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The regulatory subunit of *Escherichia coli* aspartate carbamoyltransferase may influence homotropic cooperativity and heterotropic interactions by a direct interaction with the loop containing residues 230–245 of the catalytic chain

(site-specific mutagenesis/allosterism/loop movement/domain closure)

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ABSTRACT **A recent x-ray structure of aspartate carbamoyltransferase (carbamoyl-phosphate: L-aspartate carbamoyltransferase, EC 2.1.3.2) with phosphonoacetamide bound [Gouaux, J. E. & Lipscomb, W. N. (1990) *Biochemistry* 29, 389–402] shows an interaction between Asp-236 of the catalytic chain and Lys-143 of the regulatory chain. Asp-236 is part of the loop containing residues 230–245 (240s) of the catalytic chain that undergoes a significant conformational change between the tight and the relaxed states of the enzyme. Furthermore, side-chain interactions between the 240s loop and other portions of the enzyme have been shown to be important for the low activity and low affinity of the tight state and the high activity and high affinity of the relaxed state. To determine whether the intersubunit link between Lys-143 of the regulatory chain and Asp-236 of the catalytic chain is important for either homotropic cooperativity and/or the heterotropic interactions in aspartate carbamoyltransferase, site-specific mutagenesis was used to replace Asp-236 with alanine. The mutant enzyme exhibits full activity and a loss of both homotropic cooperativity and heterotropic interactions. Furthermore, the aspartate concentration at half the maximal observed specific activity is reduced by ≈8-fold. The mutant enzyme exhibits normal thermal stability but drastically altered reactivity toward *p*-hydroxymercuribenzoate. The catalytic subunit of the mutant and wild-type enzymes have very similar properties. These results, in conjunction with previous experiments, suggest that the intersubunit link involving Asp-236 is involved in the stabilization of the 240s loop in its tight-state position and that the regulatory subunits exert their effect on the catalytic subunits by influencing the position of the 240s loop.**

Aspartate carbamoyltransferase (carbamoyl-phosphate: L-aspartate carbamoyltransferase, EC 2.1.3.2) from *Escherichia coli* is an allosteric enzyme showing positive homotropic interactions with its substrates aspartate and carbamoyl phosphate (1, 2). The enzyme is heterotropically inhibited by CTP and UTP (1, 3) and activated by ATP (1). Thus, in *E. coli* pyrimidine metabolism is subject to control by modification of the activity of aspartate carbamoyltransferase by the end products of the purine and pyrimidine pathways. Additional metabolic control is also achieved by regulating the amount of aspartate carbamoyltransferase in the cell by an attenuation mechanism (4–6).

The holoenzyme (M_r 310,000) is composed of (*i*) two catalytic subunits (M_r 100,000) each composed of three identical chains and (*ii*) three regulatory subunits (M_r 34,000) composed of two identical polypeptide chains. With x-ray crystallography, the quaternary and tertiary structures of the enzyme have been determined, and the differences between structures corresponding to the tight (T) and relaxed (R) states (7) have been documented (8–13). On the quaternary level, the major difference between the T and R states is an expansion of the holoenzyme by 12 Å along the molecular 3-fold axis along with rotations of the catalytic subunits about the 3-fold axis and rotations of the regulatory subunits about their respective 2-fold axes. These quaternary changes are accompanied by alterations in the tertiary structure of both the catalytic and regulatory subunits (12, 14). In particular, the gap between the two domains of the catalytic chain closes, helping to create the active-site pocket, while the gap between the two domains of the regulatory chain expands somewhat. In the T-to-R transition not only do the domains of the catalytic chain move together, but there is also a major reorientation of a loop composed of residues 230–245, the 240s loop. These tertiary and quaternary conformational changes involve the breaking of specific interactions that appear to stabilize the T state and are replaced by other interactions that appear to stabilize the R state.

The reorientation of the 240s loop is intimately involved in the quaternary conformational change. In the T state, side chains of residues in the 240s loop are involved in interactions between the upper and lower catalytic subunits (C1–C4). (The catalytic chains C1, C2, and C3 comprise the upper catalytic subunit, whereas the chains C4, C5, and C6 comprise the lower catalytic subunit. Chain C4 is below C1, while C5 and C6 are below chains C2 and C3, respectively.) These intersubunit interactions are lost after the quaternary structural rearrangement with the formation of new intrachain interactions in the R state. Site-specific mutagenesis studies have revealed that side chains of residues in the 240s loop are important for stabilizing both the T and R states of the enzyme (15–18). A recent x-ray diffraction study of the enzyme in the presence of phosphonoacetamide (19) as well as the structure of a mutant version of the enzyme (20) has revealed the presence of a salt link between Asp-236 of the catalytic (c) chain (Asp-236c) and Lys-143 of the regulatory (r) chain (Lys-143r), which occurs only in the T state of the

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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; $[S]_{0.5}^{CP}$, carbamoyl phosphate concentration at half maximal observed specific activity; $[S]_{0.5}^{Asp}$, aspartate concentration at half maximal observed specific activity; holoenzyme, entire aspartate carbamoyltransferase molecule composed of two catalytic subunits and three regulatory subunits; 240s loop, a loop in the catalytic chain corresponding to residues 230–245. The notation used to name the mutant enzymes is, for example, Asp-236c→Ala enzyme. The wild-type amino acid and location within the catalytic (c) or regulatory (r) chain of carbamoyltransferase is indicated to the left of the arrow, while the new amino acid is indicated to the right of the arrow.

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enzyme. To test the functional importance of the interaction between Asp-236c and Lys-143r for the properties of the enzyme and the importance of the catalytic–regulatory interface for both cooperativity and heterotropic interactions, we have constructed a mutant version of the enzyme with Asp-236c replaced by alanine. Here we report the properties of this mutant version of aspartate carbamoyltransferase.

EXPERIMENTAL PROCEDURES

Materials. Agar, ampicillin, L-aspartate, *N*-carbamoyl-L-aspartate, carbamoyl phosphate, and potassium dihydrogen phosphate were purchased from Sigma. The carbamoyl phosphate was purified before use by precipitation from 50% (vol/vol) ethanol and stored desiccated at −20°C (1). Electrophoresis-grade acrylamide, agarose, urea, Tris, and enzyme-grade ammonium sulfate were obtained from ICN.

Construction of the Asp-236c→Ala Mutation by Site-Specific Mutagenesis. The substitution of alanine for Asp-236 of the catalytic chain of aspartate carbamoyltransferase was accomplished by site-specific mutagenesis using the method of Zoller and Smith (21) with the modifications described (16, 22). Single-stranded DNA from 10 candidates was isolated and sequenced by the dideoxy chain-termination method (23) using *Thermus aquaticus* (*Taq*) DNA polymerase from United States Biochemical. Six of the candidates gave the sequence corresponding to the mutation. A small fragment of the gene containing the mutation was removed with restriction enzymes and inserted into a plasmid that had the corresponding section of the wild-type gene removed (24). The mutation was verified a second time, after construction of the plasmid, employing single-stranded DNA copied from the plasmid by using the helper phage M13K07 (25).

Aspartate Carbamoyltransferase Assay. The carbamoyltransferase activity was measured at 25°C by either the colorimetric (26) or the pH-stat method (27). All colorimetric assays were performed in duplicate, and the data points shown in the figures are the average.

Data Analysis. The analysis of the steady-state kinetic data was carried out as described by Silver *et al.* (28). Data points were fit by a nonlinear least-squares procedure to either the Hill equation or the Michaelis–Menten equation, incorporating a term for substrate inhibition when necessary (29). The analysis of the structural data, based on the three-dimensional coordinates of the CTP–enzyme complex (11), the *N*-phosphonoacetyl-L-aspartate (PALA)–enzyme complex (10), the phosphonoacetamide–enzyme complex (19), and the enzyme complexed with carbamoyl phosphate plus succinate (13) was accomplished with the program FRODO (Department of Biochemistry, Rice University) on an Evans and Sutherland PS390 graphics display interfaced to a MicroVAX Q5 computer.

Other Methods. Oligonucleotide synthesis, enzyme purification, and determination of protein concentration were as described (24).

RESULTS

Effects of the Mutation on the Kinetic Properties of the Enzyme with Respect to Aspartate and Carbamoyl Phosphate. Fig. 1*A* shows the aspartate saturation curves for the wild-type and Asp-236c→Ala holoenzymes. The mutant enzyme shows a slight elevation in specific activity, a dramatically low aspartate concentration at half maximal observed specific activity ($[S]_{0.5}^{Asp}$) of 1.4 mM, compared to the wild-type enzyme value of 11.8 mM, and loss of cooperativity (Table 1). The carbamoyl phosphate concentration of half maximal observed specific activity ($[S]_{0.5}^{CP}$) is reduced by ≈3-fold compared with the wild-type enzyme, and again the saturation kinetics of the mutant enzyme do not exhibit cooperativity (Fig. 1*B* and Table 1).

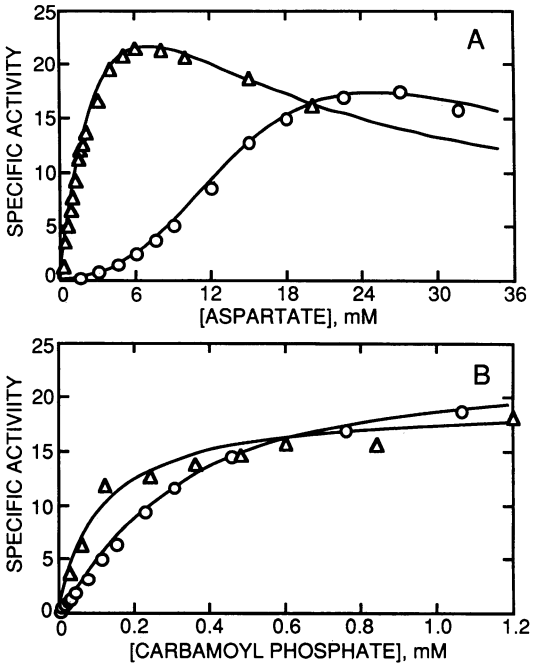

FIG. 1. Aspartate and carbamoyl phosphate saturation curves for the wild-type and the mutant holoenzymes done at 25°C in 50 mM Tris acetate buffer (pH 8.3). (*A*) Aspartate saturation curves are shown for the wild-type (○) and the Asp-236c→Ala (△) enzymes. The carbamoyl phosphate concentration was held constant at 4.8 mM. (*B*) Carbamoyl phosphate saturation curves for wild-type (○) and Asp-236c→Ala (△) enzymes. Aspartate concentration was held constant at 25 mM and 8 mM for wild-type and Asp-236c→Ala enzymes, respectively. Specific activity is reported in units of mmol of *N*-carbamoyl-L-aspartate formed per hr per mg of protein.

Influence of the Allosteric Effectors. Neither ATP nor CTP exerted any effect on the rate of the enzyme-catalyzed reaction either at the $[S]_{0.5}^{Asp}$ or at 3× $[S]_{0.5}^{Asp}$ (Fig. 2). Preliminary equilibrium binding studies with tritiated CTP indicate that the CTP still binds to the mutant enzyme (data not shown).

Effect of PALA on the Mutant Holoenzyme. PALA inhibits the mutant enzyme as it does the wild-type enzyme at concentrations of aspartate low relative to the $[S]_{0.5}^{Asp}$, but there is no intervening activation as observed with the wild-type enzyme at lower concentrations of PALA (Fig. 3).

Relative Rates of Reaction of Wild-Type and Asp-236c→Ala Enzymes with *p*-Hydroxymercuribenzoate in the Presence and Absence of PALA and Carbamoyl Phosphate. The wild-type enzyme reacts with *p*-hydroxymercuribenzoate with a second-order rate constant at 35 $M^{-1}\cdot s^{-1}$ at 25°C in 40 mM potassium phosphate buffer (pH 7.0), which increases ≈5-fold to 167 $M^{-1}\cdot s^{-1}$ in the presence of PALA. In contrast, the Asp-236c→Ala enzyme has a 15-fold higher rate constant (530 $M^{-1}\cdot s^{-1}$) than the wild-type enzyme in the absence of

Table 1. Kinetic parameters for the wild-type and the mutant holoenzymes

	Maximal velocity, mmol·hr^{-1}·mg^{-1}	$[S]_{0.5}^{Asp}$, mM	n_H^{Asp}	$[S]_{0.5}^{CP}$, mM	n_H^{CP}
Wild type	17.2	11.8	2.2	0.29	1.3
Asp-236c→Ala	21.6	1.4	1.0	0.11	1.0

The data and experimental conditions used to determine the parameters in this table are presented in the legend to Fig. 1. Maximal velocity and Hill coefficients (n_H) were calculated by a nonlinear least-squares procedure with a modified Hill equation that incorporates a term for substrate inhibition (29).

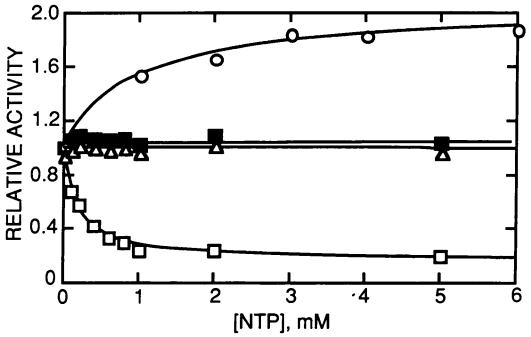

FIG. 2. ATP activation and CTP inhibition of the wild-type and the Asp-236→Ala holoenzymes at concentrations of aspartate equal to their $[S]_{0.5}^{Asp}$. Assays were done at 25°C in 50 mM Tris acetate buffer (pH 8.3). Carbamoyl phosphate concentration was held constant at 4.8 mM. For wild-type and Asp-236→Ala enzymes, aspartate concentration was held constant at 12 mM and 1.4 mM, respectively. ATP effect on wild-type (○) and Asp-236c→Ala (■) enzymes. CTP effect on wild-type (□) and Asp-236c→Ala (△) enzymes.

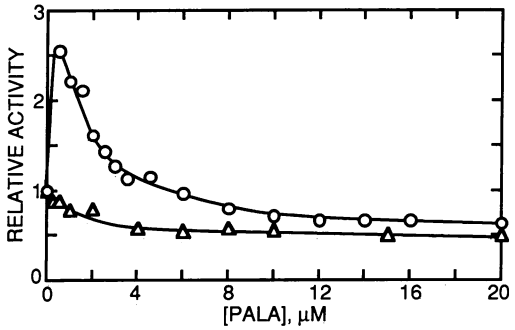

FIG. 3. Effect of PALA on the activity of wild-type and Asp-236c→Ala holoenzymes at 25°C in 50 mM Tris acetate (pH 8.3). Carbamoyl phosphate was held constant at 4.8 mM, and the aspartate concentration used for each enzyme is shown in parentheses. Data are shown for wild-type (2 mM) (○) and Asp-236c→Ala (0.23 mM) (△) enzymes.

PALA, that is halved in the presence of PALA (261 $M^{-1}\cdot s^{-1}$), giving a rate constant 1.5-fold larger than that for the wild-type enzyme in the presence of PALA.

Thermal Stability of the Wild-Type and Mutant Enzymes. Heating the aspartate carbamoyltransferase holoenzyme first causes an elevation in activity, due to the dissociation of the more active catalytic subunit, that is followed by a loss in activity, from denaturation. Therefore, the dissociation can be monitored by heating the holoenzyme for a fixed period of time over a range of increasing temperatures. The temperature at which half the enzyme dissociates has been used to determine whether mutations alter the strength of the interface between the catalytic and regulatory subunits (30). The temperatures at which the Asp-236→Ala and the wild-type enzymes half dissociate are identical (data not shown).

Kinetic Properties of the Asp-236c→Ala and Wild-Type Catalytic Subunits. The Asp-236c→Ala catalytic subunit exhibits ≈63% of the maximal activity of the wild-type enzyme, a 44% reduction in K_m^{Asp}, and a K_m^{CP} ≈10-fold larger than the wild-type catalytic subunit, although the K_d of carbamoyl phosphate for the mutant catalytic subunit is comparable with that of the wild-type catalytic subunit (Table 2). The K_i for PALA is 4-fold larger for the mutant catalytic subunit, whereas the K_i for succinate is ≈3-fold lower (Table 2). Although not identical in all properties, the Asp-236→Ala catalytic subunit is remarkably similar to the wild-type catalytic subunit. This similarity in properties would be expected because the Asp-236c interaction with Lys-143r does not occur in the isolated catalytic subunit.

DISCUSSION

A comparison of the x-ray structures of aspartate carbamoyltransferase in the T and R states has identified a loop of the catalytic chain, the 240s loop, that undergoes a significant conformational change between the T and R structures (10–12). The 240s loop is stabilized in its T-state position by one set of interactions, which, for the most part, are lost in the R state and are replaced by another set of interactions that stabilize the position of the 240s loop in the R state. The R-state position of the 240s loop not only stabilizes the enzyme in its R-state structure but also either positions residues or stabilizes the position of residues involved in binding aspartate. Site-specific mutagenesis experiments have confirmed that many of these 240s loop interactions are critical for not only the stability of the T and R states, but also for aspartate binding and catalysis (17, 18, 33).

Analysis of a recent x-ray structure of the enzyme complexed with phosphonoacetamide, an analog of carbamoyl phosphate, reveals two important interactions with the 240s loop that had not been previously observed in structures of the wild-type enzyme: Lys-143r forms a salt link with Asp-236c, and Lys-84 from one catalytic chain (C2) forms a salt link with Glu-233 of another catalytic chain (C1).

The structure of the enzyme with phosphonoacetamide bound (19) may actually better represent the kinetic T state of the wild-type enzyme for two reasons. (*i*) This structure was determined at pH 7.0, whereas many earlier studies were performed at lower pH, where the enzyme has reduced activity and cooperativity. (*ii*) Because of the ordered binding of the substrates, the cooperative aspartate saturation curve obtained by steady-state kinetics is evidence of an enzyme–carbamoyl phosphate complex in a T state converting to an enzyme–carbamoyl phosphate–aspartate complex in an R state. To test the functional importance of the interaction between Asp-236c and Lys-143r, we have constructed a mutant version of the enzyme with Asp-236c replaced by alanine.

Without the Asp-236c–Lys-143r Link the Enzyme Is in an R-Like Functional State. An interaction with Lys-143r cannot

Table 2. Kinetic parameters for the wild-type and the mutant catalytic subunits

	Maximal velocity,* mmol·hr^{-1}·mg^{-1}	K_m^{Asp}, mM	K_m^{CP}, mM	K_d for carbamoyl phosphate,† mM	K_i for succinate,‡ mM	K_i for PALA,§ μM
Wild type	23	6.0	0.02	0.03	0.46	0.01
Asp-236c→Ala	14.6	3.4	0.26	0.03	0.17	0.05

Saturation-curve data were gathered at 25°C in 50 mM Tris acetate buffer (pH 8.3). The carbamoyl phosphate concentration was held constant at 4.8 mM for aspartate saturation curves, and aspartate concentration was held constant at 30 mM and 6 mM for wild-type and Asp-236c→Ala catalytic subunits, respectively, for carbamoyl phosphate saturation curves.

*Maximal observed specific activity.

†K_d for carbamoyl phosphate was determined kinetically by the method of Porter *et al.* (31).

‡K_i for succinate was determined kinetically by the method of Porter *et al.* (31).

§K_i for PALA was determined kinetically by the method of Collins and Stark (32).

form with alanine at position 236c. Nevertheless, the Asp-236→Ala enzyme exhibits no reduction in maximal activity; this amino acid substitution eliminates homotropic cooperativity (n_H = 1.0) and significantly reduces the $[S]_{0.5}^{Asp}$, suggesting that the aspartate affinity of the mutant holoenzyme has increased. PALA does not activate the mutant enzyme, although it is inhibited by relatively low concentrations of PALA, providing additional evidence that the mutant enzyme has no residual cooperativity. The properties of the Asp-236c→Ala enzyme are very similar to a modified form of aspartate carbamoyltransferase "locked" in the R state by chemical cross-linking (34).

CTP and ATP do not affect the activity of the Asp-236c→Ala enzyme even at relatively high concentrations of nucleotide. The fact that CTP still binds to the Asp-236c→Ala enzyme indicates that the loss of nucleotide effects is the result of an alteration in the heterotropic mechanism in the mutant enzyme and not the inability of the mutant enzyme to bind the nucleotides. The loss of heterotropic effects is further evidence that the Asp-236c→Ala enzyme is in an R-like functional state. A similar loss of nucleotide effects was also observed for the enzyme cross-linked in the R state, mentioned above (34).

The identical heat stability of the Asp-236c→Ala and wild-type enzymes indicates that the replacement of Asp-236c by alanine does not significantly weaken the overall interface between the catalytic and regulatory chains. However, the altered rates of reaction of *p*-hydroxymercuribenzoate does indicate that the local environment around the regulatory chain sulfhydryl groups has been altered by this amino acid substitution. This is not unexpected because Cys-138r and Cys-141r are coordinated to the zinc, and both are very close to Lys-143r, the residue that interacts with Asp-236c.

Preliminary experiments using analytical gel chromatography indicate a decrease in the chromatographic partition coefficient of the unligated Asp-236c→Ala enzyme compared to unligated wild-type enzyme, although the decrease in the partition coefficient is not as great as is observed upon the binding of PALA to the wild-type enzyme (D. S. Burz and N. H. Allewell, unpublished observations). These results indicate that the Asp-236c→Ala enzyme is not in the T structural state.

The kinetic and structural properties of the Asp-236→Ala enzyme are very similar to the properties of another enzyme with a mutation in the 240s loop, Glu-239→Gln. In the T state of the wild-type enzyme, Glu-239c of one catalytic chain (C1) interacts with both Lys-164c and Tyr-165c of the opposite catalytic chain (C4). In the R state, these interactions are lost, and a new set of interactions are formed between Glu-239c (C1) and Lys-164c and Tyr-165c of the same catalytic chain (C1). The Glu-239→Gln holoenzyme exhibits a reduced $[S]_{0.5}^{Asp}$, no homotropic cooperativity, and a loss of heterotropic interactions (35). Low angle x-ray scattering (P. Vachette and E.R.K., unpublished observations) as well as x-ray crystallographic (20) experiments on the Glu-239→Gln enzyme indicate that the unligated enzyme is no longer in the T structural state. Furthermore, carbamoyl phosphate, which is saturating in all kinetic experiments, is sufficient to convert the Glu-239→Gln enzyme into the R structural state, where the interaction between Asp-236c and Lys-143r cannot form (P. Vachette and E.R.K., unpublished observations). Because the Glu-239→Gln and Asp-236→Ala enzymes have nearly identical kinetic properties, loss of either the C1–C4 intersubunit interactions involving Glu-239c or the C1–R4 intersubunit interactions involving Asp-236c is sufficient to destabilize the T-state position of the 240s loop, which, in turn, results in an alteration in quaternary structure. The intersubunit contacts involving Lys-143r and Asp-236c are even more complex than mentioned above (10–12, 19), although these other interactions are more tenuous in the x-ray data. For example, Lys-143r interacts with Ser-131c and Glu-109c in one half of the asymmetric unit but not in the other half. However, in the other half of the asymmetric unit there is an interaction between Lys-143r and Asn-111r. In a similar manner, Asp-236c (C1) interacts with Ser-131c (C4) in half of the asymmetric unit but not the other. The loss of this possible C1–C4 interaction may in itself destabilize the T state.

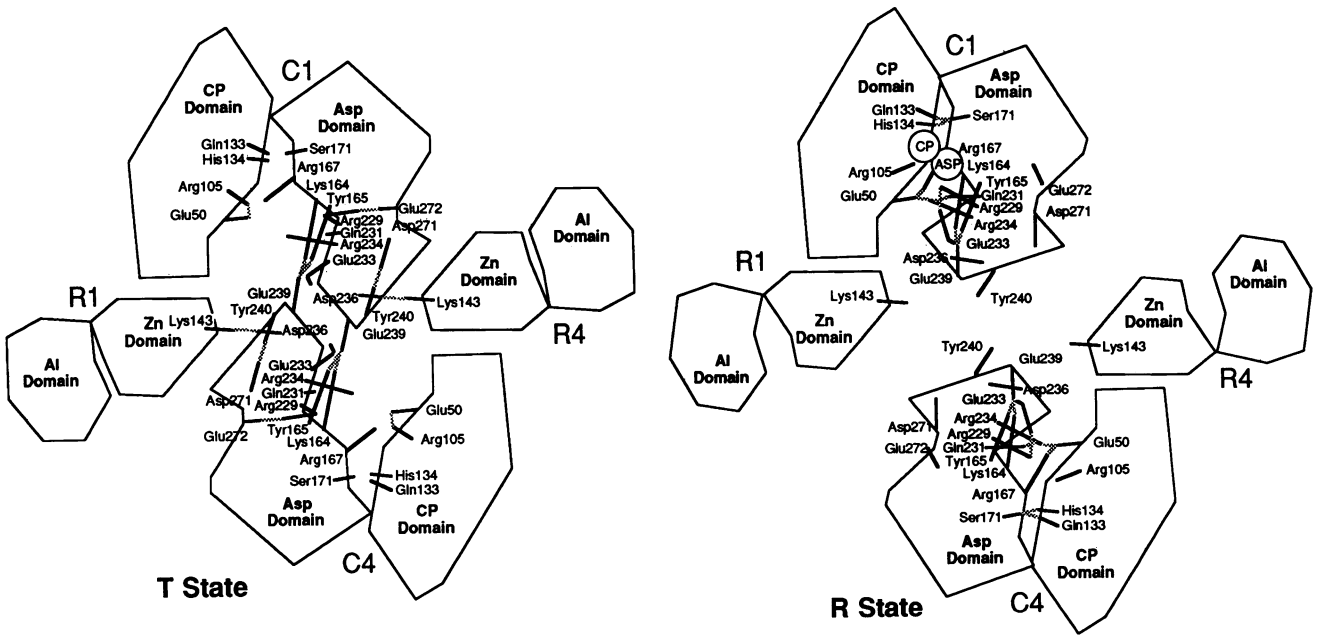

FIG. 4. The results of this study allow a new interaction to be added to those already proposed to stabilize the T and R states of aspartate carbamoyl-transferase (12, 14, 38). In the T state (*Left*), the link between Lys-143r and Asp-236c helps stabilize the 240s loop in its domain open conformation. In the R state (*Right*), the enzyme has undergone the allosteric transition, the domains of the catalytic chain are closed, and the link between Lys-143r and Asp-236c is lost as the holoenzyme elongates along the 3-fold axis. All interactions shown are seen in the x-ray structures and have also been implicated in the enzyme function by site-specific mutagenesis experiments.

Previous results reveal yet another intersubunit interaction that stabilizes the position of the 240s loop. Eisenstein *et al.* (36) have reported that the Asn-111r→Ala enzyme exhibits a complete loss of homotropic and heterotropic properties, and these authors have speculated about the importance of the C1–R4 interface for stabilization of the T state. However, they also suggest that loop movements are unnecessary to explain their results. These authors have overlooked the fact that Asn-111r interacts directly with the 240s loop in the region of the protein near the Asp-236c–Lys-143r interaction (11). The destabilization of the position of the 240s loop by the replacement of Asn-111r by alanine could equally well explain their observations.

The Glu-233c to Lys-84c Link Is Partially Responsible for the T State Having Low Activity and Low Affinity. Analysis of the structure of the phosphonoacetamide–enzyme complex also reveals a possible interaction between Glu-233c of the 240s loop and Lys-84c of an adjacent catalytic chain in the same subunit. If this interaction really occurs under physiological conditions, it may serve to keep both of these residues out of the active site in the T state. Analysis of site-specific mutations at Glu-233c (18) and Lys-84c (37) reveals that both of these residues are critical for activity. In the R state, Glu-233c forms a salt link with Arg-229c that helps to position Arg-229c to interact with the β-carboxylate of aspartate, whereas Lys-84c moves into the active site and interacts with a phosphate oxygen of carbamoyl phosphate as well as the α- and β-carboxylates of aspartate. (The interactions with aspartate are based upon analogy with the structures of the enzyme with either PALA or carbamoyl phosphate and succinate bound.) Site-specific mutagenesis experiments suggest that the link between Glu-233c (C1) and Lys-84c (C2) would not only help to stabilize the T state but would be partially responsible for the low affinity and low activity of the T state.

The 240s Loop Is Important for Heterotropic as Well as Homotropic Interactions. The 8-fold decrease in $[S]_{0.5}^{Asp}$, the complete loss of homotropic cooperativity, and elimination of heterotropic interactions suggest that the Asp-236c–Lys-143r interaction between the 240s loop of the catalytic chain and the regulatory chain may be functioning to both transmit the heterotropic signals between the subunits and to influence directly homotropic cooperativity. How is this accomplished? The position of the 240s loop is a critical factor in determining the allosteric state of aspartate carbamoyltransferase. In the T state the 240s loop is orientated so as to prevent formation of the high-activity high-affinity aspartate binding site by restricting the positions of specific side chains like Lys-84c, Arg-105c, Arg-229c, and Gln-231c (see Fig. 4). Thus, the T state has low activity and low affinity. For the concerted allosteric transition to take place, the 240s loop reorients as the two domains of the catalytic chain close together, allowing Lys-84c to move into the active site and allowing Glu-233c and Arg-234c to position Arg-229c and Glu-231c, respectively, to interact with aspartate, thus creating the high-affinity high-activity aspartate binding site (see Fig. 4) (14, 18, 33, 35). The decrease in the $[S]_{0.5}^{Asp}$ of the Asp-236→Ala enzyme can best be explained by a reorientation of the 240s loop allowing residues like Glu-233 and Arg-234 to position active-site residues such as Arg-229 and Gln-231 correctly for the binding of aspartate. Support for this conclusion comes from the analytical gel chromatography experiments that indicate that the Asp-236→Ala holoenzyme has undergone a structural change toward the R state. These results, in conjunction with previous experiments, suggest that the intersubunit link involving Asp-236c is involved in the stabilization of the 240s loop in its T-state position and that the regulatory subunits exert their effect on the catalytic subunits by influencing the position of the 240s loop and provide an explanation for cooperativity in the holoenzyme and lack of cooperativity in the catalytic subunit of aspartate carbamoyltransferase.

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