Storer, A. C., & Carey, P. R. (1985) *Biochemistry 24*, 6808-6818.
Storer, A. C., Ozaki, Y., & Carey, P. R. (1982) *Can. J. Chem. 60*, 199-209.
Warshel, A. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 5250-5254.
Welch, G. R., Somogyi, B., & Damjanovich, S. (1982) *Prog. Biophys. Mol. Biol. 39*, 109-146.

Relationship between Domain Closure and Binding, Catalysis, and Regulation in *Escherichia coli* Aspartate Transcarbamylase†

Moncef M. Ladjimi,‡ Steven A. Middleton, Kerry S. Kelleher, and Evan R. Kantrowitz*
Department of Chemistry, Boston College, Chestnut Hill, Massachusetts 02167
Received May 20, 1987; Revised Manuscript Received September 10, 1987

ABSTRACT: Previous evidence, from both crystallographic and biochemical studies, has indicated that profound tertiary and quaternary changes in the structure of *Escherichia coli* aspartate transcarbamylase occur upon the binding of the bisubstrate analogue *N*-(phosphonoacetyl)-L-aspartate (PALA). In particular, within a single catalytic polypeptide chain, the aspartate binding domain relocates closer to the carbamyl phosphate binding domain, thereby resulting in a major reorganization of the interface between the two domains. Among the new interactions, salt bridges between Glu-50 and both Arg-167 and Arg-234 are formed. In the present study, site-directed mutagenesis is used to replace Glu-50 by glutamine in the catalytic chain. The Michaelis constant for aspartate of the mutant catalytic subunit is about 10-fold higher and the turnover number 10-fold lower than their respective couterparts in the wild-type catalytic subunit, whereas the dissociation constant for carbamyl phosphate is almost unchanged. For the holoenzyme, this substitution results in an 8-fold decrease in the specific activity, a 20-fold increase in the aspartate concentration that gives half of the maximal velocity, and a loss of cooperativity for both substrates. However, the mutant enzyme is not "frozen" in a low-affinity–low-activity conformation since PALA stimulates the activity severalfold and induces an increase in the sulfhydryl reactivity analogous to that of the wild-type enzyme. Together these results indicate that the stabilization of the aspartate binding domain near the carbamyl phosphate binding domain, through specific interdomain bridging interactions, is necessary for the high-affinity–high-activity configuration of the active site. The importance of these salt bridges for the homotropic and heterotropic interactions in aspartate transcarbamylase is discussed.

Escherichia coli aspartate transcarbamylase (EC 2.1.3.2) catalyzes the committed step of the pyrimidine biosynthesis pathway, the formation of *N*-carbamyl-L-aspartate from carbamyl phosphate and L-aspartate. The enzyme, used as a model system to study the molecular mechanisms of allosteric regulation [see reviews by Gerhart (1970), Jacobson and Stark (1973), Schachman (1974), and Kantrowitz et al. (1980a,b)], exhibits positive cooperativity for both substrates (Gerhart & Pardee, 1962; Bethell et al., 1968), and its activity is inhibited by CTP and activated by ATP, the end products of the pyrimidine and purine pathways, respectively. The enzyme is composed of three regulatory dimers (i.e., regulatory subunits) and two catalytic trimers (i.e., catalytic subunits). The regulatory subunit binds CTP and ATP but is devoid of catalytic activity while the isolated catalytic subunit exhibits no homotropic cooperativity and is insensitive to the allosteric effectors. The active sites, three per catalytic subunit, are shared between adjacent catalytic chains within the trimer (Monaco et al., 1978; Robey & Schachman, 1985; Krause et al., 1985, Wente & Schachman, 1987). The amino acid sequences of the catalytic and regulatory polypeptide chains have been determined (Weber, 1968; Konigsberg & Henderson, 1983; Hoover et al., 1983; Schachman et al., 1984), and information about the three-dimensional structure of the unliganded enzyme (Honzatko et al., 1979, 1982; Ke et al., 1984) and the enzyme liganded with the bisubstrate analogue *N*-(phosphonoacetyl)-L-aspartate (PALA)[1] (Ladner et al., 1982; Krause et al., 1985, 1987) is available at atomic resolution.

The kinetic and physicochemical properties of the enzyme can be rationalized in terms of a concerted transition between two alternate conformational states in equilibrium (Gerhart & Schachman, 1968; Hammes & Wu, 1971; Griffin et al., 1973; Kirshner & Schachman, 1973; Gibbons et al., 1976; Howlett & Schachman, 1977; Johnson & Schachman, 1980; Foote & Schachman, 1985). However, the two-state model (Monod et al., 1965) does not appear to satisfactorily account for all of the experimental data. In particular, ample evidence indicates that the homotropic and heterotropic interactions proceed by different mechanisms (Kerbiriou & Hervé, 1972, 1973; Kantrowitz et al., 1977; Kerbiriou et al., 1977; Kan-

† This work was supported by a grant from the National Institutes of Health (GM26237). E.R.K. thanks the Camille and Henry Dreyfus Foundation for a fellowship.
‡ Present address: Laboratoire d'enzymologie, CNRS, 91190 Gif-sur-Yvette, France.

[1] Abbreviations: PALA, *N*-(phosphonoacetyl)-L-aspartate; T and R states, tight and relaxed states of the enzyme having low and high affinity, respectively, for the substrate; pHMB, *p*-(hydroxymercuri)-benzoate; $[S]_{0.5}$, substrate concentration at half the maximal observed specific activity; Tris, tris(hydroxymethyl)aminomethane; Gln-50 enzyme, mutant enzyme with glutamine substituted in place of glutamic acid at position 50 in the catalytic chain of aspartate transcarbamylase; holoenzyme, entire aspartate transcarbamylase molecule composed of two catalytic subunits and three regulatory subunits.

0006-2960/88/0427-0268$01.50/0

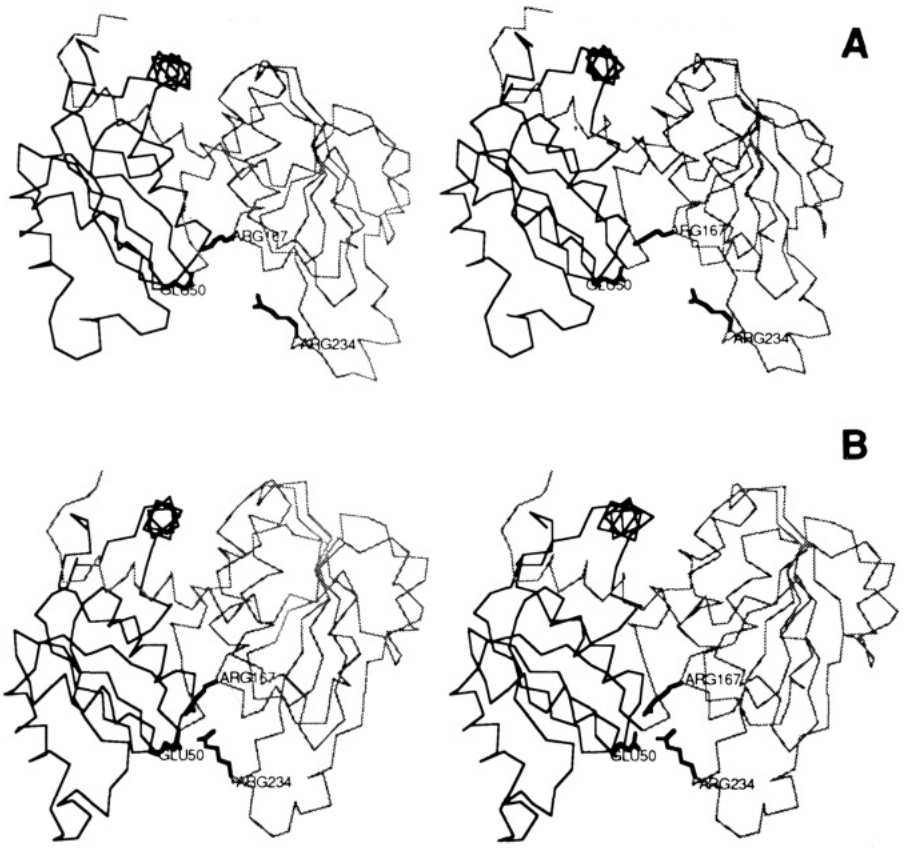

FIGURE 1: Stereoview showing the α-carbon trace of a single unliganded (A) and PALA-liganded (B) catalytic polypeptide chain. The carbamyl phosphate binding domain (dark) corresponds to the N-terminal moiety and the aspartate binding domain (light) to the C-terminal moiety of the catalytic chain. Glu-50 (carbamyl phosphate binding domain) and Arg-167 and Arg-234 (aspartate binding domain), far apart in the unliganded enzyme, are not interacting but become involved in a core interaction in the PALA-liganded enzyme. Among these three residues, only Arg-167 is directly involved in PALA binding. The PALA molecule has been omitted for clarity. The unliganded and PALA-liganded crystallographic data are from Ke et al. (1984) and Krause et al. (1987), respectively.

trowitz & Lipscomb, 1977; Enns & Chan, 1978, 1979; Chan & Enns, 1979). It has been shown that the effectors ATP and CTP do not act directly on the equilibrium between the different quaternary states but rather act through tertiary displacements involving a "site by site" local effect (Thiry & Hervé et al., 1978; Tauc et al., 1982; Hervé et al., 1985). Furthermore, a recent report shows that even the effects of ATP and CTP can be uncoupled (Ladjimi et al., 1985), suggesting that activation and inhibition do not correspond to opposite displacements of the same conformational equilibrium. Although the association of the local and concerted structural changes provides a sensitive switching mechanism for a variety of metabolic conditions, and thus can be understood on a cellular level, these structural changes need to be explained on the molecular level.

The occurrence of a local conformational change within the catalytic subunit has been demonstrated by a variety of physical techniques. Evidence from ultraviolet difference spectroscopy (Collins & Stark, 1969, 1971), difference sedimentation (Kirshner & Schachman, 1971, 1973), and circular dichroism studies (Griffin et al., 1973) has shown that upon the addition of succinate (in the presence of carbamyl phosphate) or PALA the catalytic subunit adopts a more contracted or isometric conformation. From fluorescence spectroscopy (Kempe & Stark, 1975) and NMR studies (Roberts et al., 1976), it has been suggested that this local change may be important in catalysis and in the global conformational change of the holoenzyme (Kirschner & Schachman, 1973; Jacobson & Stark, 1975; Howlett et al., 1977). More recently, the three-dimensional structure of the enzyme complexed with PALA was solved to a resolution of 2.9 Å (Krause et al., 1985) and subsequently refined to 2.5 Å (Krause et al., 1987). The binding site of PALA was then established, and the comparison between the unliganded and liganded structures revealed that, among other rearrangements, the aspartate binding domain[2] relocates closer to the carbamyl phosphate binding domain,[2] resulting in the formation of new bridging interactions between the domains (Krause et al., 1985, 1987; Figure 1). Although the movement of the aspartate binding domain appears necessary to achieve the closure of the active site, the relevance of such motion for catalysis is not evident. It is also not clear whether the PALA binding mode in the crystal resembles the binding of the natural substrates in the activated complex.

In order to correlate the structural changes with the catalytic and allosteric properties of the enzyme, we decided to perturb two major interdomain bridging interactions that are formed only after the quaternary conformational change, namely, Glu-50–Arg-167 and Glu-50–Arg-234 (Figure 1). Using site-directed mutagenesis, we have engineered a protein containing a glutamine in place of glutamic acid at position 50 in the catalytic chain. This substitution should prevent these two salt bridges from occurring but still leaves the possibility for hydrogen-bonding interactions, thus allowing us to probe the relationship between the local and global conformational change. Using this approach, we show that it is possible to obtain information on the functional basis of the structural events at the active site and the coupling of these structural

[2] The aspartate and carbamyl phosphate binding domains of the catalytic chain have previously been referred to as the equatorial and polar domains, respectively (Monaco et al., 1978; Honzatko et al., 1982; Krause et al., 1987).

events to the allosteric behavior of the enzyme.

Experimental Procedures

Materials. ATP, CTP, carbamyl phosphate, *N*-carbamyl-L-aspartate, agar, agarose, ampicillin, L-aspartate, potassium dihydrogen phosphate, *p*-(hydroxymercuri)benzoate, and Tris were purchased from Sigma Chemical Co. The carbamyl phosphate was purified by precipitation from 50% (v/v) ethanol and stored desiccated at −20 °C (Gerhart & Pardee, 1962). Enzyme-grade ammonium sulfate was purchased from Schwarz/Mann and casamino acids were purchased from Difco. *Escherichia coli* strain U39a was obtained from J. Wild, Texas A&M University. Restriction endonucleases were obtained from either U.S. Biochemicals or New England Biolabs and used according to the supplier's recommendations. T4 DNA ligase, the Klenow fragment of DNA polymerase I, and T4 polynucleotide kinase were products of U.S. Biochemicals. NA45 paper used for isolation of DNA fragments from agarose gels was purchased from Schleicher & Schuell.

Methods. (*i*) *Oligonucleotide Synthesis.* The oligonucleotide required for the site-directed mutagenesis and the sequencing primers were synthesized on an Applied Biosystems 381A DNA synthesizer.

(*ii*) *Wild-Type and Mutant Enzyme Purification.* Wild-type and Gln-50 aspartate transcarbamylase were isolated as described by Nowlan and Kantrowitz (1985), from *E. coli* strain EK1104 (F⁻ *ara*, *thi*, Δ*pro-lac*, Δ*pyrB*, *pyrF*±, *rpsL*), containing the plasmid pEK2 (Smith et al., 1986) or pEK42, respectively.

(*iii*) *Determination of Protein Concentration.* Concentrations of pure wild-type holoenzyme and isolated catalytic subunit were determined by absorbance measurements at 280 nm with extinction coefficients of 0.59 cm^2/mg and 0.72 cm^2/mg, respectively (Gerhart & Holoubek, 1967). The protein concentrations of the mutant holoenzyme and isolated subunits were determined by the method of Lowry et al. (1951).

(*iv*) *Aspartate Transcarbamylase Assay.* The transcarbamylase activity was measured at 25 °C by either a colorimetric (Pastra-Landis et al., 1981) or pH-stat method (Wu & Hammes, 1973). pH-stat assays were carried out with a Radiometer TTT80 titrator and an ABU80 autoburet. All colorimetric assays were performed in duplicate, and the data points shown in the figures are the average.

(*v*) *Data Analysis.* The analysis of the steady-state kinetic data was carried out as previously described by Silver et al. (1983). The analysis of the structural data, based on the three-dimensional coordinates of the unliganded (Ke et al., 1984) and PALA-liganded enzyme (Krause et al., 1985), was accomplished with the program PS300 FRODO (Department of Biochemistry, Rice University) on an Evans & Sutherland PS300 interfaced to a VAX 11/750.

(*vi*) *Reaction of Wild-Type and Mutant Enzymes with pHMB.* The measurements of the rates of reaction with the wild-type and the mutant enzymes with pHMB were performed according to the method of Gerhart and Schachman (1968).

Results

Construction of Gln-50 Mutant Aspartate Transcarbamylase by Site-Directed Mutagenesis. The replacement of glutamic acid by glutamine at position 50 in the catalytic chain was accomplished by site-directed mutagenesis according to the method of Zoller and Smith (1982) with one modification. After the fill-out and ligation steps, the M13 RF was transformed into HB2154 [F′ *ara*, *thi*, Δ*pro-lac*, mutL::Tn10(tet^r)/F′ *proAB*, *lacI*^{q}, *lacZ*ΔM15]. HB2154 is defective in DNA repair, and therefore mismatch repair is prevented (Carter et al., 1985). In order to avoid the introduction of undesirable mutations, the exposure of the M13 RF to the repair-defective background was reduced by plating the transformation mixture onto a lawn of HB2151 [F′ *ara*, *thi*, Δ*pro-lac*/F′ *proAB*, *lacI*^{q}, *lacZ*ΔM15], a repair-positive version of HB2154.

The construction of the M13 phage carrying the entire *pyrBI* operon has been described (Smith et al., 1986). Following primer extension, ligation, and transformation, the mutant phage were screened by colony blot hybridization with the same labeled oligonucleotide as probe (Carter et al., 1984). The sequence of putative mutants was determined by dideoxy sequencing (Sanger et al., 1977) with a specific primer located 62 bases from the mutation site.

Recloning and Conformation of Gln-50 Mutation. In order to overproduce the Gln-50 mutant enzyme, it was necessary to reclone the mutated *pyrB* gene onto a plasmid. A *Pst*I–*Bst*EII fragment of 758 base pairs, containing the desired mutation, was isolated from the purified M13 RF after agarose gel electrophoresis, with NA45 paper. In addition, the plasmid pEK2 (Nowlan & Kantrowitz, 1985) was cut with the same two restriction enzymes, and the larger fragment was isolated in a similar fashion. This fragment contained the vector pUC8 along with the remainder of the *pyrBI* operon. The fragment from the mutant M13 RF was then combined with the fragment from the pEK2 plasmid and treated with T4 DNA ligase. Selection was accomplished after transformation in U39a (F⁻ *ara*, *thi*, Δ*pro-lac*, Δ*pyrB*, *rpsL*), a strain that has a deletion in the *pyrBI* region. A plasmid was isolated, pEK42, which carried the desired mutation.

The entire *Pst*I to *Bst*EII fragment used in the construction of pEK42 was sequenced to ensure that no other mutations had occurred during the mutagenesis. Dideoxy sequencing was carried out with three oligonucleotide primers spaced along the fragment. Analysis of the sequence data revealed no mutations other than the desired Glu-50 to Gln change.

Kinetic Properties of Wild-Type and Gln-50 Mutant Holoenzymes. The aspartate saturation curves for the wild-type and Gln-50 holoenzymes are compared in Figure 2. The Gln-50 enzyme shows almost no activity even at high concentrations of aspartate and a hyperbolic aspartate saturation curve. Nonlinearity of the Eadie plot (Eadie, 1942) is a very sensitive indicator in revealing low cooperativity (Hensley et al., 1981). As seen in the inset to Figure 2, a straight line is observed for the kinetic data of the Gln-50 enzyme as compared to the curve obtained for the cooperative wild-type enzyme, demonstrating the absence of homotropic interactions. The lack of cooperativity in the mutant enzyme is not only limited to aspartate but is also observed with carbamyl phosphate. The sigmoidal response of the wild-type enzyme is not observed for the Gln-50 enzyme, or detected in an Eadie plot (Figure 3).

The kinetic parameters for these enzymes are presented in Table I. The replacement of Glu-50 by Gln affects equally the Hill coefficient for both substrates and results in a 20-fold increase in the $[S]_{0.5}$ for aspartate but only a 4-fold increase in the $[S]_{0.5}$ for carbamyl phosphate as compared to those of the wild-type enzyme. In addition, the mutation causes an 8-fold reduction in the maximal velocity.

Kinetic Properties of Wild-Type and Gln-50 Catalytic Subunits. The constants derived from the analysis of the steady state kinetics of the wild-type and Gln-50 mutant

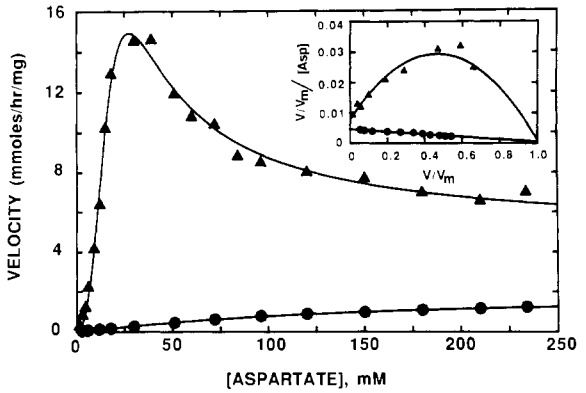

FIGURE 2: Aspartate saturation curves of the wild-type (▲) and Gln-50 mutant (●) enzymes. The reactions were carried out at 25 °C in 50 mM Tris–acetate buffer, pH 8.3, in the presence of saturating concentrations of carbamyl phosphate (4.8 mM). The curves drawn are the best fit curves calculated by a nonlinear least-squares procedure employing a modified Hill equation, which incorporates substrate inhibition for the wild-type enzyme and the Michaelis–Menten equation for the Gln-50 enzyme. (Inset) Corresponding Eadie plot for the wild-type (▲) and the mutant (●) enzymes. The V_{max} values used to calculate the V/V_{max} ratio were obtained from Table I. For the wild-type enzyme, the data that exhibited substrate inhibition were omitted.

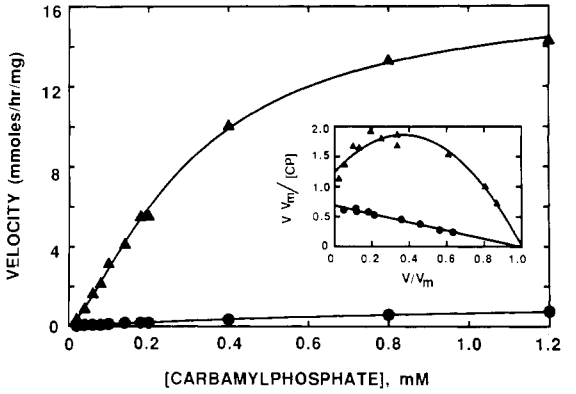

FIGURE 3: Carbamyl phosphate saturation curves of the wild-type (▲) and the Gln-50 mutant (●) enzymes. The reactions were carried out as described in Figure 2 but in the presence of 25 and 500 mM aspartate for the wild-type and mutant enzyme, respectively. (Inset) Corresponding Eadie plot. The V_{max} values used in this case are 15.6 mmol·h^{-1}·mg^{-1} for the wild-type enzyme and 1.6 mmol·h^{-1}·mg^{-1} for the mutant enzyme.

Table I: Kinetic Parameters for Wild-Type and Gln-50 Holoenzymes[a]

	V_{max} (mmol·h^{-1}·mg^{-1})	$[S]_{0.5}^{Asp}$ (mM)	$[S]_{0.5}^{CP}$ (mM)	n_H^{Asp}	n_H^{CP}
wild type	18.5	12.3	0.3	2.2 (0.16)	1.4 (0.06)
Gln-50	2.6	245.0	1.3	1.0	1.0

[a] These data are extracted from Figures 1 and 2. For the wild-type enzyme, the V_{max} and Hill coefficient (n_H) (standard errors in parentheses) were calculated by a nonlinear least-squares procedure with a modified Hill equation that incorporates substrate inhibition (Pastra-Landis et al., 1978). For the mutant enzyme, the data were fit by the same procedure to the Michaelis–Menten equation. The V_{max} values correspond to turnover numbers of 316 s^{-1} and 37 s^{-1} per active site for the wild-type and mutant enzymes, respectively.

catalytic subunits are summarized in Table II. The turnover number of the mutant catalytic subunit is about 10-fold lower, and the apparent K_m for aspartate is 10-fold higher than that for the wild-type catalytic subunit. On the other hand, it appears that the specific amino acid replacement has almost no effect on cabamyl phosphate affinity. As shown in Table II, the dissociation constant (K_D) of carbamyl phosphate for

Table II: Kinetic Parameters for Wild-Type and Gln-50 Catalytic Subunits

	k_{cat} (s^{-1})[a]	K_m^{Asp} (mM)	K_D^{CP} (μM)[b]	k_{cat}/K_m^{Asp} (s^{-1}·mM^{-1})
wild type	253	5.9	18	42.8
Gln-50	27	57.2	28	0.47

[a] The V_{max} values used to determine the k_{cat} parameter, calculated per active site, were obtained by fitting the data by nonlinear least-squares to the Michaelis–Menten equation. (V_{max} wild type 27.6 and V_{max} mutant 2.9 mmol·hr^{-1}·mg^{-1}.) [b] The dissociation constant for carbamyl phosphate was obtained by the method of Porter et al. (1969) at 25 °C and 50 mM Tris–acetate buffer (pH 8.3) and in the presence of 0.1 and 1.0 mM aspartate for the wild-type and mutant enzymes, respectively.

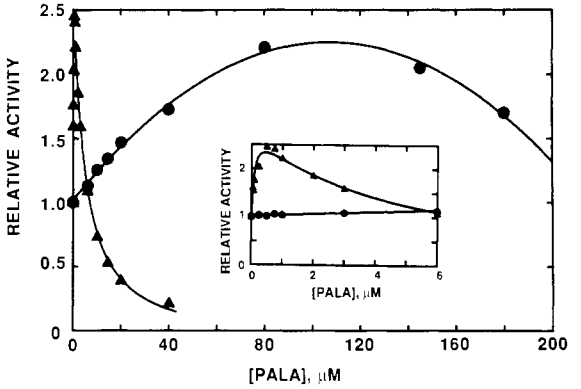

FIGURE 4: Activation of the wild-type (▲) and the Gln-50 mutant (●) enzymes by PALA at low concentrations of aspartate. The aspartate transcarbamylase activity was measured as described in the legend to Figure 2 at saturating levels of carbamyl phosphate (4.8 mM) and an aspartate concentration of approximately $^1/_6$ the K_m, that is, 2 and 40 mM for the wild-type and the mutant enzyme, respectively. The relative activity represents the ratio of activity in the absence or the presence of PALA to that in its absence. (Inset) Closeup of the region of the figure corresponding to low PALA concentrations.

the Gln-50 catalytic subunit is nearly identical with that for the wild-type catalytic subunit.

Stimulation of Enzyme Activity by the Bisubstrate Analogue PALA. Previous studies by Collins and Stark (1971) have shown that low concentrations of succinate or PALA activate the enzyme at low concentrations of aspartate. These substrate analogues are able to promote the conformational transition from the low-affinity T state to the high-affinity R state (Gerhart & Schachman, 1968; Howlett & Schachman, 1977; Blackburn & Schachman, 1977; Howlett et al., 1977). More recently, Foote and Schachman (1985) have shown that, in the reverse reaction, the binding of one PALA molecule to a T state active site was sufficient to convert all of the remaining active sites to the R conformation in the presence of pyrophosphate. Since aspartate is unable to promote the allosteric transition in the mutant holoenzyme and large concentrations of this substrate are necessary to achieve half-saturation, the replacement of Glu-50 by Gln might have locked the enzyme in a low-affinity state, making it unable to reach a conformation with higher affinity for aspartate. Alternatively, this specific amino aciid substitution could have altered the configuration of the high-affinity active site so that aspartate no longer shows preference for one state over the other. Therefore, it was of interest to determine the effect of PALA on the activity on the mutant enzyme. As shown in Figure 4, at low aspartate concentrations the activity of the mutant enzyme is stimulated at least 2-fold in the presence of low concentrations of PALA. The extent of activation is almost identical with that observed for the wild-type enzyme. However, much higher concentrations of PALA (about 200-

Table III: Reaction of Wild-Type and Gln-50 Enzymes with pHMB[a]

	k ($M^{-1}\cdot s^{-1}$) for the indicated ligand				
	none	PALA	carbamyl phosphate	succinate	carbamyl phosphate + succinate
wild type	62	250	86	67	283
Gln-50	82	320	100	80	256

[a] The reactions were carried out under the conditions described by Ladjimi and Kantrowitz (1987). PALA, carbamyl phosphate, and succinate were present at 2.5, 10, and 500 mM, respectively.

fold) were required to achieve maximal stimulation of the Gln-50 enzyme.

Conformational Changes in Wild-Type and Gln-50 Enzymes. The increase in the reactivity of the sulfhydryl groups in the presence of the active site ligands has been used as an indicator of structural changes (Gerhart & Schachman, 1968; Blackburn & Schachman, 1977; Subranami & Schachman, 1981). As shown in Table III, in the absence of ligands, the rates of sulfhydryl reactivity for the Gln-50 mutant and the wild-type enzymes are very similar. The slight increases in the reaction rates of the Gln-50 enzyme may suggest a small local effect of the mutation on the C–R interface. Upon the addition of saturating concentrations of PALA, a 4-fold increase in the reaction rate is observed for both enzymes. In the presence of a different combination of analogues, a similar result was obtained; at saturating concentrations of carbamyl phosphate and succinate, an aspartate analogue that lacks the α-amino group, the reactivity of the sulfhydryl groups of both enzymes increased at least 3-fold with respect to the rate observed in the absence of ligand. Carbamyl phosphate alone induces a small increase in the reaction rate (about 1.3-fold), analogous to that observed for the wild-type enzyme. Succinate alone has no effect, indicating that in the mutant, as in the wild-type enzyme, the effect of succinate on the rate of the reaction is measurable only in the presence of carbamyl phosphate.

Although the increase in sulfhydryl reactivity upon the addition of the active site ligands in the Gln-50 mutant enzyme supports the occurrence of structural changes, this result was obtained under saturating concentrations of ligand and does not provide any information about the quantitative relationship between the conformational change and ligand binding. Therefore, the variation of the rate constant for the pHMB reaction with respect to succinate concentration (in the presence of carbamyl phosphate) was measured for both enzymes. The increase in the reaction rate of the Gln-50 mutant and wild-type enzymes indicates that both undergo a progressive conformational change upon the addition of succinate. However, the concentration of succinate necessary to achieve half of the maximal change was at least 10-fold higher for the Gln-50 enzyme than for the wild-type enzyme (data not shown).

Influence of the Allosteric Effectors ATP and CTP on Activity of Gln-50 Mutant and Wild-Type Enzymes. The aspartate saturation curves of the Gln-50 enzyme in the absence and the presence of saturating amounts of nucleotide were determined. Analysis of the kinetic data indicates that the principal heterotropic effect of ATP and CTP is a change in the catalytic constant. The turnover numbers are 23, 110, and 37 molecules of carbamyl aspartate formed per second per active site in the presence of CTP, ATP, and no effector, respectively (data not shown). However, in the case of the wild-type enzyme, an allosteric enzyme of the K system (Monod et al., 1965), the presence of ATP and CTP affects the apparent K_m for aspartate without altering the turnover

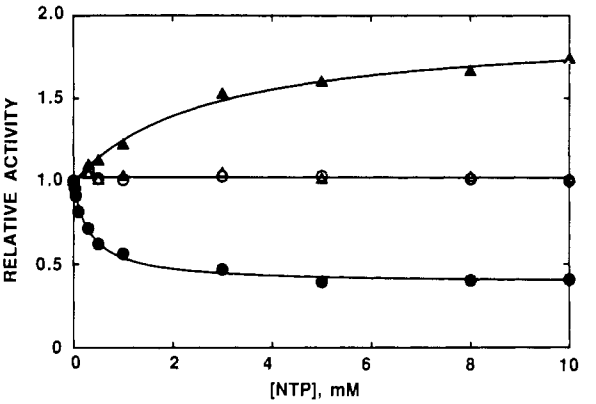

FIGURE 5: Influence of the effectors ATP and CTP on the activity of the wild-type and the Gln-50 enzymes at high concentrations of aspartate (above $2K_m$). The assays were performed as described in the legend to Figure 2, in the presence of saturating levels of carbamyl phosphate (4.8 mM) and approximately 30 and 500 mM aspartate for the wild-type and mutant enzyme, resepctively. ATP effect on the wild-type (△) and Gln-50 enzymes (▲). CTP effect on the wild-type (○) and Gln-50 enzymes (●).

number (Gerhart & Pardee, 1963, 1964).

In order to further investigate the regulatory properties of the mutant enzyme under the influence of the nucleotides, the ATP and CTP saturation curves were determined at high aspartate concentrations (above $2K_m$). As shown in Figure 5, the activity of the wild-type enzyme was independent of the concentration of the allosteric effectors, in agreement with the expected properties of a K-system enzyme, whereas the Glu-50 enzyme exhibits the characteristics of a V-system allosteric enzyme. At concentrations of substrate sufficient to convert the enzyme to the R state, ATP still stimulates the activity of the mutant enzyme by at least 2-fold and CTP reduces the activity by more than half (Figure 5).

This unusual heterotropic response, in conjunction with the lack of the homotropic cooperative interactions between the catalytic sites, led us to investigate the influence of aspartate concentration on the degree of stimulation or inhibition.[3] As shown in Figure 6A, the inhibition of the wild-type enzyme activity is strongly aspartate dependent. The maximal effect of CTP or ATP observed at low aspartate concentrations is completely reversed at high concentrations of this substrate. In contrast, the degree of stimulation and inhibition of the Gln-50 enzyme activity is much less dependent on aspartate concentrations. Even at high aspartate concentrations, a residual inhibition of 40% is observed, and the maximal stimulation reaches 200%.

Discussion

The comparison between the structures of the unliganded and PALA-liganded aspartate transcarbamylases indicates that a domain closure occurs resulting from the movement of the aspartate binding domain toward the carbamyl phosphate binding domain (Ke et al., 1984; Krause et al., 1985, 1987). This structural reorganization appears to establish the active site and seems to be stabilized by a set of interdomain bridging interactions. In order to probe the functional importance of the closure of the domains, site-directed mutagenesis was used to replace a residue that might be critical for the interdomain interaction.

Effects of Glu-50 to Gln Mutation on Substrate Binding and Catalysis. (i) Importance of Domain Closure for Binding.

[3] The percentage of stimulation or inhibition is defined as the difference in activity of the enzyme in the presence and absence of the effector, relative to the activity in the absence of effector (Ladjimi et al., 1985).

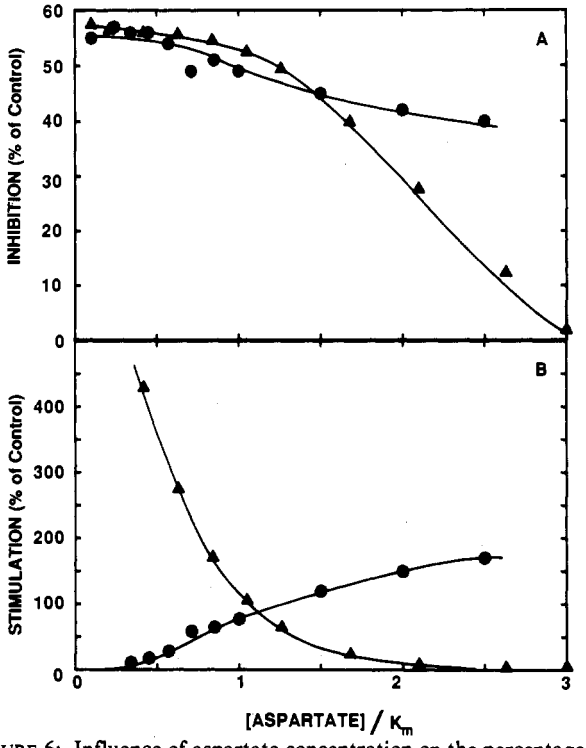

FIGURE 6: Influence of aspartate concentration on the percentage of inhibition (A) and stimulation (B) of the wild-type (▲) and Gln-50 mutant (●) enzymes. The assays were performed as previously described, with 6 mM ATP and 1 mM CTP. The percent stimulation or inhibition was calculated according to Ladjimi et al. (1985). The [aspartate]/K_m ratio for the wild-type or mutant enzyme was calculated with their respective K_m for aspartate.

The replacement of Glu-50 by Gln in the carbamyl phosphate binding domain substantially reduces the affinity of the isolated catalytic subunit for aspartate, as reflected by the 10-fold increase in the K_m. However, there is almost no effect on carbamyl phosphate binding as shown by the similarity of the dissociation constants between the mutant and wild-type catalytic subunits, 28 μM and 18 μM, respectively. On the other hand, the affinities of the mutant holoenzyme for carbamyl phosphate and aspartate are reduced by a factor of 4 and 20, respectively, relative to that of the wild-type enzyme. The greater impact on aspartate affinity as compared to carbamyl phosphate affinity, even though the mutation is located near the phosphate binding site, can be correlated with the crystallographic information (Krause et al., 1985, 1987). In particular, when the aspartate binding domain moves closer to the phosphate binding site, closure of a chamber surrounding PALA occurs, and new salt links between Glu-50 and both Arg-167 and Arg-234 are formed (Krause et al., 1985, 1987; Figure 1). We suggest that in the mutant enzyme the replacement of Glu-50 by Gln simultaneously affects two major interdomain bridging interactions, whose presence is necessary to ensure optimal orientation of the functional groups involved in aspartate but not in carbamyl phosphate binding. This interpretation is supported by additional crystallographic evidence indicating that the α- and β-carboxylates of the aspartate moiety of PALA are bound by Arg-167 and Arg-229, respectively, two residues that are driven into the active site by the movement of the aspartate binding domain. In contrast, most of the residues involved in the binding of the phosphonate moiety of PALA are not affected by the closure of the domains (Ke et al., 1984; Krause et al., 1985), and the binding of the phosphonate group of PALA to the R form is similar to the binding of pyrophosphate to the T form (Honzatko & Lipscomb, 1982; Krause et al., 1985; Voltz et al., 1986).

(*ii*) *Importance of Domain Closure for Catalysis.* The observation that catalysis in the mutant is not completely abolished suggests that Glu-50 is not required for catalysis itself but for its optimization. In fact, the replacement of Glu-50 by Gln results in a reduction of k_{cat}/K_m by about 100-fold (Table II), which corresponds to a weakening of the binding energy of the transition state by 2.7 kcal/mol. Thus, the establishment of the Glu-50–Arg-167 and the Glu-50–Arg-234 interactions following the domain closure would allow the active site to adopt a structure more complementary to the transition state than to the substrates in the ground state [see review by Jencks (1975), Fersht et al. (1985), and Wells and Fersht (1985)]. Such a structural change would then have a functional basis and would be used to lower the activation energy of the reaction and increase the catalytic rate as suggested by Collins and Stark (1971) and Jacobson and Stark (1973).

Although Glu-50 does not interact directly with PALA in the active site, its replacement by Gln reduces the maximal velocity of the holoenzyme by at least 7-fold. This result suggests that the stabilization of the aspartate binding domain near the carbamyl phosphate binding domain through the Glu-50–Arg-167 and Glu-50–Arg-234 salt bridges is necessary not only to achieve the correct binding of the α- and β-carboxylates of aspartate but also to bring aspartate closer to carbamyl phosphate in order to maximize the number of productive collisions. The interpretation, which associates the domain closure within a single catalytic chain with the high-affinity–high-activity state of the active site, is consistent with the fact that PALA is able to activate the mutant enzyme severalfold, while aspartate in the presence of carbamyl phosphate is not. Indeed, the PALA molecule, in which the bond between the CO of the phosphonate moiety and the NH of the aspartate moiety is already formed, has been identified as a possible transition-state analogue (Collins & Stark, 1971; Jacobson & Stark, 1973; Moody et al., 1979; Voltz et al., 1986). A very recent molecular modeling study has revealed that the phosphate and the carboxylates of a putative tetrahedral intermediate are bound to the enzyme, in an orientation similar to the phosphonate and carboxylates of PALA, respectively (Gouaux et al., 1987). Hence, it is not surprising that PALA is able to trigger a high-affinity–high-activity configuration of the active site by pulling the aspartate binding domain toward the carbamyl phosphate binding domain and inducing the same change in the unoccupied sites. In contrast, aspartate is not able to promote the allosteric transition in the mutant enzyme, presumably because the van der Waals repulsions between the nitrogen of aspartate and the carbon atom of carbamyl phosphate, which oppose the domain closure (Hammes et al., 1971; Jacobson & Stark, 1973), are not overcome by the establishment of the interdomain salt bridge interactions as is the case for the wild-type enzyme.

Effects of Glu-50 to Gln Mutation on Regulation of Aspartate Transcarbamylase Activity. (*i*) *Homotropic Cooperative Interactions.* The replacement of Glu-50 by Gln, which reduces aspartate affinity and catalysis, also results in a complete loss of cooperativity for aspartate and carbamyl phosphate. Hyperbolic saturation curves would be observed if the lack of the interdomain bridging interactions locked the enzyme in the low-affinity T state. However, this possibility has been ruled out since PALA, at low concentrations, activates the mutant enzyme to nearly the same extent as it does for the wild-type enzyme, indicating the persistence of interacting sites. In addition, succinate and carbamyl phosphate together, or PALA alone, induce structural changes in the

Gln-50 catalytic–regulatory subunit interface identical with those observed for the wild-type enzyme. The 4-fold increase in the pHMB reaction rate, upon the addition of saturating concentrations of active site ligands, is observed for both the wild-type and Gln-50 mutant enzymes (see Table III). Nevertheless, in the mutant enzyme a destabilization of the R state in favor of the T state cannot be excluded since at least a 10-fold increase in succinate concentration is necessary to achieve half of the maximal structural change in the catalytic–regulatory subunit interface. This is based on the assumption that in the mutant enzyme the structural change in the catalytic–regulatory subunit interface upon the binding of succinate is correlated, as in the wild-type enzyme, with the allosteric transition (Gerhart & Schachman, 1968; Blackburn & Schachman, 1977). A destabilization of the R state as the result of the Glu-50 to Gln change is consistent with the fact that the Glu-50–Arg-167 and Glu-50–Arg-234 interactions are only formed in the PALA-liganded enzyme (Krause et al., 1985) and that their absence in the Gln-50 enzyme reduces the affinity for both substrates. On the other hand, when the quaternary constraints are released as is the case for the mutant catalytic subunit, the affinity for aspartate is improved only by factor of 4 and never reaches that of the R-like wild-type catalytic subunit (Table II); this argues in favor of an altered R state active site in the Gln-50 holoenzyme. If we assume that the Gln-50 enzyme is close to the T state (K_m = 245 mM) and the wild-type catalytic subunits are close to the R state (K_m = 5.9 mM), then the affinity of aspartate would have to be about 40 times greater for the R state in order to shift the equilibrium according to a strict two-state model (Monod et al., 1965). Since, in the mutant, the ratio of affinities for aspartate between the holoenzyme (K_m = 245 mM) and the isolated catalytic subunit (K_m = 57.2 mM) is only 4, it would appear that the Glu-50 to Gln replacement drastically reduces the affinity of the Gln-50 R state for aspartate, so that preferential binding to this form is diminished and cooperativity is no longer kinetically detected. Hence, the lack of homotropic cooperative interactions between the catalytic sites in the mutant holoenzyme would be the simultaneous result of a stabilization of the T state over the R state and a loss of the binding capability of the latter relative to the former.

These results are strikingly similar to those obtained with the native enzyme when aspartate is replaced by either cysteinesulfinate (Foote et al., 1985) or L-alanosine (Baillon et al., 1985). With either of these β-substituted analogues of aspartate, the wild-type enzyme shows low activity and low affinity and no longer exhibits cooperativity in spite of the fact that activation by PALA is still observed. The residues that are found to interact with the β-carboxylate of PALA, Arg-229 and Gln-231 (Krause et al., 1985, 1987; Voltz et al., 1986; Gouaux et al., 1987), are actually brought into the active site by the displacement of the aspartate binding domain, which in turn is stabilized in position by the Glu-50–Arg-167 and Glu-50–Arg-234 interdomain interactions. Thus, provided that the binding of the aspartate moiety of PALA is similar to that of aspartate, the interactions of the β-carboxylate of aspartate with specific residues from the aspartate binding domain must be coupled to the establishment of the interdomain bridging interactions and the conversion of the enzyme to the high-affinity–high-activity R state. Therefore, it is not surprising that a modification affecting one part or the other of this coupled mechanism would result in the expression of the same functional features, whether the modification is located on the substrate or on the enzyme. Together these observations suggest that domain closure within a single catalytic chain occurs upon aspartate binding and that this closure is linked to the quaternary conformational change that activates the enzyme.

(*ii*) *Heterotropic Interactions.* Despite the absence of homotropic cooperative interactions between the catalytic sites, the Gln-50 mutant enzyme is still sensitive to the effectors ATP and CTP, providing additional evidence for the uncoupling of the conformational changes involved in the homotropic and heterotropic interactions. Yet, the heterotropic response in the mutant enzyme is markedly different from that observed for the wild-type enzyme. For the Gln-50 enzyme, ATP and CTP seem to affect the turnover number more than the apparent K_m for aspartate. Even at aspartate concentrations above $2K_m$, the activity of the Gln-50 enzyme is stimulated by a factor of 2 and inhibited by more than half by ATP and CTP, respectively (see Figure 5). Thus, the replacement of Glu-50 by Gln reveals the mixed K and V character of the enzyme, in agreement with the suggestion made above that the two extreme forms in the mutant differ only to a small extent in their affinity for aspartate.

The results of the influence of aspartate on the extent of stimulation and inhibition (Figure 6) suggest a possible explanation for the heterotropic effects. At low aspartate concentrations, the wild-type and Gln-50 mutant enzymes show the same percent inhibition by CTP. In contrast, the substitution of Gln for Glu renders the enzyme insensitive to ATP. Thus, CTP would stabilize the open configuration of the active site regardless of what amino acid is at position 50, since in such a structure the interdomain salt bridging interactions are absent. Moreover, this is consistent with the finding that the structures of unliganded and CTP-liganded enzymes are essentially identical (Honzatko et al., 1982; Honzatko & Lipscomb, 1982). Conversely, ATP would stabilize the closed configuration of the active site, which now requires the interdomain salt bridging interaction. This stabilization of the active site is achieved in the wild-type enzyme, and the maximal activation is ensured at low aspartate concentrations. However, this is not the case for the mutant where the "active site lock" ensuring a high-activity state is lost, thus making the enzyme almost insensitive to ATP. As mentioned above, increasing the concentration of aspartate promotes the high-affinity–high-activity state through the displacement of the aspartate binding domain and the stabilization of this domain closer to the carbamyl phosphate binding domain by the Glu-50–Arg-167 and Glu-50–Arg-234 interdomain salt bridges. Consequently, at high aspartate concentrations, the effect of CTP and ATP can be completely reversed in the wild-type enzyme but not completely in the Gln-50 enzyme. In the case of the mutant enzyme, maximal turnover is not achieved, and additional nucleotide effects are observed.

The interpretation that CTP stabilizes the open conformation of the active site, whereas ATP or aspartate and carbamyl phosphate stabilize the closed conformation (panels A and B of Figure 1, respectively), is in complete agreement with results from difference spectroscopy and temperature jump experiments (Harrison & Hammes, 1973; Wu & Hammes, 1973), indicating that the enzyme–effector complex can exist in two different conformations, with ATP stabilizing one conformation and CTP favoring the other conformation.

Thus, domain closure in aspartate transcarbamylase appears to be required for the establishment of the high-affinity–high-activity state and seems to be linked to the activation of the enzyme, suggesting that the local change at the active site triggers the global conformational change. The results

presented here, along with those from Middleton and Kantrowitz (1986) and from the crystallographic data, allow us at this time to propose a basic model explaining the transmission of conformational changes throughout the molecule. However, we decided first to create additional amino acid substitution mutants in order to delineate certain features of this model and to determine the interactions necessary for the stabilization of the R state with the natural substrates. The results of this investigation and a model for the concerted allosteric transition in aspartate transcarbamylase are presented in the following paper (Ladjimi & Kantrowitz, 1988).

ACKNOWLEDGMENTS

We thank W. N. Lipscomb for providing the X-ray coordinates and J. Foote for helpful suggestions about the manuscript.

REFERENCES

Baillon, J., Tauc, P., & Hervé, G. (1985) *Biochemistry 24*, 7182–7187.

Bethell, M. R., Smith, K. E., White J., S., & Jones, M. E. (1968) *Proc. Natl. Acad. Sci. U.S.A 60*, 1442–1449.

Blackburn, M. N., & Schachman, H. K. (1977) *Biochemistry 16*, 5084–5090.

Carter, P. J., Winter, G., Wilkinson, A. J., & Fersht, A. R. (1984) *Cell (Cambridge, Mass.) 38*, 835–840.

Carter, P. J., Bedouelle, H., & Winter, G. (1985) *Nucleic Acids Res. 13*, 4431–4443.

Chan, W. W.-C., & Enns, C. A. (1979) *Can. J. Biochem. 57*, 798–805.

Collins, K. D., & Stark, G. R. (1969) *J. Biol. Chem. 244*, 1869–1877.

Collins, K. D., & Stark, G. R. (1971) *J. Biol. Chem. 246*, 6599–6605.

Eadie, G. S. (1942) *J. Biol. Chem. 146*, 85–93.

Enns, C. A., & Chan, W.-C. (1978) *J. Biol. Chem. 253*, 2511–2513.

Enns, C. A., & Chan, W.-C. (1979) *J. Biol. Chem. 254*, 6180–6186.

Fersht, A. R., Shi, J.-P., Knill-Jones, J., Lowe, D. M., Wilkinson, A. J., Blow, D. M., Brick, P., Carter, P., Waye, M. M. Y., & Winter, G. (1985) *Nature (London) 314*, 235–238.

Foote, J., & Schachman, H. K. (1985) *J. Mol. Biol. 186*, 175–184.

Foote, J., Lauritzen, A. M., & Lipscomb, W. N. (1985) *J. Biol. Chem. 260*, 9624–9629.

Gerhart, J. C. (1970) *Curr. Top. Cell. Regul. 2*, 275–325.

Gerhart, J. C., & Pardee, A. B. (1962) *J. Biol. Chem. 237*, 891–896.

Gerhart, J. C., & Pardee, A. B. (1963) *Cold Spring Harbor Symp. Quant. Biol. 28*, 491–496.

Gerhart, J. C., & Pardee, A. B. (1964) *Fed. Proc., Fed. Am. Soc. Exp. Biol. 23*, 727–735.

Gerhart, J. C., & Holoubek, H. (1967) *J. Biol. Chem. 242*, 2886–2892.

Gerhart, J. C., & Schachman, H. K. (1968) *Biochemistry 7*, 538–552.

Gibbons, I., Ritchey, J. M., & Schachman, H. K. (1976) *Biochemistry 15*, 1324–1330.

Gouaux, J. E., Krause, K. L., & Lipscomb, W. N. (1987) *Biochem. Biophys. Res. Commun. 142*, 893–897.

Griffin, J. H., Rosenbusch, J. P., Blout, E. R., & Weber, K. K. (1973) *J. Biol. Chem. 248*, 5057–5062.

Hammes, G. G., & Wu, C. W. (1971) *Biochemistry 10*, 1051–1057.

Hammes, G. G., Porter, R. W., & Stark, G. R. (1971) *Biochemistry 10*, 1046–1050.

Harrison, L. W., & Hammes, G. G. (1973) *Biochemistry 12*, 1395–1400.

Hensley, P., Yang, Y. R., & Schachman, H. K. (1981) *J. Mol. Biol. 152*, 131–152.

Hervé, G., Moody, M. F., Tauc, P., Vachette, P., & Jones, P. T. (1985) *J. Mol. Biol. 185*, 189–199.

Honzatko, R. B., & Lipscomb, W. N. (1982) *J. Mol. Biol. 160*, 265–286.

Honzatko, R. B., Monaco, H. L., & Lipscomb, W. N. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 5105–5109.

Honzatko, R. B., Crawford, J. L., Monaco, H. L., Ladner, J. E., Edwards, B. F. P., Evans, D. R., Warren, S. G., Wiley, D. C., Ladner, R. C., & Lipscomb, W. N. (1982) *J. Mol. Biol. 160*, 219–263.

Hoover, T. A., Roof, W. D., Folterman, K. F., O'Donovan, G. A., Bencini, D. A., & Wild, J. R. (1983) *Proc. Natl. Acad. Sci. U.S.A. 80*, 2462–2466.

Howlett, G. J., & Schachman, H. K. (1977) *Biochemistry 16*, 5077–5083.

Howlett, G. J., Blackburn, M. N., Compton, J. G., & Schachman, H. K. (1977) *Biochemistry 16*, 5091–5099.

Jacobson, G. R., & Stark, G. R. (1973) *Enzymes (3rd Ed.) 9*, 225–308.

Jacobson, G. R., & Stark, G. R. (1975) *J. Biol. Chem. 250*, 6852–6860.

Jencks, W. P. (1975) *Adv. Enzymol. Relat. Areas Mol. Biol. 43*, 219–410.

Johnson, R. S., & Schachman, H. K. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 1995–1999.

Kantrowitz, E. R., & Lipscomb, W. N. (1977) *J. Biol. Chem. 252*, 2873–2880.

Kantrowitz, E. R., Jacobsberg, L. B., Landfear, S. M., & Lipscomb, W. N. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 111–114.

Kantrowitz, E. R., Pastra-Landis, S. C., & Lipscomb, W. N. (1980a) *Trends Biochem. Sci. (Pers. Ed.) 5*, 124–128.

Kantrowitz, E. R., Pastra-Landis, S. C., & Lipscomb, W. N. (1980b) *Trends Biochem. Sci. (Pers. Ed.) 5*, 150–153.

Ke, H.-M., Honzatko, R. B., & Lipscomb, W. N. (1984) *Proc. Natl. Acad. Sci. U.S.A. 81*, 4027–4040.

Kempe, T. D., & Stark, G. R. (1975) *J. Biol. Chem. 250*, 6861–6869.

Kerbiriou, D., & Hervé, G. (1972) *J. Mol. Biol. 64*, 379–392.

Kerbiriou, D., & Hervé, G. (1973) *J. Mol. Biol. 78*, 687–702.

Kerbiriou, D., Hervé, G., & Griffin, J. (1977) *J. Biol. Chem. 252*, 2881–2890.

Kirshner, M. W., & Schachman, H. K. (1971) *Biochemistry 10*, 1919–1925.

Kirshner, M. W., & Schachman, H. K. (1973) *Biochemistry 12*, 2997–3004.

Konigsberg, W. H., & Henderson, L. (1983) *Proc. Natl. Acad. Sci. U.S.A. 80*, 2467–2471.

Krause, K. L., Voltz, K. W., & Lipscomb, W. N. (1985) *Proc. Natl. Acad. Sci. U.S.A. 82*, 1643–1647.

Krause, K. L., Voltz, K. W., & Lipscomb, W. N. (1987) *J. Mol. Biol. 193*, 527–553.

Ladjimi, M. M., & Kantrowitz, E. R. (1987) *J. Biol. Chem. 262*, 312–318.

Ladjimi, M. M., & Kantrowitz, E. R. (1988) *Biochemistry* (following paper in this issue).

Ladjimi, M. M., Ghellis, C., Feller, A., Cunin, R., Glansdorff, N., Pierard, A., & Hervé, G. (1985) *J. Mol. Biol. 186*, 715–724.

Ladner, J. F., Kitchell, J. P., Honzatko, R. B., Ke, H.-M., Voltz, K. W., Kalb (Gilboa), A. J., Ladner, R. C., & Lipscomb, W. N. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*, 3125-3128.
Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951) *J. Biol. Chem. 193*, 265-275.
Middleton, S. A., & Kantrowitz, E. R. (1986) *Proc. Natl. Acad. Sci. U.S.A. 83*, 5866-5870.
Monaco, H. L., Crawford, J. L., & Lipscomb, W. N. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 5276-5280.
Monod, J., Wyman, J., & Changeux, J.-P. (1965) *J. Mol. Biol. 12*, 88-118.
Moody, M. F., Vachette, P., & Foote, A. M. (1979) *J. Mol. Biol. 133*, 517-532.
Nowlan, S. F., & Kantrowitz, E. R. (1985) *J. Biol. Chem. 260*, 14712-14716.
Pastra-Landis, S. C., Evans, D. R., & Lipscomb, W. N. (1978) *J. Biol. Chem. 253*, 4624-4630.
Pastra-Landis, S. C., Foote, J., & Kantrowitz, E. R. (1981) *Anal. Biochem. 118*, 358-363.
Porter, R. W., Modebe, M. O., & Stark, G. R. (1969) *J. Biol. Chem. 244*, 1846-1859.
Roberts, M. F., Opella, S. G., Schaffer, M. H., Phillips, H. M., & Stark, G. R. (1976) *J. Biol. Chem. 251*, 5976-5985.
Robey, E. A., & Schachman, H. K. (1985) *Proc. Natl. Acad. Sci. U.S.A. 82*, 361-365.
Sanger, F., Nicklen, S., & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 5463-5467.
Schachman, H. K. (1974) *Harvey Lect. 68*, 67-113.
Schachman, H. K., Pauza, C. D., Navre, M., Karels, M. J., Wu, L., & Yang, Y. R. (1984) *Proc. Natl. Acad. Sci. U.S.A. 81*, 115-119.
Silver, R. S., Daigneault, J. P., Teague, P. D., & Kantrowitz, E. R. (1983) *J. Mol. Biol. 168*, 729-745.
Smith, K. A., Nowlan, S. F., Middleton, S. A., O'Donovan, C., & Kantrowitz, E. R. (1986) *J. Mol. Biol. 189*, 227-238.
Subramani, S., & Schachman, H. K. (1981) *J. Biol. Chem. 256*, 1255-1262.
Tauc, P., Leconte, C., Kerbiriou, D., Thiry, L., & Hervé, G. (1982) *J. Mol. Biol. 155*, 155-168.
Thiry, L., & Hervé, G. (1978) *J. Mol. Biol. 125*, 515-539.
Voltz, K. W., Krause, K. L., & Lipscomb, W. N. (1986) *Biochem. Biophys. Res. Commun. 136*, 822-826.
Weber, K. K. (1968) *Nature (London) 218*, 1116-1119.
Wells, T. N. C., & Fersht, A. R. (1985) *Nature (London) 316*, 656-657.
Wente, S. R., & Schachman, H. K. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 31-35.
Wu, C.-W., & Hammes, G. G. (1973) *Biochemistry 12*, 1400-1408.
Zoller, M. J., & Smith, M. (1982) *Nucleic Acid Res. 10*, 6487-6500.

A Possible Model for the Concerted Allosteric Transition in *Escherichia coli* Aspartate Transcarbamylase As Deduced from Site-Directed Mutagenesis Studies†

Moncef M. Ladjimi‡ and Evan R. Kantrowitz*
Department of Chemistry, Boston College, Chestnut Hill, Massachusetts 02167
Received May 20, 1987; Revised Manuscript Received September 10, 1987

ABSTRACT: Aspartate transcarbamylase is stabilized in a low-affinity–low-activity state exhibiting no cooperativity by selective perturbation of the Glu-50–Arg-167 and Glu-50–Arg-234 interdomain salt bridges. Similarly, a high-affinity–high-activity state of the enzyme, retaining a significant amount of cooperativity, is obtained by perturbation of the interaction between Tyr-240 and Asp-271. In this work, we show that the rupture of the link between Tyr-240 and Asp-271 in the enzyme already lacking the interdomain salt bridges regenerates the homotropic cooperative interactions between the catalytic sites and substantially increases the activity and affinity of the enzyme for aspartate. These results suggest a possible relationship between these two sets of interactions for the establishment of the cooperative behavior of the enzyme. Another mutation, Glu-239 to Gln, introduced to perturb the Glu-239–Lys-164 and Glu-239–Tyr-165 interactions between the two catalytic subunits, is sufficient to "lock" the enzyme in the R state. These observations emphasize the importance of the interactions at the interface between the catalytic trimers in maintaining the T state of the enzyme and shed light on the role played by this pathway in the communication of homotropic cooperativity between the different sites. A model including all these findings, as well as the interactions stabilizing the T state or the R state in the presence of the natural substrates, is proposed. In this model, it is suggested that domain closure induced by aspartate binding to one active site triggers a concerted structural change that promotes the domain closure of the remaining active sites, primarily by rupture of the interactions between the catalytic subunits.

A major conclusion that has emerged from kinetic and physicochemical studies of *Escherichia coli* aspartate transcarbamylase (EC 2.1.3.2) is that a concerted transition occurs between two alternate conformational states upon the binding of asparate. In this fashion, the extent of the quaternary change during the conversion from the constrained to the relaxed state need not be directly linked to the degree of ligand saturation (Monod et al., 1965; Howlett & Schachman, 1977; Blackburn & Schachman, 1977; Howlett et al., 1977). However, the structural basis of this transition remained unknown until the structure of the enzyme complexed with the bisubstrate analogue *N*-(phosphonoacetyl)-L-aspartate (PALA)[1] became available (Krause et al., 1985, 1987). The

† This work was supported by a grant from the National Institutes of Health (GM26237). E.R.K. thanks the Camille and Henry Dreyfus Foundation for a fellowship.
‡ Present address: Laboratoire d'enzymologie, CNRS, 91190 Gif-sur-Yvette, France.

0006-2960/88/0427-0276$01.50/0