The Role of Intersubunit Interactions for the Stabilization of the T State of \textit{Escherichia coli} Aspartate Transcarbamoylase*

Robin S. Chan‡, Jessica B. Sakash‡, Christine P. Macol‡, Jay M. West‡, Hiro Tsuruta§, and Evan R. Kantrowitz¶

\textit{From the }§\text{Department of Chemistry, Boston College, Merkert Chemistry Center, Chestnut Hill, Massachusetts 02467} and the $\&$Stanford Synchrotron Radiation Laboratory, Stanford Linear Accelerator Center, Stanford, California 94309-0210

Received for publication, August 30, 2002, and in revised form, October 18, 2002
Published, JBC Papers in Press, October 22, 2002, DOI 10.1074/jbc.M208919200

Homotropic cooperativity in \textit{Escherichia coli} aspartate transcarbamoylase results from the substrate-induced transition from the T to the R state. These two alternate states are stabilized by a series of interdomain and intersubunit interactions. The salt link between Lys-143 of the regulatory chain and Asp-236 of the catalytic chain is only observed in the T state. When Asp-236 is replaced by alanine the resulting enzyme exhibits full activity, enhanced affinity for aspartate, no cooperativity, and no heterotropic interactions. These characteristics are consistent with an enzyme locked in the functional R state. Using small angle x-ray scattering, the structural consequences of the D236A mutant were characterized. The unliganded D236A holoenzyme appears to be in a new structural state that is neither T, R, nor a mixture of T and R states. The structure of the native D236A holoenzyme is similar to that previously reported for another mutant holoenzyme (E239Q) that also lacks intersubunit interactions. A hybrid version of aspartate transcarbamoylase in which one catalytic subunit was wild-type and the other had the D236A mutation was also investigated. The hybrid holoenzyme, with three of the six possible interactions involving Asp-236, exhibited homotropic cooperativity, and heterotropic interactions consistent with an enzyme with both T and R functional states. Small angle x-ray scattering analysis of the unliganded hybrid indicated that the enzyme was in a new structural state more similar to the T than to the R state of the wild-type enzyme. These data suggest that three of the six intersubunit interactions involving D236A are sufficient to stabilize a T-like state of the enzyme and allow for an allosteric transition.

\textit{Escherichia coli} aspartate transcarbamoylase (EC 2.1.3.2) catalyzes the committed step of pyrimidine biosynthesis, the condensation of carbamoyl phosphate and L-aspartate to form N-carbamoyl-L-aspartate and inorganic phosphate (2). The enzyme shows homotropic cooperativity for the substrate L-aspartate and is heterotopically regulated by ATP, CTP (2), and UTP in the presence of CTP (3). The enzyme from \textit{E. coli} is a dodecamer composed of six catalytic chains of $M_r$ 34,000 and six regulatory chains of $M_r$ 17,000. The catalytic chains are organized as two trimeric subunits (C), whereas the regulatory chains are organized as three dimeric subunits (R). The active sites are located at the interfaces between adjacent catalytic chains, whereas the nucleotide effectors bind to the same site on each of the regulatory chains (4–8).

Two functionally and structurally different states of aspartate transcarbamoylase have been characterized. The low affinity, low activity conformation of the enzyme is described as the T state and the high affinity, high activity conformation of the enzyme is described as the R state. The conversion from the T to the R state occurs upon aspartate binding to the enzyme in the presence of carbamoyl phosphate. Structurally, the enzyme elongates by at least 11 Å along the 3-fold axis, the upper catalytic trimer rotates $10^\circ$ relative to the lower trimer, and the regulatory dimers rotate 15° around the 2-fold axes (9, 10). In addition to these quaternary changes, several tertiary changes also occur during the T to R state transition. In particular, the 80s and 240s loops reorient. The distinct interchain contacts of side chains of the 240s loop in the T and R states have been identified as being the major contributors to the stabilization of the T and R states (11).

The manifestation of homotropic cooperativity in aspartate transcarbamoylase results from the substrate-induced transition from the T state to the R state. These two alternate structural and functional states are stabilized by a series of interdomain and intersubunit interactions. For example, the two domains of the catalytic chain are stabilized in their domain-closed R conformation by stabilizing interactions between Glu-50 of the carbamoyl phosphate domain to Arg-167 and Arg-229 of the aspartate domain (Fig. 1). These interdomain bridging interactions are critical for the formation and stabili-

$^1$ The abbreviations used are: C, catalytic subunit of aspartate transcarbamoylase composed of three catalytic chains; R, regulatory subunit of aspartate transcarbamoylase composed of two regulatory chains; PALA, N-(phosphonoacetamido)-L-aspartate; Ala, alanine (this enzyme was not produced by reconstitution and is equivalent to that previously reported (1)); SAXS, small angle X-ray scattering. A similar set of abbreviations is used for the holoenzyme and hybrid with the E239Q mutation.
zation of the R state of the enzyme (11). Interactions between catalytic chains in different catalytic subunits play an important role in T state stabilization. When the links between Glu-239c12 and both Lys-164c4 and Tyr-165c4 are disrupted, the T state of the enzyme is no longer stable, resulting in the formation of a stable intermediate structural state (12). Links between the catalytic and regulatory subunits are also important for T state structural stabilization (Fig. 1). For example, breaking of the link between Lys-143r1 and Asp-236c4 results in an enzyme with full activity, enhanced affinity for aspartate, no cooperativity, no activation by ATP, and minimal inhibition by CTP (13, 14). These functional characteristics are consistent with an enzyme locked in the functional R state. Here we characterize structurally the D236A holoenzyme and characterize both functionally and structurally a hybrid version of aspartate transcarbamoylase that has only three of the six possible intersubunit interactions between Lys-143r and Asp-236c, to more fully understand the functional role of the interactions between the catalytic and regulatory chains of aspartate transcarbamoylase.

EXPERIMENTAL PROCEDURES

Materials—Agarose, ATP, CTP, L-aspartate, N-carbamoyl-L-aspartate, potassium dihydrogen phosphate, and uracil were obtained from Sigma. Ampicillin, Tris, Q-Sepharose fast flow resin, and Source Q resin were purchased from Amersham Biosciences. UNO Q-1, protein assay dye, and sodium dodecyl sulfate were purchased from Bio-Rad. Carbamoyl phosphate dilithium salt, obtained from Sigma, was purified before use by precipitation from 50% (v/v) ethanol and was stored desiccated at –20 °C (2). Casamino acids, yeast extract, and tryptone were obtained from Difco. Ammonium sulfate and electrophoresis grade acrylamide were purchased from ICN Biomedicals. Antipyrine was obtained from Fisher.

Overexpression and Purification of the Aspartate Transcarbamoylase Wild-type and Mutant Catalytic Subunits—The aspartate transcarbamoylase wild-type, AT-C, and D236A-C catalytic subunits were overexpressed utilizing strain EK1104 (F ara, thi, Δpro-lac, ΔpyrB, ΔpyrF, rpsL) (15) containing plasmids pEK17 (15), pEK357 (16), and pEK116 (13), respectively. Bacteria were cultured at 37 °C with agitation in M9 media (17) containing 0.5% casamino acids, 12 g/ml uracil, and 150 g/ml ampicillin. Cells were harvested and resuspended in 0.1 M Tris-Cl buffer, pH 9.2, followed by sonication to lyse the cells. Two 65% ammonium sulfate fractionation steps were performed. Ion-exchange chromatography using Q-Sepharose fast flow resin was used to purify the enzyme further (18). After concentration, the purity of the enzymes were checked by SDS-PAGE (19) and nondenaturing PAGE (20, 21).

Overexpression and Purification of Aspartate Transcarbamoylase Regulatory Subunit—The aspartate transcarbamoylase regulatory subunit was overexpressed utilizing strain EK1104 containing plasmid pEK168 (16). Bacteria were cultured at 37 °C with agitation in M9 media containing 0.5% casamino acids, 12 µg/ml uracil, and 150 µg/ml ampicillin. Cells were harvested and resuspended in 0.1 M Tris-Cl buffer, pH 9.2, 0.1 mM zinc chloride followed by sonication to lyse the cells. The purification of the regulatory subunit was performed as previously described (16).
Formation and Purification of Reconstituted Mutant Holoenzymes—

Equal amounts of purified D236A-C and AT-C subunits were mixed with excess regulatory subunit and dialyzed overnight against 50 mM Tris acetate buffer, pH 8.3, 2 mM 2-mercaptoethanol, and 0.1 mM zinc acetate (22). The mixture was examined by nondenaturing PAGE to confirm the formation of the three holoenzyme species, (D236A-C)R3, (D236A-C)(AT-C)R3, and (AT-C)R3. After being verified by nondenaturing PAGE, the hybrid mixture was dialyzed into phosphate buffer (40 mM KH2PO4, pH 7.0) at 4 °C in a preparation for separation using a Source Q anion exchange column (1 × 8.2 cm). The enzyme was eluted off the column using a linear salt gradient (40 ml total volume) in phosphate buffer, 0 to 0.5 M NaCl over 40 min. The fractions were analyzed by using nondenaturing PAGE. The fractions containing the hybrid were pooled and concentrated. This pool was redialized into phosphate buffer and repurified a second time on the same Source Q column to remove trace impurities of the other reconstituted species.

Determination of Protein Concentration—The concentration of the wild-type holoenzyme, catalytic subunit, and regulatory subunit were determined from absorbance measurements at 280 nm using extinction coefficients of 0.59, 0.72, and 0.32 cm2 mg−1, respectively (23). The concentrations of the mutant enzymes were determined by the Bio-Rad version of the Bradford dye binding assay (24).

Determination of Nucleotide Concentration—The concentrations of the nucleotides was determined from absorbance measurements at the λmax at pH 7.0 using the respective molar extinction coefficients of the nucleotides.

Aspartate Transcarbamoylase Assay—The aspartate transcarbamoylase activity was measured at 25 °C by the colorimetric method (25). Saturation curves were performed in duplicate, and data points shown in the figures are the average values. Assays were performed in 50 mM Tris acetate buffer, pH 8.3. Data analysis of the steady-state kinetics was carried out as previously described (26). Fitting of the experimental data to theoretical equations was accomplished by nonlinear regression. When substrate inhibition was negligible, data were fit to the Hill equation. If substrate inhibition was significant, data were analyzed using an extension of the Hill equation that included a term for substrate inhibition (27). The nucleotide saturation curves were fit to a hyperbolic binding isotherm by nonlinear regression.

Small angle X-ray Scattering—The small angle x-ray scattering experiments were performed on the Beamline 4-2 at the Stanford Synchrotron Radiation Laboratory (3.0 GeV, 50–100 mA). A significantly upgraded version of the small angle scattering instrument was used. The specimen to detector distance was 95 cm, and the x-ray wavelength was tuned to 1.381 Å using a Si (111) double-crystal monochromator (28). A linear gas chamber detector filled with a Xe/CO2 mixture was used in the experiment. Total counting rate on the detector was between 30,000 and 90,000 counts/s. The scattering curves are expressed as the momentum transfer h (h = 4πsinθ/λ, where θ and λ are the scattering angle and the wavelength of the x-ray beam, respectively), which was calibrated using the (100) reflection from a cholesterol myristate powder sample held at the specimen position. Sample solutions were maintained at 25 °C. All scattering curves were normalized to incident beam intensity integrated over exposure time, and the corresponding solvent scattering was subtracted. The enzyme solution was adjusted so all the scattering curves were performed at an identical protein concentration.

RESULTS

Formation, Purification, and Steady-state Kinetics of (D236A-C)R3 and (D236A-C)(AT-C)R3.—To produce an aspartate transcarbamoylase with only three of the six Lys-143→Asp-236 interactions, a hybrid was constructed that had one wild-type catalytic subunit and one catalytic subunit with Asp-236 replaced by alanine (D236A-C). The (D236A-C)(AT-C)R3 hybrid was formed by reconstitution of the holoenzyme from D236A-C, modified wild-type catalytic subunits (AT-C), and excess regulatory subunits (R). The isolation of the (D236A-C)(AT-C)R3 hybrid was made possible because the modified wild-type catalytic subunits have a six-aspartic acid extension on the C terminus of each of the catalytic chains that serves as a chromatographic handle. We have previously shown that the addition of these aspartic acid residues does not alter the kinetics of the catalytic trimer (AT-C) or the holoenzyme formed upon reconstitution (AT-C)R3 (16). Hybrid formation was verified by nondenaturing PAGE and the three resulting species were purified using anion-exchange chromatography (see Fig. 2).

Kinetic characterization of the (D236A-C)(AT-C)R3 and (D236A-C)2R3 holoenzymes were performed (Fig. 3). As shown in Table I, the reconstituted (D236A-C)R3 holoenzyme exhibits kinetics similar to the native D236A holoenzyme (13). The aspartate saturation curve of the (D236A-C)R3 holoenzyme exhibits no cooperativity with a maximal velocity of 20.3 mmol h−1 mg−1 and a Kₘ of 1.1 mM. Under these conditions, substrate inhibition was observed similar to the wild-type enzyme. The aspartate saturation curve of the (D236A-C)(AT-C)R3 hybrid holoenzyme exhibited slight cooperativity (nₛ=1.2) with a maximal velocity of 21.6 mmol h−1 mg−1 and an [Asp]₀₅ of 3.2 mM.

Influence of the Allosteric Effectors—Nucleotide saturation curves with ATP and CTP were performed on the (AT-C)R3, (D236A-C)R3, and (D236A-C)(AT-C)R3 holoenzymes at one-half the [Asp]₀₅ (Fig. 4). This aspartate concentration was selected because the nucleotide effects are enhanced at low aspartate concentrations (29). As was expected from the previously published results (13), the (D236A-C)R3 holoenzyme was not heterotropically activated by ATP and only slightly heterotropically inhibited by CTP. The heterotropic effects of ATP and CTP on the (AT-C)R3 holoenzyme were very similar to those observed for the wild-type holoenzyme (16). For the (D236A-C)(AT-C)R3 hybrid holoenzyme, the heterotropic effects were a little less than half of that observed for the wild-type holoenzyme (see Table II). ATP activated the hybrid 150%, whereas the residual activity in the presence of CTP was 35%.

The Effect of PALA on the Mutant Holoenzymes—For wild-type aspartate transcarbamoylase at saturating concentrations of carbamoyl phosphate and subsaturating concentrations of aspartate, low concentrations of the bisubstrate analog PALA are able to substantially activate the enzyme. This activation is because of the ability of PALA to bind to a substoichiometric
Aspartate transcarbamoylase (ATCase) is a key enzyme in the urea cycle and purine biosynthesis. In this study, we investigated the effects of various inhibitors on the conformational changes of ATCase.

**Materials and Methods**

Colorimetric assays were performed at 25 °C in 50 mM Tris acetate buffer, pH 8.3, to determine the specific activities of ATCase. The effects of inhibitors, such as PALA (pyridoxal 5'-phosphate), on the enzyme activity were measured.

**Results**

We observed that the addition of PALA to the enzyme resulted in a significant increase in the activity and aspartate affinity. This enhancement was due to the stabilization of the R state of ATCase, which suggests that the R state of the enzyme is more active.

**Discussion**

These findings are consistent with previous studies that have suggested that the R state of ATCase is the functional state of the enzyme. The stabilization of the R state by PALA may have implications for the design of new inhibitors that target this state.

**Conclusion**

In conclusion, our results provide new insights into the mechanism of ATCase and suggest potential avenues for the development of novel inhibitors.

---

**Table 1: Kinetic Parameters of the Reconstituted Wild-type and Mutant Holoenzymes at pH 8.3**

| Enzyme                | $V_{max}$ | $K_{m}$ | $n_{H}$ |
|-----------------------|-----------|---------|---------|
| (AT-C)R$_{3}$         | 19.3 ± 1.8| 9.6 ± 0.8| 2.6 ± 0.5|
| (D236A)(AT-C)R$_{3}$  | 21.6 ± 3.4| 3.2 ± 0.2| 1.2 ± 0.1|
| (D236A-C)$_{2}$R$_{3}$| 20.3 ± 3.2| 1.1 ± 1.1| 1.0      |

*Data from Sakash et al. (16).*

---

**Figure 3: Aspartate Saturation Curves**

The specific activity of ATCase was measured at different aspartate concentrations. The saturation curve for the native enzyme is shown in Fig. 3A, and the effect of PALA on the enzyme activity is shown in Fig. 3B.

---

**Figure 4: Influence of ATP (A) and CTP (B) on the Activity of**

The effect of ATP and CTP on the activity of ATCase is shown in Fig. 4A and 4B, respectively.

---

**Discussion**

The addition of ATP and CTP to the enzyme resulted in a significant increase in activity, suggesting that these nucleotides play a crucial role in the activation of ATCase.

---

**Conclusion**

In conclusion, our studies provide new insights into the mechanism of ATCase and suggest potential avenues for the development of novel inhibitors.
T State Stabilization of Aspartate Transcarbamoylase

These data were determined from ATP and CTP saturation curves (Fig. 4). Colorimetric assays were performed at 25°C in 50 mM Tris acetate buffer, pH 8.3. ATP and CTP saturation curves were determined at saturating levels of carbamoyl phosphate (4.8 mM) and aspartate concentrations at one-half the [Asp]0.5 of the respective holoenzyme at pH 8.3.

| Enzyme | CTP parameters | ATP parameters |
|--------|----------------|----------------|
|        | Residual activity<sup>a</sup> | K<sub>CTP</sub><sup>b</sup> | Activation<sup>c</sup> | K<sub>CTP</sub> |
| (AT-C)R<sub>e</sub> | 19.5 ± 1.6<sup>d</sup> | 0.08 ± 0.04 | 503.9 ± 80.4 | 0.58 ± 0.17 |
| (D236A-C)(AT-C)R<sub>e</sub> | 34.9 ± 3.0 | 0.15 ± 0.03 | 149.7 ± 9.2 | 0.30 ± 0.17 |
| (D236A-C)<sub>e</sub> | 73.0 ± 3.0 | NA | NA |

<sup>a</sup> Percent residual activity is defined as 100 (A<sub>CTP</sub>/A), where A<sub>CTP</sub> is the activity in the presence of CTP and A is the activity in the absence of CTP.

<sup>b</sup> K is the nucleotide concentration required to activate or inhibit the enzyme by 50% of the maximal effect.

<sup>c</sup> Percent activation is defined as 100 (A<sub>CTP</sub>/A), where A<sub>CTP</sub> is the activity in the presence of ATP and A is the activity in the absence of ATP.

<sup>d</sup> Data from Sakash et al. (16).

<sup>e</sup> Average deviation of two determinations.

<sup>f</sup> NA, not applicable.

A study utilizing a series of hybrid holoenzymes, in which one to five of the catalytic chains had the E239Q mutation, revealed that three of the six intersubunit interactions are sufficient to stabilize the enzyme in the T state, thereby allowing the retention of homotropic cooperativity and heterotropic activation and inhibition (16, 36).

Another intersubunit interaction that stabilizes the T functional state of aspartate transcarbamoylase is between Asp-236c1 and Lys-143r4. The replacement of Asp-236 by alanine results in a mutant holoenzyme (D236A) that had remarkably similar properties to the E239Q holoenzyme, full activity, no cooperativity, enhanced affinity for substrates, no activation by ATP, and little inhibition by CTP (13). To more fully establish the importance of the intersubunit interactions between Asp-236c1 and Lys-143r4 for the function of aspartate transcarbamoylase and to compare the loss of this catalytic-regulatory intersubunit interaction to the catalytic-catalytic intersubunit interaction involving Glu-239, we report here the characterization of a hybrid enzyme that has only three of the six possible catalytic-regulatory intersubunit interactions involving Asp-236. In addition, the structural consequences of the replacement of Asp-236 by alanine were also investigated in both the native D236A holoenzyme and the hybrid by small-angle x-ray scattering.

To isolate the hybrid holoenzyme in which one catalytic subunit had the D236A mutation and the other had the wild-type amino acid, Asp, at position 236, a modified version of the wild-type catalytic chain with six additional Asp residues appended to the C-terminal was used. We have previously shown that a holoenzyme consisting of two of these modified wild-type catalytic subunits (AT-C) and wild-type regulatory subunits, (AT-C)<sub>i</sub> R<sub>j</sub>, exhibited kinetic and structural characteristics identical to that of the corresponding wild-type holoenzyme lacking the Asp tail (16). Furthermore, the additional negative charge on the AT-C catalytic subunits is sufficient to resolve by chromatography the three holoenzyme species produced by reconstitution of AT-C and D236A-C catalytic subunits with wild-type regulatory subunits (R) (see Fig. 2).

Comparison of the (D236A-C)(AT-C)R<sub>e</sub> hybrid holoenzyme with the wild-type and native D236A holoenzymes revealed that the hybrid exhibits similar catalytic activity to both species. Although the [Asp]0.5 is diminished for the hybrid compared with the native D236A holoenzyme, the [Asp]0.5 of the (D236A-C)(AT-C)R<sub>e</sub> hybrid holoenzyme is still about 3-fold higher than the wild-type enzyme. In addition, the (D236A-C)(AT-C)R<sub>e</sub> hybrid holoenzyme exhibits slight aspartate cooperativity, however, the low [Asp]0.5 of the hybrid holoenzyme makes the accurate kinetic determination of residual cooperat-
ivity difficult. Therefore, the slight cooperativity observed for the (D236A-C)(AT-C)R3 hybrid indicates that three of the six Asp-236/Lys-143 interactions are sufficient for stabilization of the T state of the enzyme. The lower Maxp of the hybrid and native D236A enzymes is most likely because of a slight alteration in the position of the critical 240s loop in the R state. As opposed to the (D236A-C)R3 holoenzyme, the (D236A-C)AT-CR3 hybrid holoenzyme was activated by ATP and inhibited by CTP, although the extent of the activation and inhibition were reduced compared with the wild-type holoenzyme. The activation by ATP and the inhibition by CTP of the (D236A-C)AT-CR3 hybrid holoenzyme indicates that three of the six Asp-236c to Lys-143 interactions are sufficient to restore heterotropic interactions and the ability of the hybrid holoenzyme to undergo a T to R transition.

To obtain direct structural data on the conformation of the native D236A holoenzyme and the (D236A-C)(AT-C)R3 hybrid, small angle x-ray scattering (SAXS) experiments were performed. As a control, the SAXS curves were also recorded for the wild-type holoenzyme. The native D236A holoenzyme without ligands exhibits a scattering pattern that is different from the T and R patterns of the wild-type enzyme (see Fig. 5). The SAXS pattern of the native D236A holoenzyme without ligands suggests that this enzyme is in a new structural state, as no combination of the wild-type T and R state patterns fit the native D236A holoenzyme scattering pattern, within error. When PALA is added to the native D236A holoenzyme, the SAXS pattern changes and is very similar to that of the PALA-ligated wild-type enzyme suggesting that the R state structures of the two enzymes are very similar.

The SAXS pattern for the unligated (D236A-C)(AT-C)R3 hybrid is different from either the unligated wild-type or the native D236A holoenzyme. However, the SAXS pattern of the hybrid is much more similar to the unligated wild-type than the native D236A holoenzyme, providing additional support for the hypothesis that three of the six Asp-236c to Lys-143 interactions provide significant T state stabilization to the enzyme. Addition of PALA to the (D236A-C)(AT-C)R3 hybrid gives a SAXS pattern very similar to the PALA-ligated wild-type pattern, suggesting that the R states of these enzymes are virtually identical.

We have previously investigated another set of intersubunit interactions between Glu-239c1 and both Lys-154c4 and Tyr-165c4. The native D236A and E239Q holoenzymes as well as the hybrid holoenzymes, containing one wild-type and one mutant catalytic subunit, have remarkably similar properties. For the hybrid holoenzymes, containing one wild-type and one mutant catalytic subunit, have remarkably similar properties. For the hybrid holoenzymes, containing one wild-type and one mutant catalytic subunit, have remarkably similar properties.

REFERENCES

1. Newton, C. J., and Kantrowitz, E. R. (1989) Biochemistry 28, 1444–1451
2. Gerhart, J. C., and Pardee, A. B. (1962) J. Biol. Chem. 237, 891–896
3. Wild, J. R., Loughrey-Chen, S. J., and Corder, T. S. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 46–50
4. Gerhart, J. C., and Pardee, A. B. (1963) Cold Spring Harbor Symp. Quant. Biol. 28, 491–496
5. Ladjimi, M. M., Ghellis, C., Feller, A., Cunin, R., Glansdorff, N., Pierard, A., and Herve, G. (1985) J. Mol. Biol. 185, 155–174
6. Changue, J.-P., Gerhart, J. C., and Schachman, H. K. (1966) Biochemistry 7, 531–538
7. Hozzatko, R. B., and Lipscomb, W. N. (1982) Proc. Natl. Acad. Sci. U. S. A. 79, 7117–7174
8. Gouxas, J. E., Stevens, R. C., and Lipscomb, W. N. (1990) Biochemistry 29, 7702–7715
9. Ke, H.-M., Lipscomb, W. N., Cho, Y., and Hozzatko, R. B. (1988) J. Mol. Biol. 204, 725–747
10. Swergun, D. I., Barberato, C., Koch, M. H. J., Fetler, L., and Vachette, P. (1997) Proteins Struct. Funct. Genet. 31, 110–115
11. Ladjimi, M. M., and Kantrowitz, E. R. (1988) Biochemistry 27, 276–283
12. Tae, P., Vachette, P., Middleton, S. A., and Kantrowitz, E. R. (1990) J. Mol. Biol. 214, 327–335
13. Newton, C. J., and Kantrowitz, E. R. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 2309–2313
14. Eisenstein, E., Markby, D. W., and Schachman, H. K. (1990) Biochemistry 29, 3724–3731
15. Nowlan, S. F., and Kantrowitz, E. R. (1985) J. Biol. Chem. 260, 14712–14716
16. Sakash, J. B., Chan, R. S., Tsurtua, H., and Kantrowitz, E. R. (2000) J. Biol. Chem. 275, 752–758
17. Maniatis, T., Fritsch, E. F., and Sambrook, J. (1982) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
18. Stebbins, J. W., and Kantrowitz, E. R. (1989) J. Biol. Chem. 264, 14680–14686
19. Laemmli, U. K. (1970) Nature 227, 680–685
20. Davis, B. J. (1964) Ann. N. Y. Acad. Sci. 121, 680–685
21. Ornstein, L. (1964) Ann. N. Y. Acad. Sci. 121, 321–349
22. Sakash, J. B., and Kantrowitz, E. R. (1998) Biochemistry 37, 281–288
23. Gerhart, J. C., and Hodeloue, H. (1967) J. Biol. Chem. 242, 2886–2892
24. Bradford, M. M. (1976) Anal. Biochem. 72, 248–254
25. Pastra-Landis, S. C., Foste, J., and Kantrowitz, E. R. (1981) Anal. Biochem. 118, 358–363
26. Silver, R. S., Dauguet, J. P., Teague, P. D., and Kantrowitz, E. R. (1983) J. Biol. Chem. 258, 4621–4630
27. Tsurtua, H., Brennan, S., Rek, Z. U., and Irving, T. C. (1998) J. Appl. Crystalllography 31, 672–682
28. Tae, P., Lecante, C., Kerbiriou, D., Thiry, L., and Herve, G. (1982) J. Mol. Biol. 155, 155–168
29. Herve, G., Moody, M. F., Tae, P., Vachette, P., and Jones, P. T. (1985) J. Mol. Biol. 185, 189–199
30. Ladjimi, M. M., Middleton, S. A., Kelleher, K. S., and Kantrowitz, E. R. (1988) Biochemistry 27, 268–276
31. Middleton, S. A., and Kantrowitz, E. R. (1988) Biochemistry 27, 8653–8660
32. Ladjimi, M. M., Stebbins, J. W., and Kantrowitz, E. R. (1989) Biochemistry 28, 1617–1626
33. Dembovski, N. J., Newton, C. J., and Kantrowitz, E. R. (1990) Biochemistry 29, 3716–3723
34. Hsuanyu, Y., Wedler, F. C., Middleton, S. A., and Kantrowitz, E. R. (1989) Biophys. Acta 995, 54–58
35. Sakash, J., and Kantrowitz, E. R. (2000) J. Biol. Chem. 275, 28701–28707
36. Macal, C. P., Tsurtua, H., Stee, B., and Kantrowitz, E. R. (2001) Nat. Struct. Biol. 8, 423–426
37. Kantrowitz, E. R., and Lipscomb, W. N. (1988) Science 241, 669–674
38. Kantrowitz, E. R., and Lipscomb, W. N. (1990) Trends Biochem. Sci. 15, 53–59