Immunological Studies of Aspartate Transcarbamylase. I. Characterization of the Native Enzyme, Catalytic, and Regulatory Subunit Immune Systems*

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ABSTRACT: Antisera to aspartate transcarbamylase and subunits, catalytic and regulatory, have been analyzed serologically. Antisera to aspartate transcarbamylase react more effectively with aspartate transcarbamylase than with either or both of its isolated subunits. This greater complement fixing activity was attributed to the more effective lattice formation with aspartate transcarbamylase in which all of the antigenic determinants are located on one molecule. Antisera to catalytic subunit showed greater activity with catalytic subunit than with aspartate transcarbamylase. Antiregulatory subunit can also distinguish free regulatory subunit from its bound form in aspartate transcarbamylase. Dissociation of aspartate transcarbamylase by *p*-hydroxymercuribenzoate results in a decrease in serologic activity with antiaspartate transcarbamylase and a concomitant increase in complement fixing activity with anticatalytic subunit. Quantitative measurements of aspartate transcarbamylase dissociation by *p*-hydroxymercuribenzoate can be made from the decrease in complement fixation when measured with antiaspartate transcarbamylase. When aspartate transcarbamylase in 4 mM phosphate buffer is subjected to 59° and above for 5 min, its serologic activity with antiaspartate transcarbamylase decreases and its complement fixing activity with anticatalytic subunit increases.

Aspartate transcarbamylase (ATCase)[1] of *Escherichia coli* is one of the best understood of the allosteric enzymes (Gerhart and Pardee, 1962, 1964; Gerhart and Schachman, 1968; Changeux *et al.*, 1968). It catalyzes the condensation of aspartate and CAP to carbamyl aspartate which is then converted in a series of reactions into pyrimidine nucleotides (Yates and Pardee, 1956). Gerhart and Schachman (1965, 1968) have shown *E. coli* ATCase to contain at least two different types of subunits, each with a specific function. One of these subunits (catalytic subunit) catalyzes the enzymatic reaction while the other subunit (regulatory subunit) functions solely as a modifier of the catalytic subunit properties.

The complement fixing capacity of rabbit immune systems depends primarily upon the affinity of the antibodies for the individual antigenic determinants and secondarily on the subsequent aggregation of the initial antigen–antibody complexes (Hill and Osler, 1955). Changes in the structure of the protein antigen, such as alteration of the covalent sequence or disruption of covalent or noncovalent bonds may directly or indirectly alter antigenic determinants on the protein, thus decreasing the complementarity between the antigen and and its specific antibody. Even the effect of a local conformational change in only one of several antigenic determinants may be amplified by the process of antigen–antibody aggregation and lead to an alteration of the complement fixation curve. Conformational changes in multichained proteins, composed of immunologically distinct subunits, are especially sensitive to detection by antibodies. Hemoglobin and mammalian lactic dehydrogenases are two molecules that fall into this class and have been studied extensively by this technique (Reichlin *et al.*, 1963–1965; Cahn *et al.*, 1962). Alterations in structure of even relatively simple protein antigens have been detected by complement fixation (Levine, 1962; Reichlin *et al.*, 1963; Gerstein *et al.*, 1963, 1964; von Fellenberg and Levine, 1967).

It was thought that antibodies to catalytic and regulatory subunits as well as antibodies to the intact ATCase would be of advantage in detecting dissociation and possibly even conformational changes in the macromolecule when bound to various ligands. The present paper describes the immunochemical characterization of such antibodies and shows that these antibodies are sensitive indicators of dissociation.

*Publication No. 603 from the Graduate Department of Biochemistry, Brandeis University, Waltham, Massachusetts 02154, and from the Department of Biochemistry, School of Medicine, University of North Carolina, Chapel Hill, North Carolina 27514. *Received July 30, 1968.* Supported in part by research grants from the National Institutes of Health (AI-01940 and HD-02148) and the National Science Foundation (GB-5354 and GB-4302). L. L. is an American Cancer Society Professor of Biochemistry (Award No. PRP-21).

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[1] Abbreviations used that are not listed in *Biochemistry 5*, 1445 (1966), are: ATCase, aspartate transcarbamylase; CAP, carbamyl phosphate; MBSA, methylated bovine serum albumin; *p*-HMB, *p*-hydroxymercuribenzoate; Isosatris, 0.01 M Tris–0.14 M NaCl (pH 7.4) containing 0.1% bovine serum albumin.

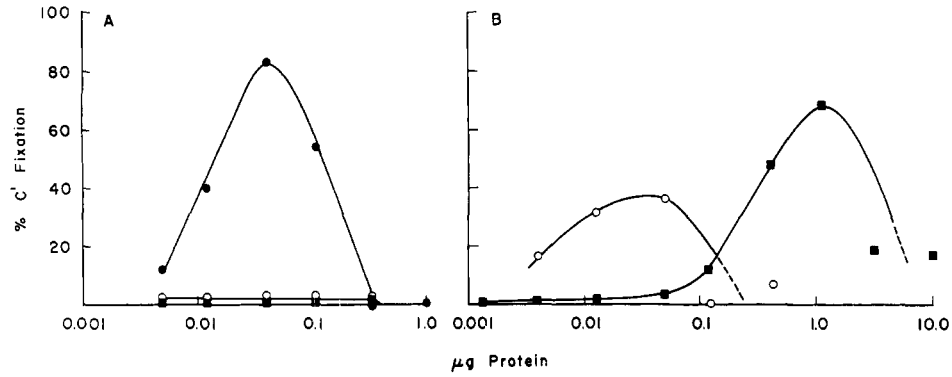

FIGURE 1: C′ fixation of ATCase (●), catalytic subunit (○), and regulatory subunit (■) with anti-ATCase diluted $^1/_{90,000}$ (A) and $^1/_{60,000}$ (B). C′ stands for complement.

Materials and Methods

The *E. coli* ATCase used for immunization of the rabbits was purified through step 5 (DEAE-Sephadex) according to Gerhart and Holoubek (1967) from extracts of the diploid strain of these authors. We are most grateful to Dr. Gerhart for providing us with this strain and details of his purification procedure through step 5 prior to their publication. This preparation of *E. coli* ATCase, assayed by the method of Gerhart and Pardee (1962), had a specific activity of 6000–7000. The enzyme was purified according to the complete procedure of Gerhart and Holoubek (1967). These preparations had a specific activity of 8600. Catalytic and regulatory subunits were obtained from these enzyme preparations by treatment with *p*-HMB followed by sucrose gradient centrifugation according to Gerhart and Schachman (1965) and also by DEAE-Sephadex chromatography (Gerhart and Holoubek, 1967). By acrylamide gel electrophoresis, both subunits so obtained were pure.

The colorimetric determination of carbamyl aspartate was a modification of the Gerhart–Pardee (1962) procedure while the enzyme incubation conditions were those of Gerhart and Pardee (1962). The enzyme reaction was stopped by addition of an equal volume of 10% perchloric acid to the incubation mixture. The enzyme activity is expressed as micromoles of carbamyl aspartate formed per hour per milligram of protein unless otherwise noted. Protein was measured by the method of Oyama and Eagle (1956) or by absorbance at 284 mμ using the absorption coefficients indicated by Gerhart and Holoubek (1967).

L-Aspartate, dilithium carbamyl phosphate, Tris, and *p*-HMB were commercial products.

Preparation of Immune Sera. IMMUNIZING ANTIGENS. ATCase, catalytic, and regulatory subunits were complexed with MBSA as described by Plescia *et al.* (1964) with the following modifications. To a solution containing 250 μg of antigen protein in 0.15 N NaCl was added 25 μl of 1% MBSA. For toepad injections, the antigen–MBSA complex (2 ml) was emulsified with an equal volume of complete Freund's adjuvant. Three weeks later, an intravenous injection of the antigen–MBSA complex in 1 ml was given and the rabbits were bled 5 days later.

ABSORPTION OF ANTICATALYTIC SUBUNIT WITH ATCASE. Anticatalytic subunit (8 ml) was incubated with 1.6 ml containing 2.2 mg of ATCase for 1 hr at 37°. The incubation was transferred to the cold (4°) for 22 hr after which it was centrifuged in Spinco Model L2 (SW 39 rotor) at 38,000 rpm for 3 hr at 0°.

Serologic Assays. Quantitative microcomplement fixation was performed according to the procedure of Levine (1967). The antisera used in the complement fixation studies were tested over a wide range of antigen concentrations and only showed a single complement fixation peak. Double diffusion was performed by the method of Ouchterlony (1949) in 1% agar containing 0.9% NaCl. The wells contained 0.1 ml of antiserum or 0.1 ml of antigen. Diffusion was allowed to continue for at least 24 hr at 2–4° unless otherwise stated.

Results

Immunochemical Analysis of the Immune Sera. ANTISERUM TO ATCASE. Antisera from two rabbits immunized with *E. coli* ATCase were tested by double diffusion in agar with crude *E. coli* extracts. Both sera showed multiple precipitating immune systems. The heterogeneity of the antisera was not surprising since the immunogen was only 75–85% pure. To identify some of the immune systems present in these antisera, a double-diffusion experiment was performed with one antiserum and four different antigens: ATCase (75–85% pure), ATCase with higher specific activity, electrophoretically pure catalytic subunit, and a crude extract of an *E. coli* mutant (strain HfrCYA-231) lacking in ATCase activity. One precipitating band was seen with the ATCase preparation of higher specific activity while two or three precipitating bands were seen with the ATCase preparation used for immunization. One band was present with the catalytic subunit. Two or three bands were observed with the crude extract lacking ATCase. The band obtained with the pure ATCase preparation formed a pattern of partial identity with the single catalytic subunit band and a pattern of nonidentity with the bands of the strain lacking ATCase.

The double-diffusion results suggest that the major antibody component of the antiserum, which formed a band of partial identity with catalytic subunit, was di-

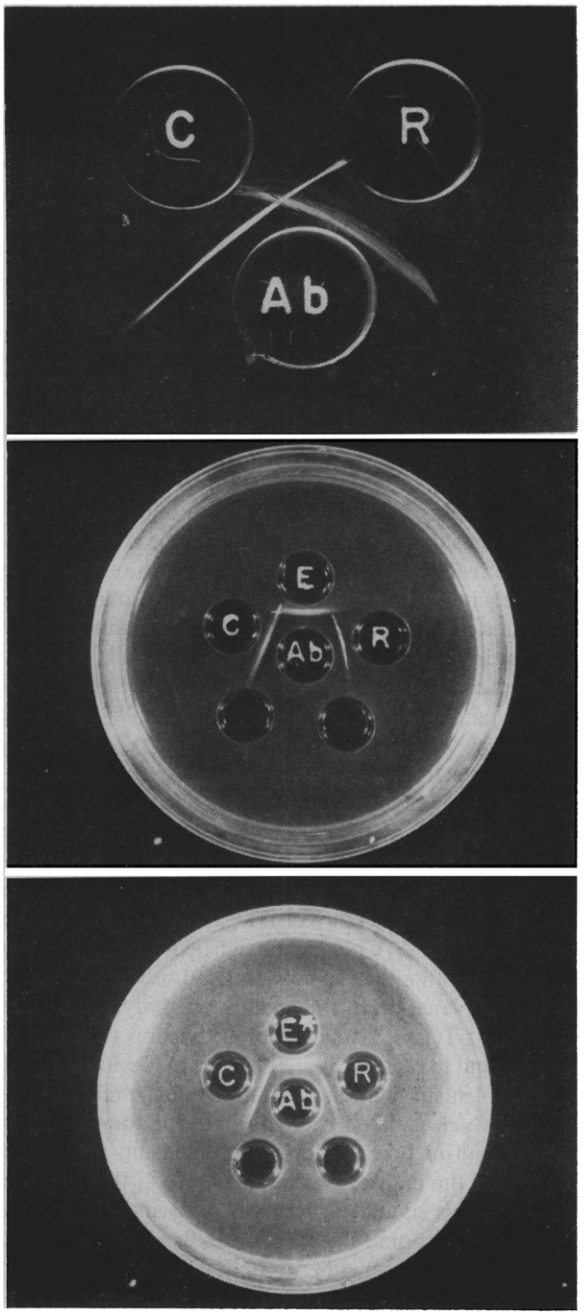

FIGURE 2: Gel diffusion analysis of ATCase (E), catalytic subunit (C), regulatory subunit (R), *p*-HMB-treated ATCase (E*) with anti-ATCase (Ab). Figure A, B, and C are the top, middle, and bottom pictures, respectively.

rected toward ATCase while the minor components were directed toward impurities present in the ATCase preparation used for immunization. At the high dilution of antiserum used in microcomplement fixation, only antibodies directed toward ATCase would be present in sufficiently high concentration to react with ATCase even in crude extracts. Various preparations of ATCase differing in purity were tested with anti-ATCase by microcomplement fixation. It was expected that complement fixation with ATCase would be dependent upon the amount of enzyme added and independent of its specific activity if the reacting antibodies were directed

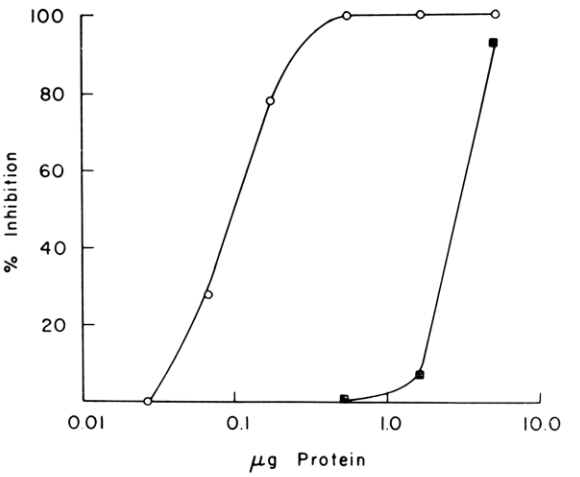

FIGURE 3: Inhibition of ATCase–anti-ATCase C′ fixation by free catalytic subunit (○) and free regulatory subunit (■). For fixation, 0.05 μg of ATCase and $^{1}/_{90,000}$ dilution of anti-ATCase were used.

toward ATCase. These expectations were confirmed experimentally. Despite the multiple immune systems observed in double-diffusion experiments, the appearance of only one peak of complement fixation over a 0.1–1.0-μg range of ATCase demonstrates the homogeneous behavior of this antiserum at high dilution.

Anti-ATCase was then tested separately with ATCase and its catalytic and regulatory subunits to determine its ability to distinguish between the three molecular species by microcomplement fixation. These data are shown in Figure 1. Whereas ATCase fixes complement at $^{1}/_{90,000}$ dilution of the antiserum, catalytic and regulatory subunits do not (Figure 1A). If the antiserum is used at higher concentrations ($^{1}/_{60,000}$), the complement fixing activity of both catalytic and regulatory subunits can be observed (Figure 1B). Thus, the macromolecule reacts more effectively with anti-ATCase.

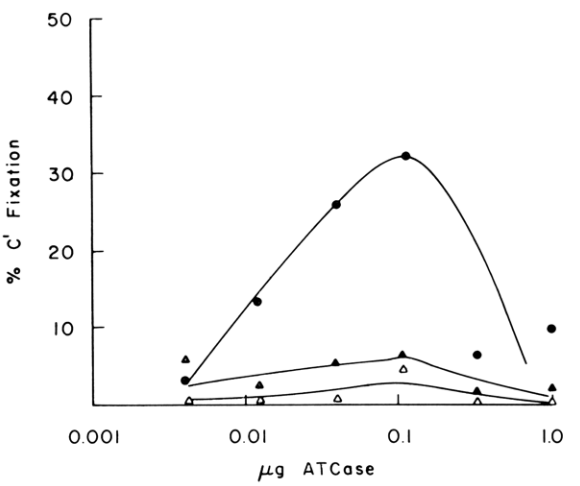

FIGURE 4: C′ fixation with mixed anticatalytic subunit, diluted $^{1}/_{18,000}$, and antiregulatory subunit, diluted $^{1}/_{2000}$ with ATCase (●), catalytic subunit (▲), and regulatory subunit (△). *p*-HMB-dissociated ATCase fixed C′ the same as regulatory subunit. C′ stands for complement.

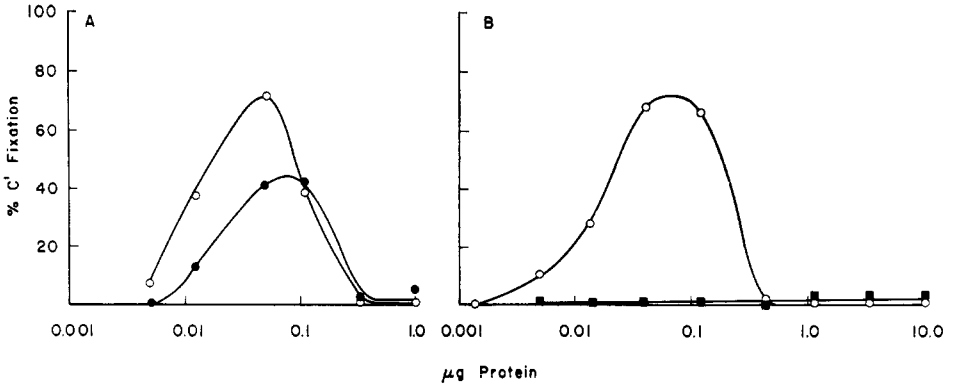

FIGURE 5: C′ fixation of anticatalytic subunit diluted $^1/_{11,000}$ (A) and ATCase-absorbed anticatalytic subunit diluted $^1/_{1200}$ (B) with catalytic subunit (○) and ATCase (●, A) (■, B). C′ stands for complement.

Anti-ATCase contains antibodies directed to antigenic determinants present on separated catalytic and regulatory subunits. Isolated, catalytic, and regulatory subunits form bands of nonidentity with antibodies in antiserum to ATCase (Figure 2A). The gel diffusion experiments (Figure 2B) with isolated catalytic and regulatory subunits and intact ATCase reacting with anti-ATCase show the anticipated reactions of partial identity. If the ATCase is dissociated with *p*-HMB into catalytic and regulatory subunits, the spurs seen with undissociated ATCase are lost. Instead, two bands are formed, one giving a pattern of identity with catalytic subunit and the other giving a pattern of identity with regulatory subunit (Figure 2C). While it is clear that anti-ATCase contains antibodies directed toward regulatory and catalytic subunits, the gel diffusion experiments give no information concerning the presence or absence of antibodies directed toward quaternary structure. Yet, as can be seen from the data in Figure 1, there is a reaction with ATCase at a $^1/_{90,000}$ dilution of anti-ATCase, a dilution with which there is no reaction with catalytic or regulatory subunit.

In order to test for antibody combining sites complementary to the quaternary structure of the native enzyme, the effect of isolated catalytic and regulatory subunit on the complementary fixation of intact ATCase was tested. It was expected that if antibodies to quaternary structure were being measured at $^1/_{90,000}$ dilution of the antiserum, the subunits would not inhibit the ATCase-anti-ATCase reaction, or if they did, the inhibition would be relatively inefficient. However, as can be seen in the data presented in Figure 3, isolated catalytic subunit is a very efficient inhibitor of the ATCase–anti-ATCase reaction. Isolated catalytic subunit (0.1 μg) inhibits the reaction of 0.05 μg of ATCase and anti-ATCase 50%. Isolated regulatory subunit also inhibits the ATCase–anti-ATCase reaction 100%, but with the regulatory subunit, 3.0 μg is required for 50% inhibition. These data suggest that the ATCase–anti-ATCase reaction at the $^1/_{90,000}$ dilution of antiserum may not be due to antibodies specific for quaternary structure, but that the macromolecular structure of ATCase is such that lattice formation with ATCase is more efficient than the lattice formation with either or both isolated subunits. To test for this explanation of the greater reactivity of ATCase with anti-ATCase, a mixture of antiserums to catalytic subunit and to regulatory subunit was made and assayed for complement fixation at dilutions of the mixed antisera in which isolated catalytic or regulatory subunits alone or in mixture do not fix complement. As can be seen from the data presented in Figure 4, this mixture of antiserums simulated that seen with anti-ATCase in that the macromolecular ATCase fixed complement more effectively than either or both isolated subunits.

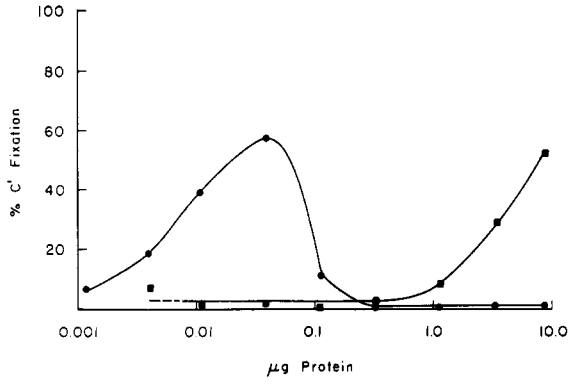

FIGURE 6: C′ fixation of antiregulatory subunit diluted $^1/_{1000}$ with ATCase (●) and regulatory subunit (■). C′ stands for complement.

ANTISERUM TO CATALYTIC SUBUNIT. The catalytic subunit, like ATCase, proved to be a good immunogen. In contrast to the marked heterogeneity of the ATCase immune sera, the catalytic subunit immune sera were found to be homogeneous by double diffusion in agar. A single band of precipitation was observed between the antisera and catalytic subunit which formed a small spur with the single band obtained with ATCase. The antisera did not form any precipitate with the extract of *E. coli* lacking ATCase activity. The small spur suggested that the anticatalytic subunit sera contained antibodies directed toward some antigenic determinants which are masked when associated with regulatory subunits, *i.e.*, in the native enzyme.

Figure 5A shows that at a $^1/_{11,000}$ dilution of the serum, the isolated catalytic subunit fixed 70% of the complement, while ATCase fixed 40%. This 30% difference in complement fixation probably reflects the antibody rep-

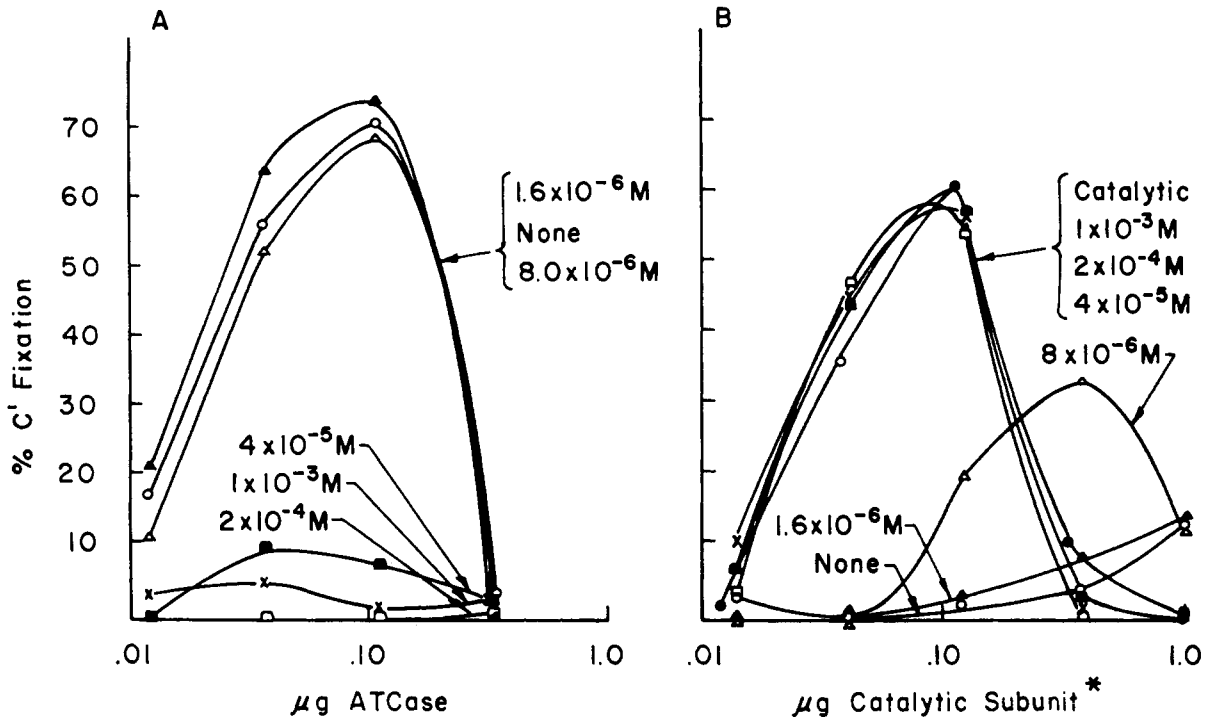

FIGURE 7: Effect of different amounts of *p*-HMB on the serologic activity of ATCase with anti-ATCase and anticatalytic subunit. (A) For dissociation, ATCase (35 μg/ml) was incubated with *p*-HMB in 0.4 M phosphate buffer (pH 7.0) at 25°. After 30 min, the incubation mixture was diluted in Isosatris to contain 0.5 μg of ATCase/ml and assayed with anti-ATCase diluted $^1/_{90,000}$ (B) For dissociation, ATCase (68 μg/ml) was incubated with *p*-HMB in 0.4 M phosphate buffer (pH 7.0) at 25°. After 30 min the incubation mixture was diluted to contain 1.5 μg of ATCase/ml assayed with ATCase-absorbed anticatalytic subunit diluted $^1/_{1200}$. Solid circles represent C′ fixation curve observed with pure catalytic subunit. *Catalytic subunit equivalent; *i.e.*, micrograms of pure catalytic subunit or micrograms of ATCase multiplied by $^2/_3$. C′ stands for complement.

resented by the small spur in the gel diffusion experiment. If antigenic determinants common to both the catalytic subunit and ATCase could be removed by absorption of the anticatalytic subunit with ATCase, the difference between the serologic activities of ATCase and catalytic unit would be accentuated. Accordingly, catalytic subunit antiserum was absorbed with ATCase. The complement fixing activities of ATCase and catalytic subunit with the absorbed antiserum are shown in Figure 5B. At a $^1/_{1200}$ dilution of the absorbed serum, 70% complement fixation was obtained with 0.1 μg of catalytic subunit, while an equivalent amount of subunit in the native ATCase molecule did not fix complement at all. With some absorbed antisera, ATCase at a tenfold higher antigen concentration did fix complement; this complement fixation with ATCase varied from 10 to 40% at peak.

ANTISERUM TO REGULATORY SUBUNIT. Regulatory subunit was not as effective an immunogen as whole ATCase or catalytic subunit. Nevertheless, antibodies were detected by double diffusion and complement fixation techniques. Only a single weak band of precipitation was observed with either ATCase or regulatory subunit and antiregulatory subunit. These bands showed a pattern of identity. When analyzed by complement fixation at a 1:1000 dilution of the antiserum, both ATCase and regulatory subunit reacted but the amounts of whole enzyme and regulatory subunit required for maximum complement fixation were strikingly different. While 0.1 μg of ATCase was sufficient to yield maximum complement fixation, 1–10 μg of regulatory subunit was required for activity with this antiregulatory subunit serum (Figure 6). While the serologic activity of ATCase–antiregulatory subunit reaction was very reproduceable, the extent of complement fixation with regulatory subunit varied from preparation to preparation depending upon its state of aggregation, although the range of regulatory subunit required for this variable complement fixation remained about 1–10 μg.

Use of Anti-ATCase and Anticatalytic Subunit to Measure Dissociation of ATCase. As shown above, both anti-ATCase and absorbed anticatalytic subunit readily distinguish native ATCase from the dissociated subunits by micro complement fixation. The usefulness of these two antisera for following changes in the quaternary structure of ATCase which must occur during the process of dissociation was then tested. Two agents were used to dissociate ATCase: *p*-HMB and heat. It was expected that as the ATCase molecules dissociated, the serologic activity of the ATCase measured with anti-ATCase would decrease. At the same time, serologic activity measured with anticatalytic subunit would increase.

EFFECT OF *p*-HMB ON THE ANTIGENIC STRUCTURE OF ATCASE. The effect of incubation of ATCase with varying concentrations of *p*-HMB (at 25° for 20 min) on the serologic activity of ATCase is shown in Figure 7. Levels of *p*-HMB equal to or greater than 4×10^{-5} M *p*-HMB resulted in the loss of serologic activity of ATCase with

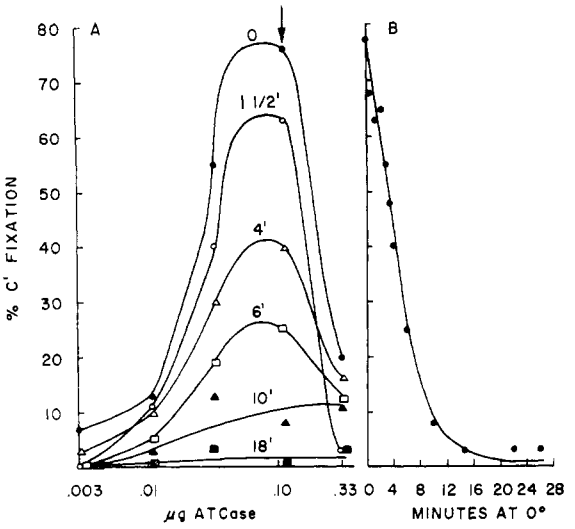

FIGURE 8: Time course of *p*-HMB dissociation of ATCase detected with anti-ATCase. ATCase (38 μg/ml) was incubated at 0° in 0.4 M phosphate buffer (pH 7.0) containing 2×10^{-4} M *p*-HMB. At the indicated time intervals, aliquots were transferred to 5 ml of cold Isosatris for C′ fixation with a $^{1}/_{90,000}$ dilution of anti-ATCase. (A) C′ fixation as a function of antigen concentration. (B) Per cent C′ fixation with 0.1 μg of ATCase as a function of time. C′ stands for complement.

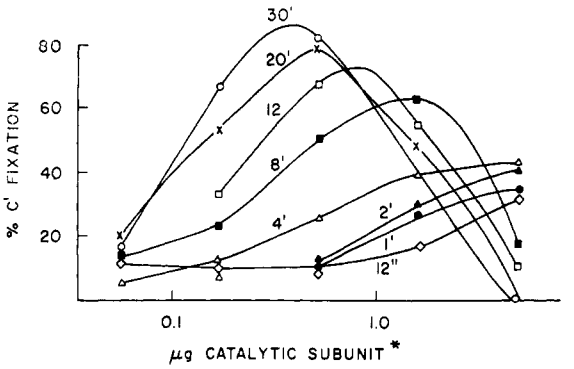

FIGURE 9: Time course of *p*-HMB dissociation of ATCase detected with anticatalytic subunit. ATCase (210 μg/ml) was incubated in 0.04 M phosphate buffer (pH 7.0) containing 2×10^{-5} M *p*-HMB. At the indicated time intervals, aliquots were transferred to 5 ml of cold Isosatris for C′ fixation with $^{1}/_{1200}$ dilution of ATCase absorbed anticatalytic subunit. *Catalytic subunit equivalent (see legend to Figure 7). C′ stands for complement.

anti-ATCase (Figure 7A). With anticatalytic subunit serum (Figure 7B), however, the appearance of catalytic subunit antigen was observed with as little as 8×10^{-6} M *p*-HMB. The products of incubation with higher concentrations of *p*-HMB were serologically identical in complement fixing activity with that obtained with purified catalytic subunit.

The rate of ATCase dissociation by *p*-HMB could also be measured with these antisera, indicating that the *p*-HMB dissociation can be "frozen" by dilution of the ATCase–*p*-HMB incubation mixture. Anti-ATCase was used to test ATCase incubated for various times with 10^{-4} M *p*-HMB (0°). The complement fixation curves are presented in Figure 8A and show that the serologic activity ATCase with this antiserum decreases with increasing exposure of ATCase to *p*-HMB. The decrease is more clearly depicted in Figure 8B, in which the complement fixation by 0.1 μg of ATCase is presented as a function of time. The unmasking of catalytic subunit antigenic determinants during incubation of ATCase with 2×10^{-5} M *p*-HMB (0°) was detected with anticatalytic subunit, as shown in Figure 9.

It has been noted that the free catalytic subunit is an effective inhibitor of the ATCase–anti-ATCase reaction. The decrease in complement fixation accompanying the dissociation of ATCase by *p*-HMB results from loss of native enzyme structure and the concomitant generation of the inhibiting products. Thus, the inhibition curve with free catalytic subunit can be used to estimate the number of native ATCase molecules remaining in a mixture of ATCase and free catalytic subunit or dissociated products.

EFFECT OF HEAT ON THE ANTIGENIC STRUCTURE OF ATCASE. The two antisera were used to measure the stability of ATCase structure after 5-min exposure to elevated temperatures. Figure 10A shows the reaction of heated ATCase with anti-ATCase. Incubation of ATCase at temperatures up to 53° had little or no detectable effect on ATCase antigenicity. After incubation at 56 and 59°, there was a slight decrease in complement fixation with anti-ATCase. After 62° incubation, the complement fixation of ATCase was markedly decreased. After 5 min at 69° the residual serologic activity was similar to that obtained in experiments with untreated catalytic subunit. When these same incubation mixtures were tested with anticatalytic subunit (Figure 10B), an increase in serologic activity was observed which approached that obtained with the untreated catalytic subunit control. Thus, both antisera detected changes in ATCase antigenicity as a function of heating.

Discussion

Rabbits, immunized with ATCase, elicit antibodies which when measured by complement fixation react more effectively with ATCase than either or both of its isolated subunits. Three possibilities must be considered in order to explain this increased serologic activity of the native enzyme. (1) There may be some antigenic determinants formed by contributions from both catalytic and regulatory subunits at points of contact between the subunits; (2) some antigenic determinants on the catalytic and regulatory chains have a different conformation in the macromolecule than in their free form; and (3) the complementarity of all the antigenic determinants in the macromolecule and the free subunits are unchanged, but these antigenic determinants are now resident on two rather than on one molecule.

Reichlin *et al.* (1964) have shown that unfractionated mixtures of interspecies molecular hybrids of hemoglobin fix complement less effectively than the parent molecules and suggested that for the most effective serologic activity, antigenic determinants must reside on the same molecule. Two lines of evidence support this ex-

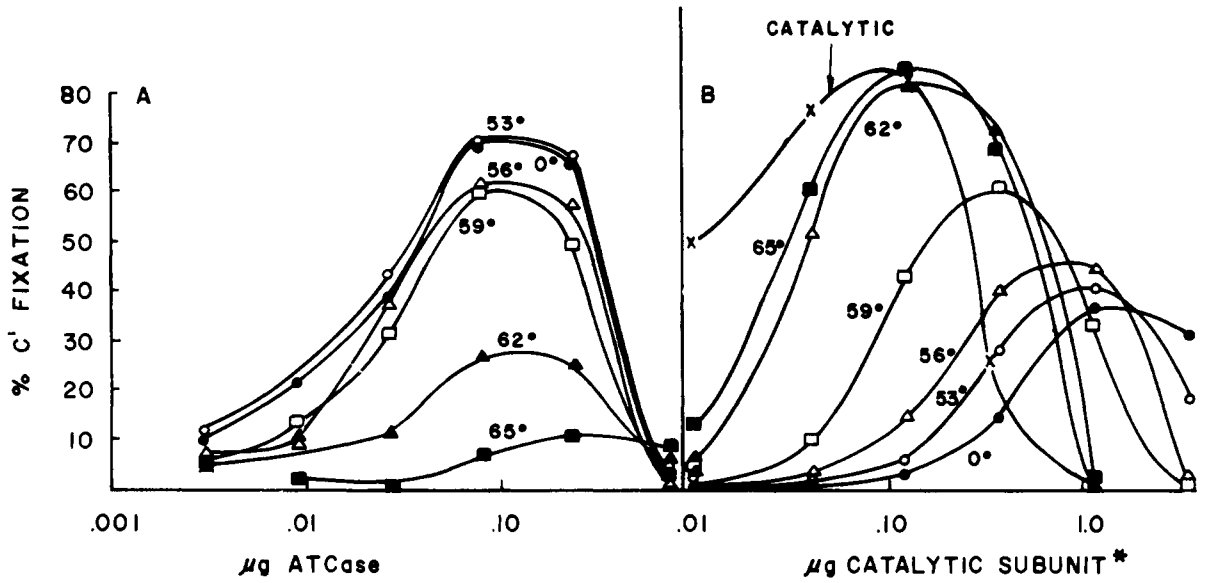

FIGURE 10: C′ fixation of anti-ATCase and anticatalytic subunit with ATCase which had been incubated at varying temperatures for 5 min. ATCase (16 μg/0.5 ml) in 4 mM phosphate buffer (pH 7.0) was immersed in a water bath at the indicated temperature for 5 min, after which 4.5 ml of cold Isosatric buffer was added. These Incubation mixtures were then diluted further for complement fixation. (A) Anti-ATCase diluted $^1/_{90,000}$. (B) ATCase-absorbed anticatalytic subunit diluted $^1/_{1200}$. Crosses represent C′ fixation curve observed with pure catalytic subunit. *Catalytic subunit equivalent (see legend to Figure 7). C′ stands for complement.

planation for the greater activity of ATCase with anti-ATCase: (1) Free catalytic or regulatory subunits completely inhibit the ATCase–anti-ATCase complement fixation (isolated catalytic subunit is a very potent inhibitor, *i.e.*, 0.1 μg of catalytic subunit/0.05 μg of ATCase yields 50% inhibition); and (2) mixtures of anti-regulatory subunit and anticatalytic subunits react more effectively with ATCase than with both free catalytic and regulatory subunits. This mixture of antisera simulates the activities of anti-ATCase with ATCase and its isolated subunits.

We have little experimental data to explain the greater activity of anticatalytic subunit toward catalytic subunit. After absorption with ATCase, the complement fixing titer of the antiserum toward catalytic subunit was reduced from $^1/_{11,000}$ to $^1/_{1200}$. This absorbed antiserum reacts much more effectively with catalytic subunit than with ATCase. The most likely explanation for the increased serologic activity of the catalytic subunit with its homologous antiserum is that some antigenic determinants on the free subunit are partially hidden when residing in the native enzyme.

Dissociation of ATCase by *p*-HMB results in a decrease in serologic activity of ATCase with anti-ATCase and concomitant increase of serologic activity with anticatalytic subunit. The relationship of this decrease in serologic activity with anti-ATCase and the generation of free catalytic subunits permits quantitative measurements of ATCase dissociation by *p*-HMB. This immunochemical method for measuring dissociation by *p*-HMB under a variety of conditions has led to the detection of conformation changes in ATCase resulting from interaction with several ligands (von Fellenberg *et al.*, 1968).

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Immunological Studies of Aspartate Transcarbamylase. II. Effect of Ligands on the Conformation of the Enzyme*

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ABSTRACT: Antibodies can be used to measure the dissociation of *Escherichia coli* aspartate transcarbamylase into its subunits (Bethell *et al.*, 1968b). The reactivity of the sulfhydryl groups with *p*-hydroxymercuribenzoate is influenced by ligands which stabilize different allosteric conformations of aspartate transcarbamylase at 25° (Gerhart, J. C., and Schachman, H. K. (1968), *Biochemistry 7*, 553). We have studied the nature of the *p*-hydroxymercuribenzoate dissociation of aspartate transcarbamylase at 0° using antibodies to detect the amount of dissociation. We have found that the *p*-hydroxymercuribenzoate at 0° depends upon the concentration of *p*-hydroxymercuribenzoate and aspartate transcarbamylase. Stereospecific ligands change the rate of *p*-hydroxymercuribenzoate dissociation. Carbamyl phosphate or the aspartate analog, succinate, added singly, increases the rate of *p*-hydroxymercuribenzoate dissociation. The maximal effects of carbamyl phosphate and succinate are additive . Carbamyl phosphate increases the binding of succinate to aspartate transcarbamylase. In phosphate buffer, aspartate, below 1 mM, also increases the rate of aspartate transcarbamylase dissociation, but, as the concentration of aspartate is raised, the enhancement of *p*-hydroxymercuribenzoate dissociation decreases and under certain circumstances aspartate transcarbamylase is stabilized against *p*-hydroxymercuribenzoate dissociation. The exact effect of aspartate is dependent upon the orthophosphate concentration. Cytidine triphosphate, adenosine triphosphate, uridine triphosphate, guanosine triphosphate, and pyrophosphate stabilize aspartate transcarbamylase toward *p*-hydroxymercuribenzoate. A direct effect of the ligands, carbamyl phosphate plus succinate or cytidine triphosphate, on aspartate transcarbamylase or its subunits was studied with antibodies to aspartate transcarbamylase, or to the catalytic subunit, or to the regulatory subunit of aspartate transcarbamylase. These studies may indicate that the major conformational effect occurs in the subunit to which the ligand binds. Some effects of urea are also discussed.

Dissociation of *Escherichia coli* ATCase[1] can be measured quantitatively using antibodies against the native enzyme and its subunits (Bethell *et al.*, 1968b). Kinetic data suggest (Gerhart and Pardee, 1962, 1964) and recent experiments demonstrate conformational changes in ATCase resulting from interaction with ligands (Gerhart and Schachman, 1968; Changeux and Rubin, 1968). In this paper we offer further evidence based on serologic studies for conformational changes in ATCase due to ligand binding. According to the predictions of Monod *et al.* (1965) substrates favor a loose, and feedback inhibitors a tight quaternary structure of an allosteric enzyme. Thus, it might be expected that the substrates would favor the dissociation of the enzyme into its subunits and the feedback inhibitor would inhibit that dissociation. In the present paper we have studied the dissociation of the native enzyme into its subunits and the effect of ligands on the dissociation.

In the preceeding paper (Bethell *et al.*, 1968b) it was demonstrated that changes in the complement fixing activities are very sensitive indicators of changes in ATCase

* Publication No. 604 from the Graduate Department of Biochemistry, Brandeis University, Waltham, Massachusetts 02154, and from the Department of Biochemistry, School of Medicine, University of North Carolina at Chapel Hill, North Carolina 27514. *Received July 30, 1968.* Supported in part by research grants from the National Institutes of Health (AI-01940 and HD-02148) and the National Science Foundation (GB 5354 and GB 7381). L. L. is an American Cancer Society Professor of Biochemistry (Award No. PRP-21).

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[1] Abbreviations used that are not listed in *Biochemistry 5*, 1445 (1966), are: ATCase, aspartate transcarbamylase; CAP, carbamyl phosphate; *p*-HMB, *p*-hydroxymercuribenzoate.