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Biochemistry

A 70-amino acid zinc-binding polypeptide from the regulatory chain of aspartate transcarbamoylase forms a stable complex with the catalytic subunit leading to markedly altered enzyme activity

(subunit interaction/allostery/conformational change/domain)

DAVID W. MARKBY*, BIN-BING ZHOU, AND H. K. SCHACHMAN†

Department of Molecular and Cell Biology and Virus Laboratory, Wendell M. Stanley Hall, University of California, Berkeley, CA 94720

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ABSTRACT **In an effort to clarify effects of specific protein–protein interactions on the properties of the dodecameric enzyme aspartate transcarbamoylase (carbamoyl-phosphate:L-aspartate carbamoyltransferase, EC 2.1.3.2), we initiated studies of a simpler complex containing an intact catalytic trimer and three copies of a fragment from the regulatory chain. The partial regulatory chain was expressed as a soluble 9-kDa zinc-binding polypeptide comprising 11 amino acids encoded by the polylinker of pUC18 fused to the amino terminus of residues 84–153 of the regulatory chain; this polypeptide includes the zinc domain detected in crystallographic studies of the holoenzyme. In contrast to intact regulatory chains, the zinc-binding polypeptide is monomeric in solution because it lacks the second domain responsible for dimer formation and assembly of the dodecameric holoenzyme. The isolated 9-kDa protein forms a tight, zinc-dependent complex with catalytic trimer, as shown by the large shift in electrophoretic mobility of the trimer in nondenaturing polyacrylamide gels. Enzyme assays of the complex showed a hyperbolic dependence of initial velocity on aspartate concentration with V_{max} and K_m for aspartate ≈50% lower than the values for free catalytic subunit. A mutant catalytic subunit containing the Lys-164 → Glu substitution exhibited a striking increase in enzyme activity at low aspartate concentrations upon interaction with the zinc domain because of a large reduction in K_m upon complex formation. These changes in functional properties indicate that the complex of the zinc domain and catalytic trimer is an analog of the high-affinity *R* ("relaxed") state of aspartate transcarbamoylase, as proposed previously for a transiently formed assembly intermediate composed of one catalytic and three regulatory subunits. Conformational changes at the active sites, resulting from binding the zinc-containing polypeptide chains, were detected by difference spectroscopy with trinitrophenylated catalytic trimers. Isolation of the zinc domain of aspartate transcarbamoylase provides a model protein for study of oligomer assembly, communication between dissimilar polypeptides, and metal-binding motifs in proteins.**

The assembly of oligomeric proteins is a multistep process including folding of individual polypeptides, formation of structural and functional domains within chains, and association of folded chains into multimeric proteins. For the regulatory enzyme, aspartate transcarbamoylase (ATCase; carbamoyl-phosphate:L-aspartate carbamoyltransferase, EC 2.1.3.2) from *Escherichia coli*, the process is particularly complex because the enzyme is composed of six catalytic (c) polypeptide chains organized as two catalytic (C) trimeric subunits and six regulatory (r) polypeptide chains organized as three regulatory (R) dimeric subunits (1–6). Hence, assembly of the holoenzyme, designated as C_2R_3 or c_6r_6, involves independent folding of r and c chains, their self-association to form R dimers and C trimers, respectively, followed by a series of mixed-association reactions to yield intact ATCase (7). In effect, the three R dimers serve as "cross-links" between the two C trimers, producing an allosteric enzyme that exhibits cooperativity with regard to both substrates, carbamoylphosphate and aspartate, as well as inhibition by CTP and activation by ATP (8). These allosteric properties (9) are attributable to a ligand-promoted global conformational change in the enzyme from a compact, low-activity T ("taut") state to a swollen, more active R ("relaxed") state (10–12). Accompanying this alteration in quaternary structure are changes in interchain interactions (13) as well as structural and functional changes in various polypeptide chains (14–17). Because of the multiplicity, strength, and interdependence of the protein–protein contacts in ATCase, which collectively yield a stable enzyme capable of existing in two alternative forms, it has been difficult to determine effects of individual interchain interactions on the conformations and functions of adjoining chains. This difficulty could be circumvented, in part, by studying a stable complex containing only one C trimer and bound r chains. As shown here, a zinc-binding polypeptide chain of 70 amino acids from the carboxyl-terminal region of the r chain interacts with C trimer to form a stable complex having strikingly altered enzyme activity.

A complex containing one C trimer and three R dimers has been described (18–22) in studies of the assembly of ATCase from C trimers and R dimers. This complex, CR_3 or c_3r_6, which is formed rapidly in mixtures containing low concentration of C trimers and a large excess of R dimers, was detected by changes in enzyme activity, by hybridization techniques, and by sedimentation velocity experiments (19, 20). The enzymatic properties of CR_3 differ from those of both free C trimer and intact ATCase (20). Like free subunit but unlike holoenzyme, CR_3 exhibits Michaelis–Menten kinetics and is insensitive to regulation by nucleotide effectors. However, K_m of CR_3 for aspartate is ≈25% that of C trimer, and V_{max} of CR_3 is ≈50% that of free C trimer. These properties led to the suggestion that CR_3 is an analog of the high-affinity R state of ATCase (20). Because CR_3 is formed only at extremely low concentrations of C trimer with a large excess of R dimers and is only marginally stable, only limited experiments can be conducted with it.

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Abbreviations: ATCase, aspartate transcarbamoylase; c, catalytic polypeptide chain; C, catalytic subunit; r, regulatory polypeptide chain; R, regulatory subunit; PALA, *N*-phosphonacetyl-L-aspartate; TNBS, 2,4,6-trinitrobenzenesulfonate; C_{TNP}, trinitrophenylated C subunit.

*Present address: Department of Pharmacology, University of California, San Francisco, CA 94143-0450.
†To whom reprint requests should be addressed.

Structural considerations suggest an alternative approach using modified r chains to examine c–r interactions. Each r chain is composed of two structural domains (23), the amino-terminal allosteric domain that binds nucleotide effectors and the carboxyl-terminal zinc domain that binds a structural zinc ion required for assembly of holoenzyme (6, 24). The interface between adjacent c and r chains involves only side chains from the zinc domain of the r chain, whereas interchain interactions within the R dimer are confined to the allosteric domain (16, 17, 23). Hence, two different approaches appear feasible for forming a stable complex containing only one C trimer and bound r chains (or partial chains). Modifications could be made in intact r chains that prevent dimer formation but do not disturb the zinc domain. Alternatively, the r chain could be cut either proteolytically or by genetic manipulation to permit isolation of the zinc domain.

In the present work, zinc domain of the r chain was expressed as a separate protein using recombinant DNA techniques. Based on the high affinity of r chain for zinc and the ability to reconstitute ATCase (1), we anticipated that isolated zinc domain would associate with C trimer to form a stable complex. Analysis of association of the zinc domain with C trimer permits examination of the affinity and cooperativity of c–r interactions along with conformational and functional changes in the individual proteins.

EXPERIMENTAL PROCEDURES

Construction of Plasmids for Expression of Zinc Domain. A zinc-containing polypeptide fragment was expressed as a fusion protein containing 11 amino acids encoded by the polylinker of pUC18 (25) followed by residues 84–153 of the r chain. In initial experiments, zinc domain was expressed under control of the *lac* promoter by cloning the *Hin*cII–*Sph* I fragment of pPYRB11 (26) into the *Sma* I–*Sph* I sites of pUC18 to generate pUC18-Zn2, which was transformed into *E. coli* strain HB101-4442 (27). In subsequent experiments, a T7 RNA polymerase-based expression system was used to produce large amounts of protein. (*i*) Part of *pyrI* formed by digestion of pPYRB3 (28) with *Sal* I and *Hin*cII was cloned into the *Sma* I/*Sal* I sites of a modified M13mp18 vector containing an *Nde* I site at the initiation codon of LacZ′. (*ii*) The *Nde* I–*Bgl* II fragment of the resulting plasmid was cloned into the *Nde* I/*Bam*HI sites of pT7-7 (29) to generate pT7-7-Zn2, which was transformed into BL21(DE3)pLysS (30) for expression of zinc domain.

Protein Purification. ATCase was prepared from *E. coli* strain TR 4363 transformed with pPYRB3. C and R subunits were isolated after treatment of ATCase with neohydrin. C subunit containing the substitution of glutamic acid for Lys-164 was provided by Eric Johnson (University of California at Berkeley) from mutant enzyme (31). For purification of zinc domain, BL21(DE3)pLysS transformed with pT7-7-Zn2 was grown to mid-logarithmic phase in Luria broth medium containing ampicillin at 50 μg/ml and chloramphenicol at 30 μg/ml and induced with 0.4 mM isopropyl β-D-thiogalactoside for 2 hr. Cells were harvested by centrifugation and resuspended in Mes buffer (20 mM 2[*N*-morpholino]ethanesulfonic acid, adjusted with KOH to pH 6.5, containing 1 mM 2-mercaptoethanol and 0.1 mM zinc acetate). Also 0.1 mM phenylmethanesulfonyl fluoride was present to inhibit proteolysis. Crude extract was prepared by two freeze-thaw cycles, treatment with DNase and RNase, and centrifugation. The extract was applied to an S-Sepharose fast flow column equilibrated with Mes buffer, and adsorbed proteins were eluted with a linear gradient of 0–0.5 M KCl in Mes buffer. Fractions were assayed for zinc domain by monitoring complex formation with C subunit using nondenaturing electrophoresis and for purity by SDS/PAGE. Pooled fractions were concentrated by using an Amicon ultrafiltration cell equipped with a YM3 membrane. The zinc domain was purified further by gel filtration over a Sephadex G-50 Superfine column followed by ultrafiltration.

Other Methods. PAGE of native proteins and the C trimer–zinc domain complex was done with 7.5% separating gels without stacking gels. Gels were stained either for total protein using Coomassie brilliant blue R250 or for ATCase activity (32). Electrophoresis of denatured proteins was done with SDS/13.5% polyacrylamide gels.

Enzyme activity was measured at 30°C in 0.1 M Mops (4-morpholinepropanesulfonic acid) adjusted to pH 7.0 with KOH and containing 2 mM 2-mercaptoethanol, 0.1 mM zinc acetate, 4 mM carbamoyl phosphate, and various concentrations of L-aspartate (33). V_{max} and K_m were determined by nonlinear least-squares analysis. A dissociation constant (K_d) for the C subunit–zinc domain complex was estimated from enzyme activity as a function of the concentration of zinc domain by assuming noncooperative association to three independent sites and the binding of one zinc domain affecting only a single active site.

Wild-type C trimer was modified with 2,4,6-trinitrobenzenesulfonate (TNBS) (34) to incorporate a sensitive spectral probe at the active sites of C trimer, yielding a trinitrophenylated C subunit (C_{TNP}) that was used in spectral studies with a Cary model 118 double-beam spectrophotometer. Spectral changes due to binding zinc domain were measured as a difference spectrum.

RESULTS

Detection of Zinc Domain in Crude Extracts. Because principal contacts between R and C subunits in ATCase are localized at the zinc-containing region of the r chain and estimates for dissociation of one R dimer from CR_3 (20, 22) yielded values of K_d between 10^{-7} and 10^{-8} M, we expected that zinc domain would form a stable complex with C trimer at low protein concentrations (μg/ml). Although dissociation would occur during electrophoretic separation of various interacting species, the complex should be detectable by a gel-shift assay because of alteration of the mobility of C trimer as a result of its binding zinc-containing polypeptide chains in the extract.

The electrophoresis patterns in Fig. 1 from gels stained for ATCase activity demonstrate that mixing purified C subunit with a crude extract from cells expressing the zinc domain

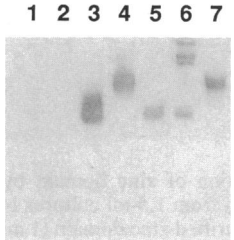

FIG. 1. Detection of zinc domain by shift in electrophoretic mobility of C trimer in nondenaturing polyacrylamide gels. Crude extracts of HB101-4442 transformed with either pUC18 or pUC18-Zn2 were prepared from 1.5-ml cultures by freeze–thaw lysis in 50 μl of 0.1 M Tris·HCl, pH 8.0/1 mM 2-mercaptoethanol/0.1 mM zinc acetate. Ten microliters of each extract was incubated for 5 min at 23°C with or without 30 ng of C trimer, and samples were analyzed by gel electrophoresis and then stained for ATCase activity (35). Lanes: 1, extract of strain transformed with pUC18; 2, extract of strain transformed with pUC18-Zn2; 3, pUC18 extract plus C trimer; 4, pUC18-Zn2 extract plus C trimer; 5, C trimer standard; 6, mixture of species including, in decreasing order of migration: C trimer C_2R_2, intact ATCase, and high molecular weight aggregates; 7, C trimer plus 25-fold molar excess of purified zinc domain.

significantly decreases the electrophoretic mobility of the active species. As seen in lane 4, the complex between C trimer and zinc domain has a mobility intermediate between free subunit (lane 5) and intact ATCase (lane 6). These experiments were done with extracts from *E. coli* strain HB101-4442 (27) from which structural genes for ATCase are deleted. Hence, no enzyme activity is detected in crude extracts of the strain transformed with pUC18-Zn2, which encodes zinc domain (lane 2) or the control vector (lane 1). Because the control vector pUC18 produces no zinc domain, there is no shift in the electrophoretic mobility of the C trimer (lane 3). This gel-shift technique provided a sensitive assay for monitoring production of zinc domain and served as a semiquantitative titration method for estimating amounts during purification.

Complex Formation Between Zinc Domain and C Trimer. High-level expression and purification of zinc-domain fusion protein were accomplished by using an inducible T7 RNA polymerase system with a protease-deficient strain. Approximately 40 mg of purified, stable protein was obtained per liter of culture. Expression of zinc domain from plasmid pT7-7-Zn2 is illustrated in Fig. 2*A* by SDS/PAGE patterns of crude extracts of BL21(DE3)pLysS. Lane 4 shows the large increase in expression of a 9-kDa protein after induction of BL21(DE3)pLysS/pT7-7-Zn2 compared to the same strain before induction (lane 3) or the host cell (lane 2). The single band in lane 5, purified zinc domain, migrates with a mobility corresponding to a protein of ≈9 kDa, consistent with the molecular weight calculated from the DNA sequence. A preliminary estimate of the molecular weight (10^4) from sedimentation equilibrium indicates that the protein is monomeric at 1 mg/ml (a value of 0.70 ml/mg for the partial specific volume was assumed).

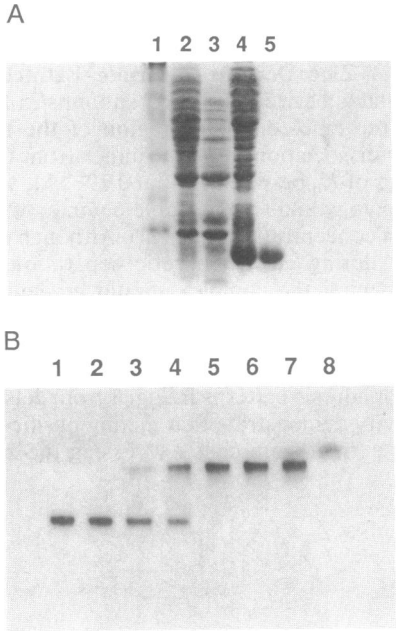

FIG. 2. (*A*) Detection of zinc domain by SDS/PAGE. Crude extracts were prepared from 1.5-ml cultures by freeze–thaw lysis in 20 μl of Mes buffer. Purified zinc domain (1 mg/ml) was in the same buffer. Five microliters of each sample was mixed with equal volume of 0.0626 M Tris, pH 6.8, containing 10% (vol/vol) glycerol, 5% (vol/vol) 2-mercaptoethanol, and 2.3% SDS, and samples were analyzed by SDS/13.5% PAGE. Lanes: 1, molecular mass standards from bottom: 14.3, 21.5, 30, 46, 69, 92.5 kDa; 2, extract of BL21(DE3)pLysS/T7-7 vector; 3, extract of BL21(DE3)pLysS/pT7-7-Zn2 before induction (minus isopropyl β-D-thiogalactoside); 4, extract of BL21(DE3)pLysS/pT7-7-Zn2 after induction with isopropyl β-D-thiogalactoside; 5, purified zinc domain. (*B*) Complex formation between purified zinc domain and C trimer. Purified zinc domain and C trimer were in Mes buffer. Experiments were done by nondenaturing gel electrophoresis with 2 μg of C trimer per lane and staining gels for total protein. Lanes: 1, C trimer alone; 2–7, C trimer plus zinc domain at molar ratios of 0, 1, 3, 4.5, 6, and 10 zinc domains per C trimer; 8, zinc domain alone.

Formation of the complex between C trimer and purified zinc domain is readily detected by PAGE of native proteins as shown in Fig. 2*B*. Increased amounts of zinc domain added to a constant concentration of C trimer cause a progressive and virtually complete conversion of C trimer into complex at a ratio of ≈4.5 molar equivalents of zinc domain per C trimer. Only two bands were observed, and no partially assembled species were detected as discrete bands of mobility intermediate between those of free C trimer and complex.

The zinc domain–C trimer complex is readily disrupted by addition of mercurials that react with the sulfhydryl groups of the zinc-containing polypeptide. The complex is reformed by the addition of 2-mercaptoethanol and zinc ions.

Changes in Enzyme Activity upon Formation of C Trimer–Zinc Domain Complex. Fig. 3*A* shows aspartate saturation curves for C trimer and the complex. A hyperbolic dependence of velocity on aspartate concentration was observed for the complex as demonstrated by the Eadie plot (data not shown), which was linear at low aspartate concentration. V_{max} for the complex was 11 μmol/μg per hr compared to 21 μmol/μg per hr for free C trimer and 12 μmol/μg per hr for intact ATCase (these values are based on the C trimer content not total protein). In addition to the decrease in V_{max} upon complex formation, there is a reduction of K_m for aspartate from 5.6 mM for free C trimer to 2.1 mM for the complex. The substantial reduction in turnover number provides a convenient measure for complex formation, as shown by the titration experiment illustrated in Fig. 3*B*. An approximate K_d of 10^{-8} M for the dissociation of one zinc-containing poly-

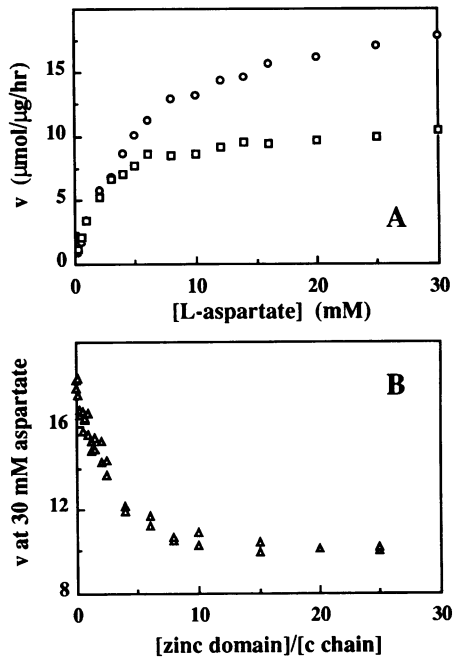

FIG. 3. Effect of zinc domain on catalytic activity of wild-type C trimer. Experiments were done with C trimer at 0.15 μg/ml. (*A*) Saturation curves of initial velocity (v) as a function of aspartate concentration. ○, C trimer alone; □, mixtures of C trimer and zinc domain at 0.8 μg/ml. (*B*) Effect of increased amounts of zinc domain on activity of C trimer. Assays were done at 30 mM aspartate (with 4 mM carbamoylphosphate) to exploit the difference in activity between C trimer and the complex.

peptide from the complex was estimated from the titration curve.

A striking example of functional changes in C trimer as a consequence of its binding the zinc domain was evident in studies of a mutant form of ATCase in which Lys-164 in the c chain was replaced by glutamic acid. The C trimer containing the Lys-164 → Glu replacement has an extremely low affinity for aspartate and the bisubstrate analog *N*-phosphonacetyl-L-aspartate (PALA) (31). In contrast, the holoenzyme exhibits a much higher affinity for both aspartate and PALA and appears, unlike wild-type ATCase, to be in the high-affinity **R** state, even in the absence of active-site ligands. Fig. 4*A* shows aspartate saturation kinetics for Lys-164 → Glu C trimer and its complex with the zinc domain. As reported (31), the curve for mutant C trimer does not attain half-saturation even at 300 mM aspartate. Upon formation of the complex, there is a dramatic increase in aspartate affinity with K_m equal to 77 mM and V_{max} equal to 4.2 μmol/μg per hr. Complex formation was examined in a titration experiment by measuring the initial velocity at a subsaturating aspartate concentration of 20 mM. As seen in Fig. 4*B*, the interaction of the mutant C trimer with zinc domain leads to ≈5-fold increase in activity because of the shift in K_m. Protein concentrations in this titration experiment were higher than those represented by Fig. 3*B* because of the low activity of the mutant protein. Hence, saturation of the mutant C trimer occurs at a lower ratio of zinc domain per c chain.

Conformational Changes in C Trimer Resulting from Interaction with Zinc Domain. A conformational change as a result of the interaction was demonstrated by using chemically modified C subunit in which both Lys-83 and Lys-84 were trinitrophenylated by treating the protein with TNBS (34). Fig. 5*A* shows the spectrum of C_{TNP} with two strong absorption bands at 345 and 420 nm. The positive and negative peaks at 340 and 425 nm in the difference spectrum in Fig. 5*B* resulting from the addition of zinc domain show clearly that a conformational change occurred in C_{TNP} leading to an alteration of the environment of the spectral probes at or near the active sites.

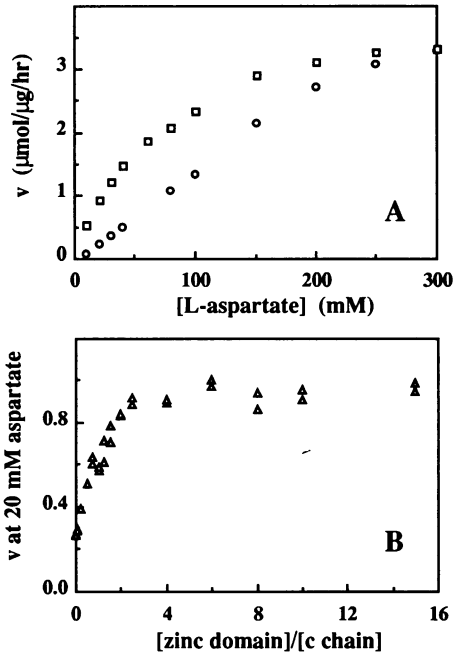

FIG. 4. Effect of zinc domain on the catalytic activity of mutant C trimer containing the Lys-164 → Glu replacement. (*A*) Saturation curves presented as initial velocity (v) as a function of aspartate concentration for mutant C trimer alone (○) and for mixtures of mutant C trimer (0.8 μg/ml) and zinc domain at 4.5 μg/ml (□). (*B*) Effect of increased amounts of zinc domain on activity of mutant C trimer containing the Lys-164 → Glu replacement. The aspartate concentration was 20 mM.

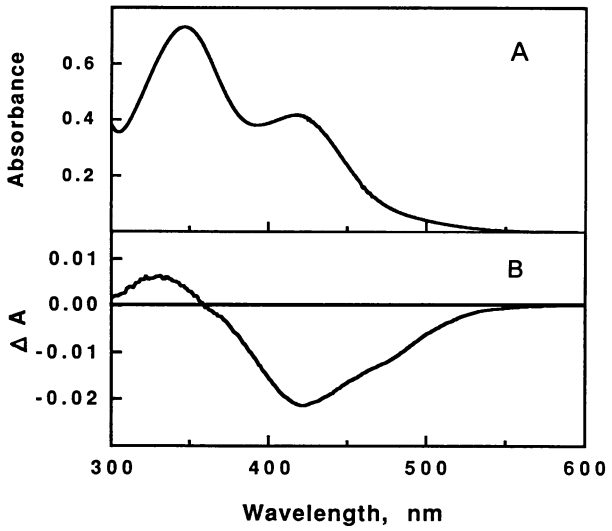

FIG. 5. Change in absorption spectrum of C_{TNP} upon formation of complex with zinc domain. Wild-type C trimer was modified with TNBS to give a derivative containing ≈2.0 trinitrophenyl groups per c chain (34). (*A*) Absorption spectrum of C_{TNP} at 1 mg/ml in a 50 mM imidazole acetate buffer, pH 7.0/2 mM 2-mercaptoethanol/0.2 mM EDTA. (*B*) Difference spectrum for interaction of C_{TNP} with zinc domain. Equal volumes of C_{TNP} (2 mg/ml) in imidazole acetate buffer and zinc domain (2.5 mg/ml in the same buffer without EDTA and containing 0.05 mM zinc acetate) were placed in separate chambers of tandem cells. The baseline was recorded, contents of the sample cell were then mixed, and difference spectrum was recorded.

DISCUSSION

The zinc domain of the r chain of ATCase was expressed efficiently in *E. coli* as a soluble 9-kDa polypeptide comprising 11 amino acids encoded by the polylinker of pUC18 fused to the amino terminus of residues 84–153 of the r chain. Approximately 16 residues encoded by *pyrI* in the expressed fusion protein are not part of the zinc domain as defined crystallographically (16, 17, 23). What effect, if any, the additional 27 amino acids have on the interaction with C trimer is not known. Because the fusion protein forms a stable complex with C subunit and the amino-terminal extension in the construct is on the opposite face of the zinc domain from that forming the r–c contact, we assume tentatively that the additional residues have little impact on the interaction and properties of the complex. Alternative DNA constructs are clearly needed to permit the isolation and study of an even smaller zinc-containing polypeptide lacking that section of the N-terminal region of the chain that is not an intrinsic part of the zinc domain and is presumably unstructured.

As shown by electrophoresis (Fig. 1 and Fig. 2*B*) and alterations in enzyme activity (Figs. 3 and 4), the zinc domain, as isolated here, forms a specific complex with C trimer. When experiments are done at concentrations ≈1 mg/ml, almost stoichiometric binding occurs, and the mobility of C trimer upon addition of the zinc-containing polypeptide attains its minimal value at a molar ratio of 4.5 zinc domain per C trimer. This value is somewhat larger than the expected ratio of 3.0, and it is not yet known whether this apparent discrepancy is attributable to damaged polypeptide incompetent for assembly or limited affinity of interaction coupled with some dissociation during the electrophoretic

separation. The dissociation constant for each chain from the complex is estimated as 10^{-8} M based on the assumption that the three binding sites on the trimer are independent and changes in activity are directly proportional to the fraction of r–c contacts formed. Because the active sites are at interfaces between chains in the C trimer (35, 36), and assembly studies (22) indicate possible cooperativity in binding of R dimers to C trimers, the estimate of K_d must be considered tentative.

The zinc domain–C trimer complex exhibits catalytic properties similar to CR_3 (20) and differs significantly from intact ATCase and free C trimer. Unlike holoenzyme, the complex exhibits a hyperbolic dependence of initial velocity on aspartate concentration, and K_m for aspartate is 50% that of C trimer. In addition, zinc domain reduces V_{max} of C trimer by one-half, to a level similar to that of intact ATCase when it is converted to the **R** state by saturating substrate concentrations (11). Because zinc domain increases the aspartate affinity of C trimer 2-fold and analysis of ATCase cooperativity indicates that the **R** state has a slightly higher affinity for aspartate analogs than C trimer (11, 37), we consider the zinc domain–C trimer complex an analog of intact ATCase in the **R** state. This inference is strengthened by results obtained with a mutant enzyme in which Lys-164 in the c chain is replaced by glutamic acid (31). The C trimer of Lys-164 → Glu ATCase exhibits a low affinity for aspartate and the bisubstrate analog PALA. However, the mutant holoenzyme has a much higher affinity for ligands, and sedimentation velocity experiments demonstrate that the enzyme exists in a swollen conformation indicative of the **R** state. Zinc domain promotes a marked decrease in K_m of Lys-164 → Glu C subunit from a value >300 mM aspartate to 77 mM aspartate, whereas the holoenzyme exhibits a K_m of 100 mM (31). The proposed analogy between the zinc domain–C trimer complex and relaxed state of holoenzyme must be subjected to further tests, such as comparing the relative affinities of various species for the ligand PALA.

It is clear that substantial changes occur at the active site of wild-type C trimer and the mutant Lys-164 → Glu C trimer when they interact with the zinc-containing polypeptide fragment of r chain. Even though the contacts between c and r chains are at a considerable distance from the active sites, the effects of association are propagated "globally," resulting in a conformational change that is detected by the change in affinity for substrates and alteration in the absorption spectrum of chromophores at the active sites. Evidence from this and previous investigations showing that the environment of the active site in isolated C trimer differs from those of either the **T** or **R** state of the holoenzyme illustrates the "soft" nature of ATCase. The free energy of interconversion between the **T** and **R** states is estimated to be only ≈3.5 kcal/mol (11, 37). Such a low energetic barrier for a conformational change from a low-activity form to a state of higher activity might be expected for a regulatory enzyme like ATCase, which must undergo alterations in response to fluctuations in levels of cellular metabolites. The availability of the zinc-containing polypeptide fragment of the r chain, which binds to C trimer, should facilitate studies of protein–protein recognition as well as structural and functional consequences of their interaction.

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