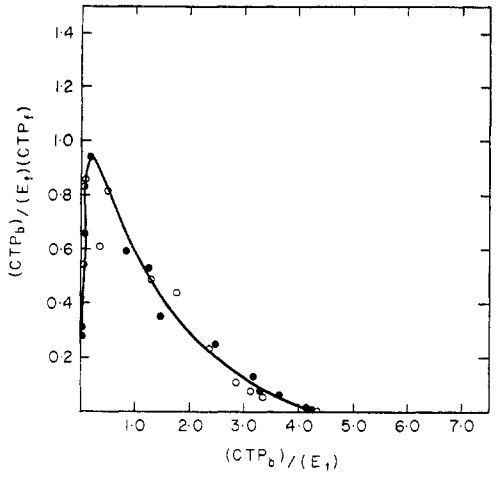

FIGURE 2: Data from Figure 1 drawn in the form of the Scatchard (1949) plot. Conditions were as described in the legend of Figure 1. The data have been fitted by eye. Units of the vertical axis are 10^{-5} M^{-1}. O,● represent the values obtained from two different sets of binding experiments.

Hill plot gives n_H values of 1.25 below 30% saturation and 0.58 above 30% saturation (Figure 6).

The concentration of CTP required to half-saturate the enzyme was estimated to be 7.5×10^{-5} M at 4° and 32.0×10^{-5} M at 23°. Obviously these values are not true association constants but are included here to indicate in a rough way that the binding of CTP decreases with increasing temperature.

Discussion

The most striking features of the binding curves are their deviations from conventional plots. Due to the complexity of the Scatchard plots obtained at both 4 and 23°, it is necessary to review the parameters associated with more conventional Scatchard plots. Plotting $[S]_b/[E]_t[S]_f$ as a function of $[S]_b/[E]_t$, where $[S]_b/[E]_t$ is the moles of ligand bound per mole of enzyme, and $[S]_f$ is the concentration of free or unbound ligand, a straight line is obtained if the sites are independent (Klotz, 1953). Deviation from a straight line is indicative of interaction between sites or dissimilarity of the sites. In Figure 7, the deviations to be expected for positive cooperativity and negative cooperativity (or dissimilarity of sites or enzyme forms) are shown. Comparing the results of Figure 2 and Figure 5 to the hypothetical plots of Figure 7, it would appear that the binding of CTP to the transcarbamylase involves a mixture of both positive and negative cooperative-like behavior.

Another striking feature of the binding data is the occurrence of at least two distinct n_H values for CTP in the Hill plots, with one change in slope occurring between 30 and 35% sat-

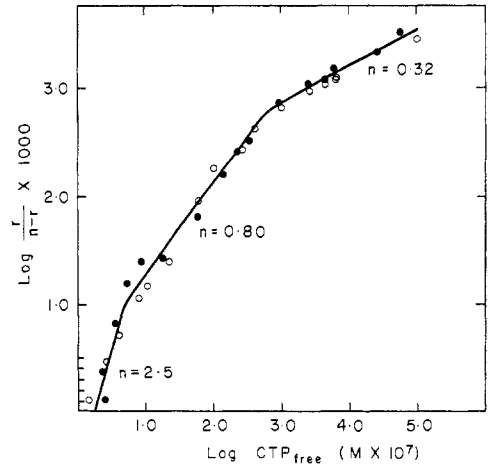

FIGURE 3: Data from Figure 1 drawn in the form of the Hill plot. Conditions were as described in the legend of Figure 1. The value n_M has been assumed to be 6.0, the theoretical value. The data have been fitted by eye. A value of 2.70 on the vertical axis represents 33% saturation. O,● represents the values obtained from two different sets of binding experiments.

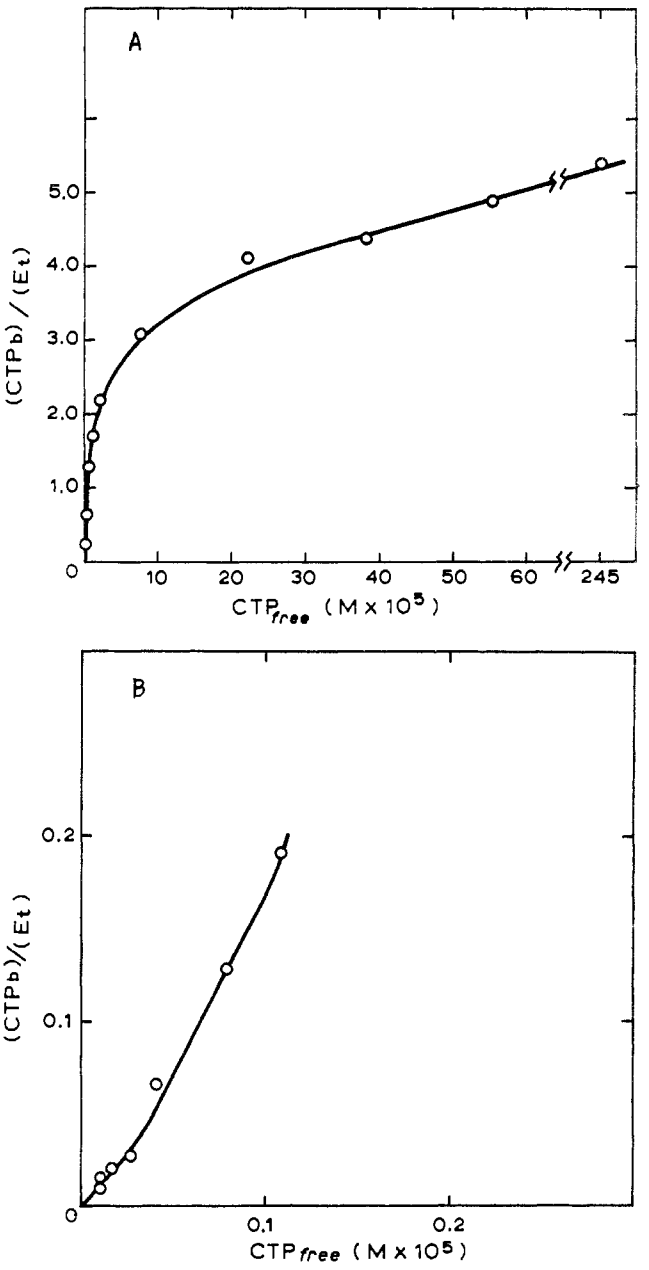

FIGURE 4: Equilibrium dialysis study of binding of CTP to the aspartate transcarbamylase of *E. coli* at 4°. Conditions: 0.04 M potassium phosphate buffer–2 mM 2-mercaptoethanol–0.2 mM EDTA (pH 7.0), 4°, 6.45×10^{-5} M aspartate transcarbamylase. (A) $\bar{Y}$ *vs.* [S] plot at high CTP concentrations; (B) $\bar{Y}$ *vs.* [S] plot at low CTP concentrations.

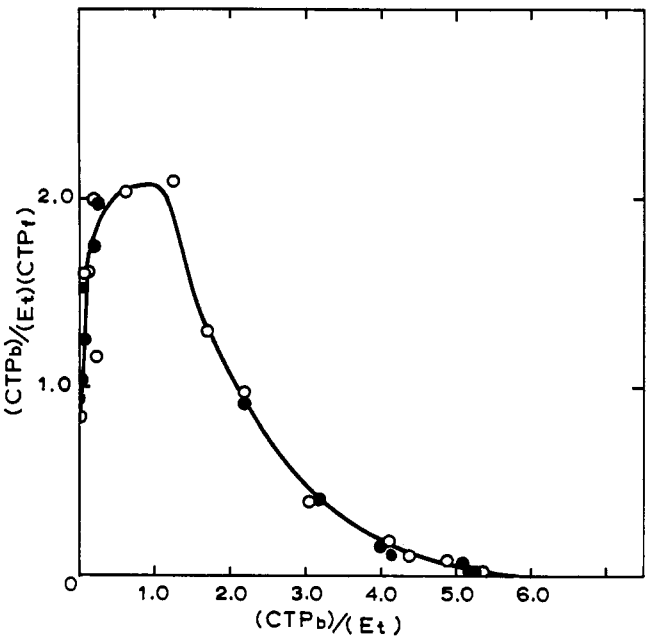

FIGURE 5: Data from Figure 4 drawn in the form of the Scatchard plot. Conditions were as described in the legend of Figure 4. The data have been fitted by eye. Units of the vertical axis are 10^{-5} M^{-1}.

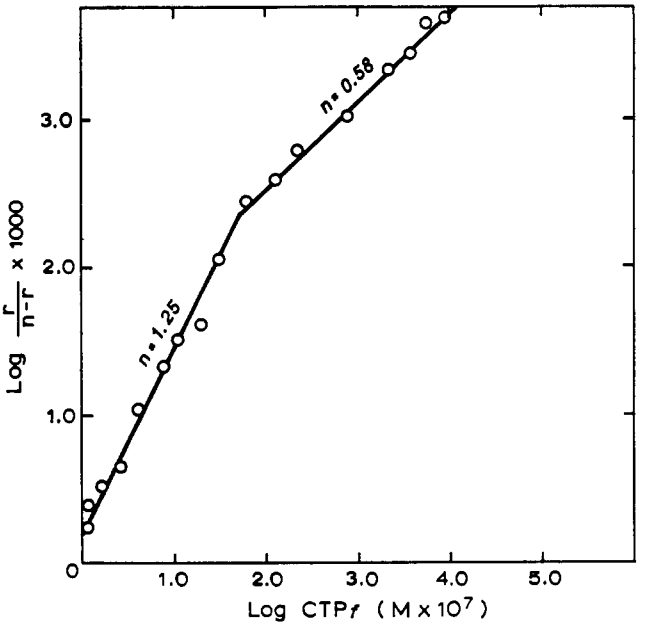

FIGURE 6: Data from Figure 4 drawn in the form of the Hill plot. Conditions were as described in the legend of Figure 4. The value of n_M has been assumed to be 6.0, the theoretical value. The data have been fitted by eye. A value of 2.70 on the vertical axis represents 33% saturation.

uration. This type of behavior is qualitatively similar to the data obtained for NAD binding to yeast glyceraldehyde-3-phosphate dehydrogenase (Cook and Koshland, 1970). The parameters associated with more conventional Hill plots have been analyzed (Cook and Koshland, 1970) with the conclusion that the break in the binding curves is a significant property of the enzyme system rather than an anomaly of the Hill representation.

The results of the binding studies could be explained by (a) a "sequential" mechanism involving both positive and negative cooperativity or (b) a mechanism assuming sites of different affinity for CTP on the native aspartate transcarbamylase molecule. Several different possibilities could give rise to the latter mechanism: (a) the presence of several forms of native aspartate transcarbamylase, *i.e.*, isozymes; (b) the presence of other enzymes in the transcarbamylase preparation which bind CTP; (c) nonidentical regulatory subunits which bind CTP with differing affinity; (d) identical regulatory subunits which are geometrically arranged within the molecule, giving rise to sites of different affinity for CTP; (e) binding of CTP to both the regulatory subunit and catalytic subunit.

In the present study, the first two possibilities have been ruled out on the basis of disc electrophoresis and cellogel electrophoresis studies which have indicated a homogeneous enzyme preparation. The possibility of nonidentical regulatory subunits has been ruled out, primarily on the basis of amino acid sequence studies of the regulatory subunit (Weber, 1968). Preliminary experiments on the binding of CTP to the isolated catalytic subunit would indicate that no significant binding occurred (Cook,[4] 1972). A geometric arrangement of identical regulatory subunits resulting in CTP binding sites of different affinity cannot easily be ruled out. One model for the transcarbamylase molecule has been proposed which infers that regulatory subunits are in contact in the native structure (Rosenbusch and Weber, 1971). The isolation of regulatory dimers after dissociation of native aspartate transcarbamylase (Gerhart and Schachman, 1965) and cross-linking studies in native aspartate transcarbamylase (Davies and Stark, 1970) indicate that pairs of regulatory chains are arranged in close proximity. However, no direct evidence is available to indicate physical association of regulatory dimers in the native enzyme. The isolation of regulatory dimers following dissociation of the transcarbamylase may be an artifact of subunit association as has been noted in the case of β-amylase reassociation (Cook and Koshland, 1969).

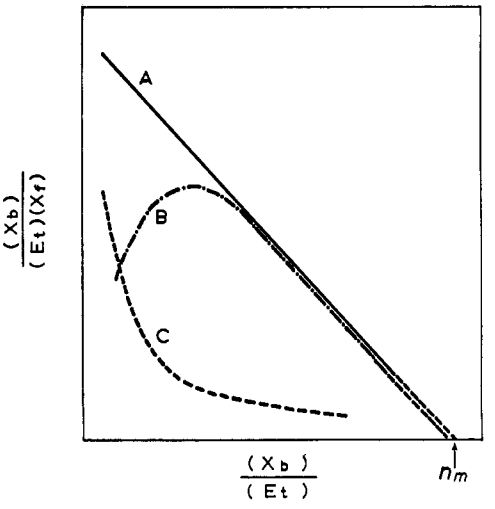

FIGURE 7: Hypothetical Scatchard plots. A represents n independent and equal sites. B represents n identical sites exhibiting positive cooperativity. C represents either n identical sites exhibiting negative cooperativity or nonidentical binding sites without cooperativity.

[4] Unpublished results.

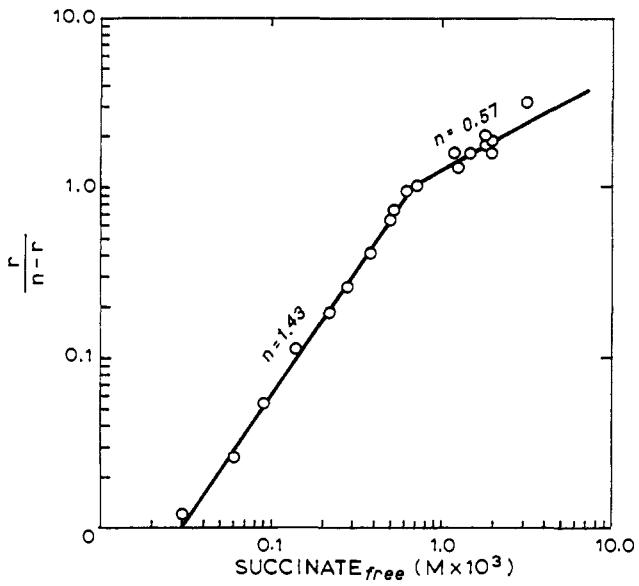

FIGURE 8: Equilibrium dialysis study of binding of succinate to aspartate transcarbamylase at 21° by Changeux *et al.* (1968) plotted in the form of the Hill plot. The value of n_M has been assumed to be 3.8. The data have been fitted by eye. A value of 1.0 on the vertical axis represents 50% saturation.

The binding data of Winlund and Chamberlin (1970) have been explained on the basis of two distinct classes of binding sites in the transcarbamylase, each class containing 2.9 independent sites. In the present manuscript, the binding data obtained at 4° at high CTP concentrations are qualitatively similar to the binding data of Winlund and Chamberlin (1970). The positive cooperativity observed in Figures 2 and 5 cannot be explained, however, by assuming preexisting sites of different affinity. The log-decay portion of the curves can be explained, however, by either dissimilar binding sites or negative cooperativity. Examining Figures 2 and 5, one must conclude that the binding sites are not independent at low CTP concentrations. The binding data of Winlund and Chamberlin do not extend below 16.6% saturation, *i.e.*, one site out of six filled. The resultant Hill plot of the data of these authors is a straight line with a n_H value of 0.54. The Hill plot presented in Figure 3 reveals greater complexity even in the range of 16–90% saturation with two n_H values (n_H = 0.80 and 0.32). The average of these two values is 0.56, in close agreement with the data of Winlund and Chamberlin who did fewer binding experiments. Figure 3 reveals a minimum of three limiting slopes with decreased interaction coefficients as CTP concentration is increased. Similar behavior is observed in binding CTP at 4° (Figure 6), but the interaction is not as marked. Very weak positive cooperativity is noted at this temperature (n_H = 1.0–1.2) with only two limiting slopes.

The model, based on two distinct classes of CTP binding sites in the transcarbamylase due to geometric arrangement, could be altered in an effort to fit the present data. The observation of positive cooperative behavior at low CTP concentration would infer that the first class of three binding sites for CTP is not independent, but interacts in a positive manner. The second class of three binding sites could be independent, *i.e.*, does not interact with increasing CTP concentrations. This model is not supported by the Hill plots which indicate a change in binding affinity after the second CTP site is filled. The position of the break in the Hill plots, which is somewhat ambiguous, is not sufficient evidence to eliminate such a model from consideration.

A second model, based on "sequential" changes of protein structure in the presence of increasing CTP concentrations, although more complex, is more consistent with the data obtained. The binding data can be qualitatively described as a mixture of positive and negative cooperative behavior with $K_1 < K_2 > K_3 > K_4 > K_5 > K_6$. This model is based primarily on the Hill representation of the data. A distinct break in the Hill plots occurs at 30–35% saturation (Figures 3 and 6). The position of the break would indicate that binding of CTP to the first two sites occurs with weak positive cooperativity while binding to the last four sites occurs with strong negative cooperativity. Qualitatively similar results were obtained for NAD binding to yeast glyceraldehyde-3-phosphate dehydrogenase (Cook and Koshland, 1970). In the case of the yeast enzyme, the binding data were fitted by computer according to the procedure described by Cornish-Bowden and Koshland (1970) in an effort to determine the individual binding constants. The theoretical binding curve which gave the best fit to the data was found to have intrinsic constants in the relationship $K_1 < K_2 > K_3 > K_4$, indicating a mixture of positive and negative cooperativity. The explanation of the results was made on the basis of sequential changes of subunit conformation induced by the binding of NAD. Although computer fits of the present data have not been attempted, the results would appear to be qualitatively similar.

The CTP binding results obtained at 23° are consistent with the proposed hypothesis of sequential changes in subunit structure. A mixture of positive and negative cooperative-like behavior was obtained as at 4°, but the affinity for CTP was reduced at the higher temperature. The original binding studies of Changeux *et al.* (1968) were attempted at 21° with the conclusion that n_M was 3.8 for CTP, which supported a tetrameric model for aspartate transcarbamylase. Our observations that binding at 23° is much weaker than at 4°, coupled with a model involving negative cooperativity, would predict that the binding of CTP to sites 5 and 6 would occur with difficulty. In fact, the highest value of $[CTP]_b/[E]_t$ obtained experimentally was 4.2 at 23° at a concentration of CTP which would be expected to completely saturate the enzyme (6×10^{-3} M). Attempts to obtain complete saturation at 23° were unsuccessful. This observation is consistent with a model predicting decreasing affinity of the binding sites for CTP.

Binding studies of CTP to the isolated regulatory dimer also reveal negative cooperative effects (Changeux *et al.*, 1968). In this case, the heterogeneous binding of CTP may be due to a negative cooperative interaction between the two subunits or to the geometric arrangement of the two subunits in the dimer, resulting in two CTP binding sites which are physically different in the absence of ligand.

If a model involving sequential changes of subunit conformation induced by ligand is valid for the transcarbamylase, similar results might be predicted for the substrates of the reaction. The binding of succinate to native aspartate transcarbamylase has been examined by Changeux *et al.* (1968). The shape of the binding curves is similar to the CTP binding data, *i.e.*, obvious sigmoidicity at low succinate concentrations and a failure to reach a maximum at high succinate. The resulting n_M value of 3.8 for succinate, determined from the Scatchard plot, is lower than the expected value of six, based on the number of catalytic subunits. It is important to note that this binding study was attempted at 21°, a temperature which may not be optimum for binding, analogous to the

binding of CTP. When the succinate binding data are plotted in the Hill plot (Figure 8), two n_H values are obtained of 1.43 below 50% saturation and 0.57 above 50% saturation. Although the results are not unambiguous, the similarity of this plot to the CTP binding data would indicate a possible mixture of positive and negative cooperative behavior in the binding of succinate to native aspartate transcarbamylase. Succinate binding has been examined with the isolated catalytic trimer, but no deviation from conventional plots was observed (Rosenbusch and Weber, 1971). This conclusion is in agreement with the earlier findings that the cooperative behavior noted with the transcarbamylase in kinetic studies is absent in the isolated catalytic trimer (Gerhart and Pardee, 1962; Porter *et al.*, 1969).

The binding of BrCTP and substrates to native aspartate transcarbamylase has been examined by the temperature-jump relaxation technique (Eckfeldt *et al.*, 1970; Hammes *et al.*, 1971; Hammes and Wu, 1971). These authors have concluded that the binding of substrates is consistent with a concerted mechanism while the binding of BrCTP may be explained by either a concerted or sequential mechanism. The conclusions from the relaxation studies on the transcarbamylase have been questioned in a recent review by Kirschner (1971), who concluded that the results to date are inconclusive for the transcarbamylase and that the concerted mechanism is too simple to account for the observed data. The use of the relaxation technique to distinguish between a concerted and sequential model for any enzyme has been questioned in a recent article by Loudon and Koshland (1972).

Considering all the evidence, it would seem that aspartate transcarbamylase is composed of six identical regulatory subunits and six identical catalytic subunits and binds CTP with a mixture of positive and negative cooperative-like behavior. Evidence has been presented that succinate may also exhibit a mixture of cooperative effects. If the transcarbamylase is indeed a homogeneous protein, such binding patterns require the general ligand-induced model. One may well ask the advantage of a mixture of these two features. In the case of CTP, an allosteric inhibitor, this behavior would provide increased sensitivity to CTP inhibition at lower CTP concentrations and decreased sensitivity at high CTP concentrations. This behavior may ensure that aspartate transcarbamylase cannot be completely inhibited even at high CTP concentrations, resulting in a continual low supply of valuable pyrimidine pathway intermediates. The explanation of both the yeast glyceraldehyde-3-phosphate dehydrogenase system and the aspartate transcarbamylase system, two well-studied but obviously unrelated enzymes, in terms of a mixture of positive and negative cooperative behavior, may indicate that this type of behavior is not a feature of a few odd enzymes, but may rather be a pervasive pattern in many. Many other enzyme systems may obey a similar mechanism when deliberate attempts are initiated to uncover such phenomena.

Several questions still remain to be answered in the aspartate transcarbamylase system. The binding of succinate to native aspartate transcarbamylase must be examined in detail at various temperatures to substantiate the prediction of mixed cooperativity. The binding of CTP must be examined in the presence of aspartate and/or carbamyl phosphate to see if any changes occur in the CTP binding plots. Finally, a more detailed initial velocity kinetic examination of the native enzyme is required to support the proposed mechanism. Such studies are currently under investigation in this laboratory.

Acknowledgment

The author acknowledges the invaluable help and advice of Miss Debbie Digges during the course of this work.

References

Boyer, S. H., Fainer, D. C., and Naughton, M. A. (1963), *Science 140*, 1228.
Bray, G. A. (1960), *Anal. Biochem. 1*, 279.
Buckman, T. (1970), *Biochemistry 9*, 3255.
Changeux, J.-P., Gerhart, J. C., and Schachman, H. K. (1968), *Biochemistry 7*, 531.
Conway, A., and Koshland, D. E., Jr. (1968), *Biochemistry 7*, 4011.
Cook, R. A., and Koshland, D. E., Jr. (1969), *Proc. Nat. Acad. Sci. U. S. 64*, 247.
Cook, R. A., and Koshland, D. E., Jr. (1970), *Biochemistry 9*, 3337.
Cornish-Bowden, A. J., and Koshland, D. E., Jr. (1970), *Biochemistry 9*, 3325.
Davies, G. E., and Stark, G. R. (1970), *Proc. Nat. Acad. Sci. U. S. 66*, 651.
Eckfeldt, J., Hammes, G. G., Mohr, S. C., and Wu, C. W. (1970), *Biochemistry 9*, 3353.
Gerhart, J. C. (1970), *Curr. Top. Cell. Regul. 2*, 275.
Gerhart, J. C., and Holoubek, H. (1967), *J. Biol. Chem. 242*, 2886.
Gerhart, J. C., and Pardee, A. B. (1962), *J. Biol. Chem. 237*, 891.
Gerhart, J. C., and Schachman, H. K. (1965), *Biochemistry 4*, 1054.
Hammes, G. G., Porter, R. W., and Stark, G. R. (1971), *Biochemistry 10*, 1046.
Hammes, G. G., Porter, R. W., and Wu, C. W. (1970), *Biochemistry 9*, 2992.
Hammes, G. G., and Wu, C. W. (1971), *Biochemistry 10*, 2150.
Kirschner, K. (1971), *Curr. Top. Cell. Regul. 4*, 167.
Kirtely, M. E., and Koshland, D. E., Jr. (1967), *J. Biol. Chem. 242*, 4192.
Klotz, I. M. (1953), *Proteins 18*, 772.
Koshland, D. E., Jr., Nemethy, G., and Filmer, D. (1966), *Biochemistry 5*, 365.
Loudon, G. M., and Koshland, D. E., Jr. (1972), *Biochemistry 11*, 229.
Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951), *J. Biol. Chem. 193*, 265.
Markus, G., McClintock, D. K., and Bussel, J. B. (1971), *J. Biol. Chem. 246*, 762.
Meighen, E. A., Pigiet, V., and Schachman, H. K. (1970), *Proc. Nat. Acad. Sci. U. S. 65*, 234.
Porter, R. W., Modebe, M. O., and Stark, G. R. (1969), *J. Biol. Chem. 244*, 1846.
Rosenbusch, J. P., and Weber, K. (1971), *J. Biol. Chem. 246*, 1644.
Scatchard, G. (1949), *Ann. N. Y. Acad. Sci. 51*, 660.
Weber, K. (1968), *Nature (London) 218*, 1116.
Wiley, D. C., and Lipscomb, W. N. (1968), *Nature (London) 218*, 1119.
Winlund, C. C., and Chamberlin, M. J. (1970), *Biochem. Biophys. Res. Commun. 40*, 43.