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The Effect of Chaotropic Ions on the Dissociation of Antigen–Antibody Complexes*

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ABSTRACT: Hydrophobic, ionic, and hydrogen bonds are probably responsible for the interaction of antigens and antibodies. Certain ions such as thiocyanate, perchlorate, and iodide (chaotropic ions), known to unfold or dissociate macromolecular structures by disrupting these bonds, have now been found to dissociate primary antigen–antibody complexes without destroying the immunospecific activity of antibody. The order of decreasing effectiveness is thiocyanate > perchlorate > iodide. Demonstrations that chaotropic ions can dissociate primary antigen–antibody complexes are provided by the recovery of functionally active antibody from influenza virus–antiinfluenza complexes, and from complexes of antibody with solid immunoadsorbents in which the antigen is covalently bonded to an inert matrix. Other experimental approaches include inhibition of specific precipitation and dissolution of specific precipitates.

The three-dimensional structure of protein molecules is maintained by numerous, relatively weak bonds formed between different parts of the molecule. Evidence accumulated during the past 10 years indicates that the nature of these bonds, their relative positions, and the conformation of the resulting structure are a direct consequence of the sequence of amino acid residues in the molecular chain (Kauzmann, 1959; Singer, 1965). In "simple" or unconjugated proteins, the possible types of such bonds are limited, and include hydrogen bonds, "hydrophobic" bonds, electrostatic attractions, and London dispersion forces. One structural role of disulfide bonds seems to be that of locking into place a single, relatively stable conformation.

A line of evidence substantiating the importance of these weak bonds in influencing the tertiary structure stems from the unfolding or disruption of macromolecules by urea, guanidine, and more recently by certain ions (chaotropic ions) (Hamaguchi and Geiduschek, 1962; von Hippel and Wong, 1964; Robinson and Jencks, 1965a,b; Nagy and Jencks, 1965; Warren and Cheatum, 1966; Warren and Peterson, 1966). Under appropriate conditions, these materials presumably break a sufficient fraction of the bonds responsible for the tertiary structure, and the molecule unfolds under the influence of thermal forces.

Antibodies and antigens also owe their conformational stability to the bond types enumerated above. The unfolding of antibody in guanidine and the refolding with recovery of antibody activity upon the removal of guanidine have been demonstrated (Whitney and Tanford, 1965), but there exists a possibility (Singer and Doolittle, 1966), that even though most of the molecule certainly is unfolded, the active site may remain intact during the guanidine treatment.

The various weak bonds mentioned above are responsible not only for the conformation of the antigen and antibody molecules themselves, but also probably furnish the binding free energy for the formation of the antigen–antibody complex (Karush, 1962). Accordingly, it may be expected that chaotropic ions would dissociate antigen–antibody complexes, possibly under conditions innocuous to the antigen and antibody molecules themselves.

* From the Division of Biochemistry, Scripps Clinic and Research Foundation, La Jolla, California. *Received December 9, 1966.* Supported by The John A. Hartford Foundation and the National Science Foundation (NSF GB-4288).

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We have investigated the effects of thiocyanate, perchlorate, and iodide ions upon the antigen–antibody reaction using the ovalbumin, bovine serum albumin, and influenza virus systems. The experiments pertain to several aspects of the antigen–antibody reaction, *viz.*, the inhibition of specific precipitation, the dissolution of specific precipitates, irreversible effects of chaotropic ions upon antibody, and the recovery of antibody activity from complexes by dissociation with chaotropic ions.

The recovery of functionally active antibody from complexes with influenza virus and with solid immunoadsorbents in which the antigen is covalently bonded to an inert matrix shows that chaotropic ions can dissociate the primary bonds between antigen and antibody. The results are pertinent to the theory of the molecular mechanism of the antigen–antibody reaction and, in addition, are of practical importance since they afford new methods for the immunospecific purification of antibody.

Experimental Section

Materials. Ovalbumin (OV)[1] (five times crystallized) and BSA crystalline were obtained from Pentex, Inc. Rabbit anti-OV was made by a course of 0.5-mg intravenous injections of OV at 1-week intervals over a period of 2 months, followed by booster injections 1 or 2 months apart. Two weeks after each booster injection, antiserum was collected and pooled.

The antibody was purified by the ammonium sulfate precipitation method (Campbell *et al.*, 1963) except that 0.55 volume of ammonium sulfate was used for each volume of serum. Rabbit anti-BSA was obtained through the courtesy of Dr. P. Minden of this institution or from Antibodies, Inc. This antibody was prepared by immunization with BSA in complete Freund's adjuvant. Influenza virus (Asian 305) and rabbit anti-influenza serum were obtained through collaboration with Cordis Laboratories.[2] Immunospecific adsorbents (Campbell *et al.*, 1951) were made by a modification of the carbodiimide method (Weliky *et al.*, 1964). The CMC (Whatman CM 11) was first coupled to PPDA with a water-soluble carbodiimide (N. Levandoski, personal communication) after which the PPDA-CMC was coupled to BSA. The amounts of PPDA and BSA combined were determined by micro-Kjeldahl analysis.

Other inorganic and organic chemicals were of CP or reagent grade, and were used without purification. A buffer solution containing 0.15 M sodium chloride, 0.01 M disodium hydrogen phosphate, and 0.005 M sodium dihydrogen phosphate is termed diluent buffer.

[1] Abbreviations used: OV, ovalbumin; BSA, bovine serum albumin; PPDA-CMC, *p*-phenylenediamine coupled to CMC; BSA-PPDA-CMC, BSA coupled to PPDA-CMC; CMC, carboxymethylcellulose.

[2] This portion of the project was supported in part under a contract between the Office of the Surgeon General and Cordis Laboratories, Miami, Fla. (contract no. DA-49-193-MD-2829), under the supervision of Dr. Seymour P. Halbert.

Methods

Quantitative Precipitin Determinations. Antibody was measured by quantitative precipitin determinations at varying antigen and constant antibody concentration. The reaction mixture (1.25–2.0 ml) was incubated for 1 hr at 37° and overnight at 4°, after which the precipitates were washed twice by centrifugation with 2-ml portions of 0.15 M NaCl, and then dissolved in 2 ml of 0.2 M NaOH. The optical density of the resulting solution was immediately read at 280 mμ, and the amount of antibody in the precipitate was calculated by assuming that all of the antigen was precipitated at equivalence. Since the extinction coefficients for antibody and for OV or BSA are not the same, optical density values outside the equivalence zone are valid for comparative purposes, but are not directly interpretable as total precipitated protein.

Preparation of Suspensions of Antigen–Antibody Precipitates. The antigen–antibody precipitates used in these experiments were prepared by mixing antigen and antibody in diluent buffer. The mixture was incubated at 37° for 1 hr and overnight at 4°. The precipitate was washed twice by centrifugation with 2-ml portions of 0.15 M NaCl and suspended in a suitable volume of 0.15 M NaCl. In typical experiments, the OV–anti-OV precipitates were prepared from 4 ml of rabbit anti-ovalbumin containing 2.2 mg of precipitable antibody/ml and 11.5 mg of total protein/ml with 0.7 mg of OV in 16 ml of diluent buffer.

The BSA–anti-BSA precipitates were prepared from 3 ml of anti-BSA containing 8.5 mg of precipitable antibody/ml and 21.4 mg of total protein/ml with 0.1 ml of BSA containing 38.3 mg of BSA/ml in 10 ml of diluent buffer.

Tests for Irreversible Effects of Chaotropic Ions upon Antibody. Solutions of the ion to be tested, contained either in diluent buffer or in sodium acetate buffer (NaOAc, 0.2 M; AcOH, 0.2 M), were added to an antibody solution. The mixture was allowed to stand 1 hr at room temperature, after which the chaotropic ion was removed by two successive 8-hr dialyses *vs.* 2 l. of diluent buffer. The ratio of outside to inside volumes during the dialysis was about 200, and the $t_{1/2}$ for the conditions used was about 2.5 hr. ($t_{1/2}$ for dialysis is taken to be the time for the concentration of a diffusible species to decrease to one-half its original concentration, when the outside concentration is zero.) In a typical experiment a mixture of 0.8 ml of concentrated buffer (1.5 M NaCl, 0.1 M Na_2HPO_4, and 0.05 M NaH_2PO_4), 1.6 ml of 10 M NaSCN, and 1.6 ml of H_2O were added with stirring to 4 ml of antiovalbumin containing 0.7 mg of precipitable antibody/ml and 11.1 mg of total protein/ml. After 1 hr the solution was dialyzed as outlined above. The reaction with Fe^{3+} was used to test for SCN^- in the outside solution. The second dialysis contained about 1% as much SCN^- as the first portion. The dialyzed antibody solution was analyzed by the quantitative precipitin test (2 M SCN^- curve in Figure 5).

Measurement of Antiinfluenza Antibody by a Light Transmission Method. The antibody solution contained

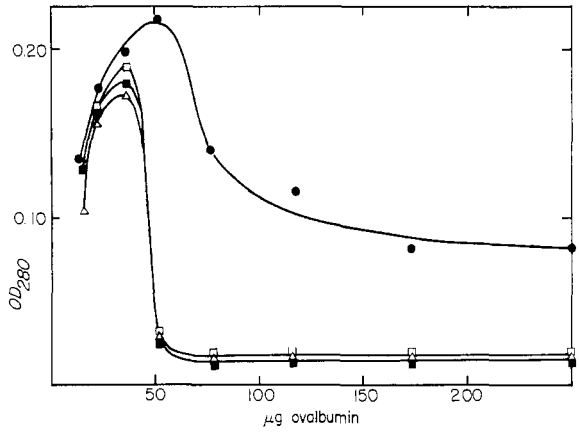

FIGURE 1: Quantitative precipitin curves showing the inhibition of precipitation of ovalbumin and rabbit antibody at pH 6.6 when thiocyanate, perchlorate, or iodide is present in the reaction mixture. Diluent buffer was also present in all instances. (●) control. (□) 0.5 M sodium iodide. (■) 0.5 M sodium perchlorate. (Δ) 0.5 M sodium thiocyanate.

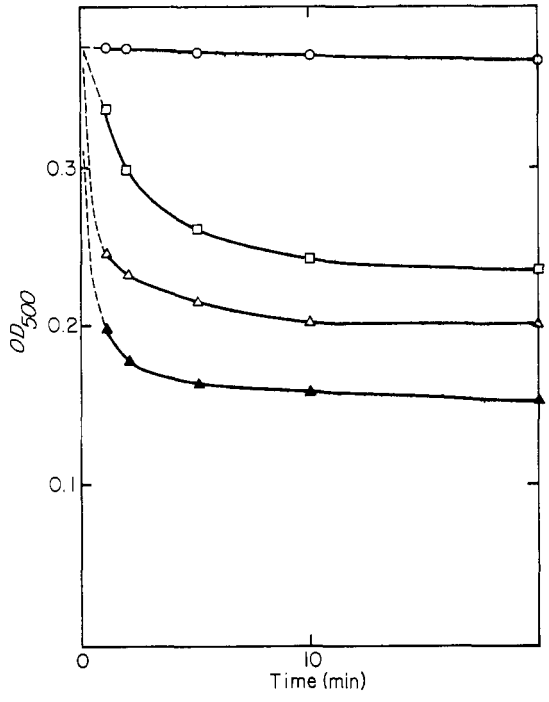

FIGURE 2: Dissolution of washed ovalbumin–antiovalbumin precipitates (optimal precipitation zone) in various ions added to diluent buffer. A small measured portion of the specific precipitate finely suspended in diluent buffer was added to the ion, and the light transmission was measured as a function of time (pH 6.2). Corrections for the effect of refractive index on the scattering have been applied as described in the text. (○) control. (□) 2 M potassium iodide. (Δ) 2 M sodium perchlorate. (▲) 2 M sodium thiocyanate.

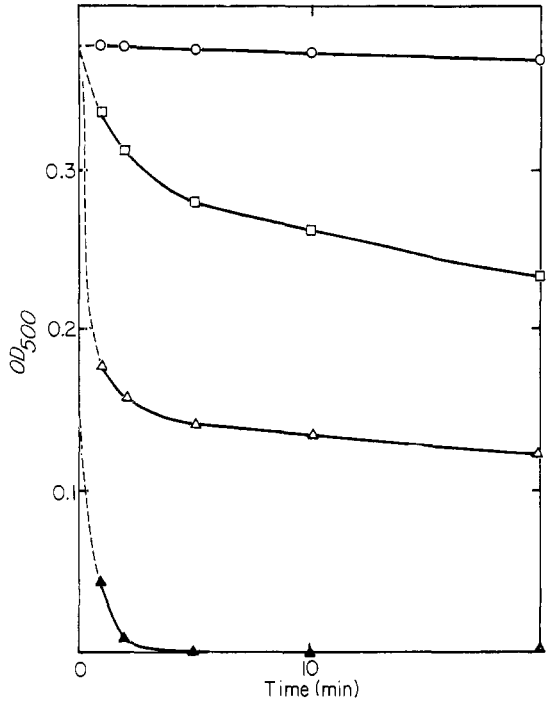

FIGURE 3: Similar to Figure 2 except ion concentrations were 4 M. (○) control (pH 6.8). (□) iodide (pH 5.6). (Δ) perchlorate (pH 5.3). (▲) thiocyanate (pH 6.0).

2 ml of 0.3 M NaCl and diluent buffer was placed in a cuvet for assay. A blank reading was taken and 50 μl of virus (OD_{260} 17 cm^{-1}) was added with stirring. The optical density at 436 mμ, being strongly sensitive to turbidity changes, was measured as a function of time. The slopes of the resulting curves are approximately proportional to antibody concentration. The high ionic strength (0.37) was found to be necessary to depress the nonspecific interaction between normal γ-globulin and influenza virus.

Immunospecific Purification of Antiinfluenza Antibody. Influenza virus (4.5 ml with OD_{260} 10.2 cm^{-1}) was mixed with 2 ml of antiinfluenza serum or with 2 ml of normal rabbit serum as a control. The solutions were incubated at 37° for 30 min and overnight at 4°. The precipitates which formed in both the normal and immune systems were centrifuged and washed twice with 2-ml portions of 0.15 M NaCl. The precipitates were dissolved in 10 ml of either (a) 4 M NaSCN containing 0.2 M NaOAc and 0.2 M AcOH (pH 4.5), or (b) 3 M NaSCN containing 0.05 M K_2HPO_4 and 0.025 M KH_2PO_4 (pH 6.0). The solutions were centrifuged at 39,000 rpm for 3 hr and filtered on Whatman No. 42 filter paper to remove all clumps of viral material. The clear solutions were dialyzed *vs.* 2 l. of diluent buffer to give, in the case of 4 M NaSCN, 12.5 ml having an OD_{280} of 0.116 for the normal and 0.351 for the immune preparation. The solutions were concentrated by vacuum ultrafiltration and tested by the light transmission method.

Immunospecific Purification of Anti-BSA on BSA-

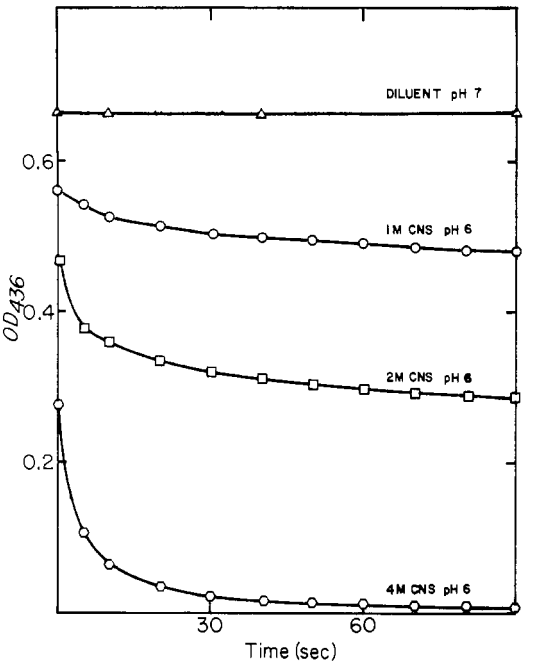

FIGURE 4: Light transmission measurements on a short-time scale showing the course of the dissolution of ovalbumin–antiovalbumin precipitates in various concentrations of sodium thiocyanate added to diluent buffer. The zero-time points for the three lower curves have been calculated by applying refractive index corrections as described in the text.

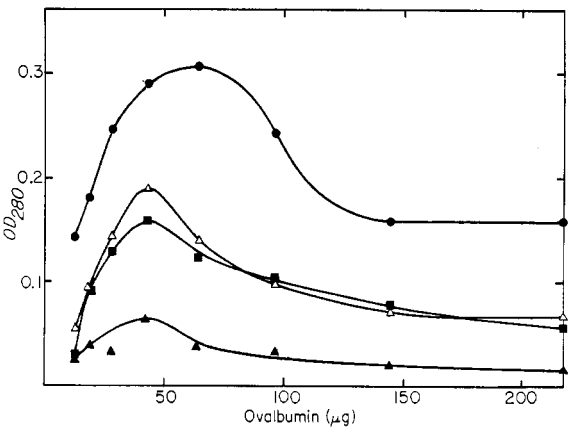

FIGURE 5: Dissolution of ovalbumin–antiovalbumin precipitates in diluent buffer with added ions. Specific precipitates were first formed at the antigen concentrations indicated along the abscissa (1 hr at 37° and overnight at 4°). The OD_{280} is proportional to the amount of washed precipitate remaining undissolved after 10 min at room temperature when suspended in the ionic concentrations shown. (●) original precipitin curve in diluent buffer. (Δ) 2 M sodium perchlorate. (■) 1 M sodium thiocyanate. (▲) 2 M sodium thiocyanate.

PPDA-CMC. Anti-BSA solution (2–9 ml) containing 4–8 mg of precipitable antibody was added to a slurry consisting of 2 g of BSA-PPDA-CMC and 20 ml of 0.15 M NaCl. The mixture was stirred at room temperature for 30 min, poured into a 1-cm glass column, and washed with 0.15 M NaCl until the OD_{280} of the effluent was less than 0.01. Eluent (20 ml) (2 M NaSCN, $NaClO_4$, or NaI in diluent buffer) was poured into the column, and the eluate was collected in a cuvet for optical density measurements.

Substantially all of the antibody appeared within the first 10 ml of eluate. This solution was dialyzed *vs.* two successive volumes of 3 l. of diluent buffer for 8 to 16 hr, respectively. Total protein in the dialyzed eluate was measured by OD_{280} and by quantitative biuret test; precipitable antibody was determined by quantitative precipitin test.

Results

Inhibition of Precipitation. The specific precipitation of ovalbumin by antiovalbumin is inhibited by thiocyanate, perchlorate, or iodide ions. The inhibition is accompanied by a shift of the optimal precipitation zone toward lower antigen concentrations (Figure 1). Figures 1, 5, and 6 employed three different antibody preparations. All experiments on any one figure were performed on the same preparation. Extinction coefficients ($E^{1\%}_{280\,m\mu}$) for antigen and antibody in 0.2 M NaOH were found to be 6.94 and 13.95 for BSA and rabbit 7S γ-globulin, respectively. Using these values, the amount of protein precipitated at the optimal precipitation zone can be calculated by assuming that all of the antigen is precipitated.

Dissolution of Precipitates. The dissolution of precipitates (reversal of precipitation) by thiocyanate, perchlorate, or iodide near neutrality (pH 5.3–6.7) as indicated on the figures was measured by light transmission (Figures 2–4). These measurements show that the decreasing order of effectiveness is $SCN^- > ClO_4^- > I^-$. By varying the pH, the rate and extent of dissolution were found to be about the same at pH 6 and 8, but considerably greater at pH 4.

The presence of the added ions increases the refractive index of the solution, which in turn will decrease the light scattering by the suspended particles. This effect was compensated for by measuring the optical density of the suspension in glycerol solutions of varying refractive index. The decreases in scattering noted were used as corrections for the data obtained in thiocyanate, perchlorate, and iodide solutions. The light scattering of the suspension in glycerol did not change during the experiment. In Figures 2 and 3, all the optical density values in the lower three curves have been multiplied by the ratio of scattering in diluent buffer to scattering in glycerol solution, so as to elevate the entire curve and to make the zero-time point the same for all. The concentration of glycerol was chosen to have the same refractive index as that of the solution of chaotropic ion. In Figure 4 the correction has been used to calculate a separate zero-

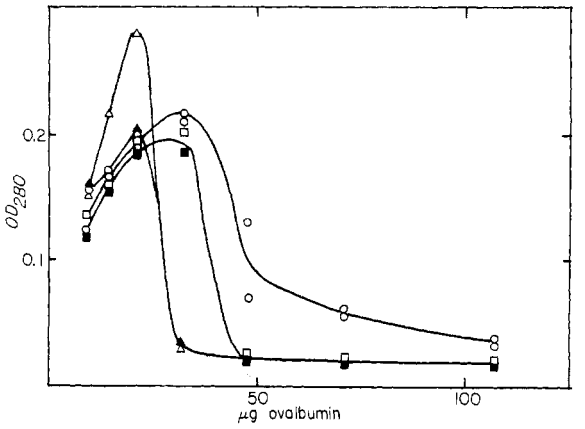

FIGURE 6: Test for irreversible alteration in antiovalbumin after 1-hr exposure at room temperature to various ions added to diluent buffer. After the 1-hr period the added ions were removed by dialysis *vs.* diluent buffer, and quantitative precipitin tests were made with the results shown. (○) control. Duplicate sets of points are shown and a smooth curve has been drawn to average the results. (□) 2 M sodium perchlorate in diluent buffer (pH 6). (■) 2 M sodium thiocyanate in diluent buffer (pH 6). The results for 2 M perchlorate and 2 M thiocyanate at pH 6 are very similar, and only one smooth curve has been drawn for both. (Δ) 2 M sodium perchlorate in acetate buffer (pH 4.5). (▲) 4 M sodium perchlorate in diluent buffer.

time point for each curve corresponding to the scattering the original suspension should have at each refractive index. No correction was applied to the control curve in any case.

The dissolution of specific complexes was also studied (Figure 5) by measuring the amount of precipitate remaining insoluble after treatment with various ionic solutions. The order of effectiveness of the ions determined by this means is the same as that found by light transmission measurements.

Tests for Irreversible Alteration of Antibody by Chaotropic Ions. Exposure of antiovalbumin to thiocyanate or perchlorate followed by removal of the ions results in irreversible effects if either the ionic concentration is too high or the pH is too low (Figure 6). Very little change is evidenced at 2 M SCN^- or ClO_4^- near neutrality except in the region of antigen excess. In 2 M ClO_4^- at pH 4.5, the beginning of additional changes can be seen. At this pH, pretreatment with 4 M concentrations of either ion produces increased amounts of precipitation when antigen is subsequently added.

Immunospecific Purification of Antiinfluenza Antibody. Dissociation of the specific precipitate formed by influenza virus and antiinfluenza antibody was carried out in 4 M SCN^- at pH 4.5, and in 3 M SCN^- at pH 6.0. The yields of immunospecifically purified antibody recovered were 70% for 4 M SCN^- and 65% for 3 M SCN^-, as assayed by the turbidity method (Figure 7). A parallel procedure starting with normal serum resulted in a preparation devoid of antiinfluenza virus antibody activity.

Immunospecific Purification of anti-BSA on BSA-PPDA-CMC. Anti-BSA antibody adsorbed on the BSA-PPDA-CMC columns was eluted in highly concentrated form with 2 M solutions of NaSCN, $NaClO_4$, and NaI in diluent buffer (Table I). No attempt was made to maximize yields. Using freshly prepared columns, 65% of the precipitable antibody present in the original antiserum was recovered by thiocyanate treatment, and 36% by perchlorate. Significant amounts, 45 and 25%, respectively, were recovered by thiocyanate or iodide elution from columns which were reused after washing with 0.15 M NaCl to $OD_{280} < 0.010$.

Discussion

The results obtained show that antigen–antibody complexes are dissociated by thiocyanate, perchlorate, or iodide ions in the region of 1–4 M near neutrality. At lower pH ranges the reaction is even more rapid, but the complications due to irreversible effects, possibly similar to those observed previously (Kleinschmidt and Boyer, 1952a,b; Turner and Boyer, 1952), are increased.

The proof that primary complexes are dissociated rests upon the demonstration that functionally active antibody can be recovered from virus–antibody complexes and from solid immunoadsorbents by treatment with chaotropic ions. The yields of antibody from immunospecific columns varied from 25 to 67%, depending upon the ion used and other experimental conditions (Table I). The purity of these preparations as judged by quantitative precipitin determinations was as high as 84%.

Recovery of antiinfluenza antibody for two different preparations was 70% using 4 M NaSCN (pH 4.5) and 65% using 3 M NaSCN (pH 6.0) as the dissociating conditions. These data largely eliminate the possibility that the dissociation was due to pH effects.

While a clear distinction must be drawn between dissolution of a specific precipitate and the dissociation of a complex, the two phenomena are probably closely related. The classical picture of the formation of antigen–antibody precipitates involves the binding together of antigen and antibody into primary complexes followed by the aggregation of the complexes by formation of further antigen–antibody bonds. The union of bivalent antibody and multivalent antigen thus allows for the formation of branching chains, giving rise to large aggregates and ultimately to precipitates. This picture points up the inherent similarity between primary complex formation and precipitate formation. The fact that univalent antibody (prepared by the papain digestion of bivalent antibody) does not form specific precipitates with antigen is in harmony with the classical picture.

The ultimate formation of a precipitate is thus seen as an indication that the association of antigen and antibody has exceeded a certain critical aggregate size.

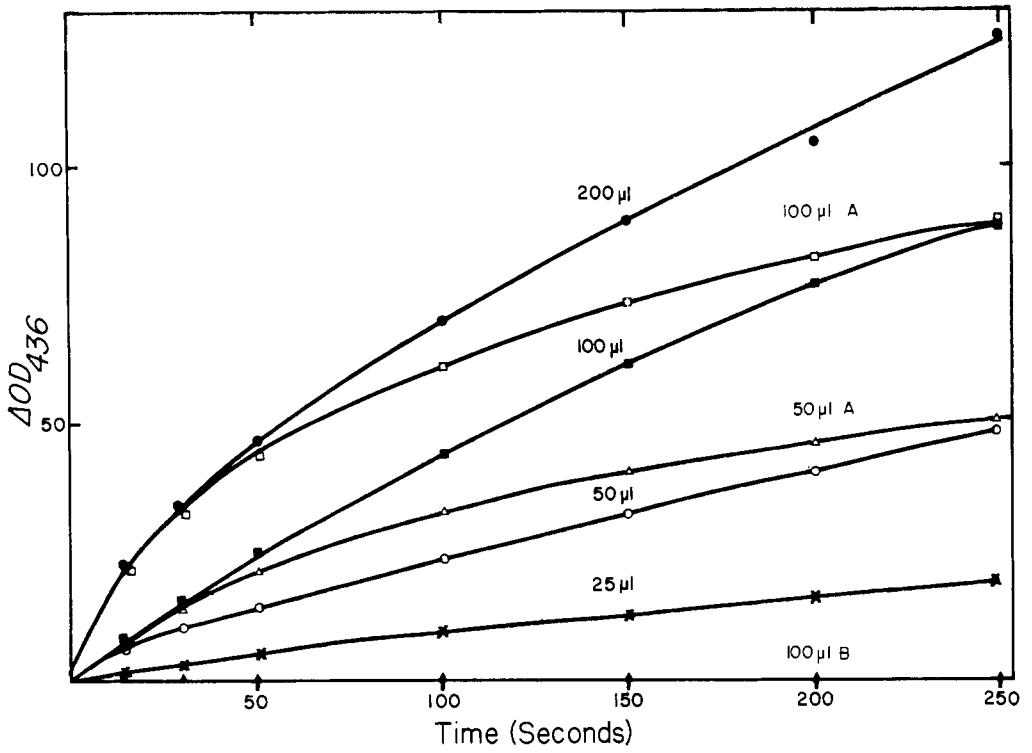

FIGURE 7: Assay of antiinfluenza antibody by the light transmission method. The four standard curves with 25, 50, 100, and 200 µl of antiserum were used to calibrate the method. Each milliliter of the sample of antibody (A) obtained by thiocyanate dissociation of the virus–antibody complex was derived from 1.4 ml of antiserum. Since the antibody concentration in the final material is approximately equal to that in the antiserum, the yield of antibody was about 70%. As a control, normal serum was also processed by the thiocyanate treatment. The resulting material (B) was inactive in the assay as shown. An additional experiment using 3 M NaSCN at pH 6.0 yielded a purified antibody in 65% yield which behaved similarly to preparation A.

TABLE 1: Purification of Anti-BSA Antibody by Immunospecific Adsorption and Elution with Chaotropic Ions.

Expt	Applied to Column (mg)		Eluting Ion (2 M, in diluent buffer)	Recovered from Eluate (mg)	
	Protein	Antibody		Protein	Antibody
I-A	60.8	7.6	SCN^-	5.6	4.7
II-A	110	4.4	SCN^-	4.2	2.9
II-B	110	4.4	ClO_4^-	2.3	1.6
III-A[a]	178	3.8	SCN^-	3.4	1.7
III-B[a]	178	3.8	I^-	2.5	1.0

[a] Adsorbent from II-A and II-B, respectively, reused after washing with 0.15 M NaCl to $OD_{280} < 0.01$.

The whole phenomenon would thus appear to be one continuous process in which the progressive steps are moderated by antigen–antibody affinity, antibody heterogeneity, and the environment. One is hard-pressed to devise an alternative model in which the forces responsible for primary complex formation are clearly different from those producing the large precipitating aggregates. An important corollary to be drawn from these ideas is that environmental agents which favor dissolution of precipitates should also favor dissociation of the primary antigen–antibody bonds. For this reason the turbidity changes indicative of the dissolution of precipitates constitute valuable qualitative guides in rapidly delineating conditions favorable to dissociation. Our results support this line of reasoning since the order of decreasing effectiveness, SCN^-

$> ClO_4^- > I^-$, has been observed for both phenomena (*cf.* Figures 1–5 and Table I).

The effect of chaotropic ions on specific precipitation (Figure 1) is to decrease the amount of precipitate and to shift the optimal precipitation zone toward lower antigen concentrations. This shift may be due to precipitation of only that antibody of somewhat higher affinity for the antigen. This fraction of antibody, being less than the total observed in the control curve, requires less antigen for precipitation.

In contrast, exposure of antibody to high concentrations of chaotropic ions, especially at low pH, followed by removal of the ions by dialysis resulted in increased precipitation when antigen was added. This effect is at present unexplained.

In Figures 2–4 the dissolution of the precipitate appears to reach an equilibrium within a few minutes. As the ionic concentrations are increased, or in going from iodide to perchlorate to thiocyanate, the equilibrium levels become lower and the reaction rates higher. Some irreversible effects are produced in antiovalbumin by exposure to 2 M SCN^- or ClO_4^- at pH 6, and these effects are accentuated at lower pH ranges. However, antiinfluenza antibody activity was recovered to an extent of 70%, even at 4 M SCN^- at pH 4.5 (Figure 7). The detailed shape of the precipitin curve is probably a more sensitive indication of alteration than merely observing the amount of antibody precipitated at equivalence. Possibly not all antibodies have the same stability in solutions of chaotropic ions, as might be expected on the basis of the different amino acid compositions and sequences in different antibody molecules. The recovery of antiinfluenza and anti-BSA antibodies in highly concentrated form demonstrates the usefulness of dissociation by chaotropic ions in immunospecific purifications. The results obtained in this study show that the effectiveness of ions in reversing the antigen–antibody reaction increases in the order $I^- < ClO_4^- < SCN^-$, which is the same order found by others for the disruption of the tertiary structure of proteins.

The mechanism of action of the chaotropic ions on macromolecules generally, and upon antigen–antibody complexes in particular, probably includes several types of effects. (1) The addition of ions may be expected to exert effects by electrostatic shielding, thus weakening the interaction between charged groups. (2) There is a classical salting-out effect at the high ionic strengths employed, which should tend to promote folding-up of the protein molecule and to decrease its solubility. (3) The binding of ions such as iodide and thiocyanate by BSA is well known (Scatchard and Black, 1949; Scatchard *et al.*, 1950). While serum albumins are perhaps unique in their ability to bind a wide variety of ions and neutral molecules, other proteins probably also bind, but to a smaller extent. At the high ionic concentrations used in our work, even weak binding might give rise to important effects. For example, binding usually will produce an alteration in net charge. In addition, groups or sites normally involved in maintaining tertiary structure or in forming intermolecular bonds (antigen–antibody complexes) could be masked by the bound ions. If this occurs, ion binding could lead to unfolding or dissociation. (4) This factor, probably of major importance, is the effect of chaotropic ions on hydrophobic bond formation. The free energy of formation of hydrophobic bonds (Kauzmann, 1959) is believed to be derived chiefly from an increase in entropy when organic groupings are withdrawn from aqueous media into organic surroundings. The enthalpy change for the transfer of benzene from liquid benzene to water is near zero, while the unitary entropy change is about −14 eu. These facts have been interpreted as indicating a high degree of order in the water structure surrounding the benzene molecule. The negative entropy change makes the unitary free-energy change positive, and causes apolar groups to cluster together and to leave the water phase (hydrophobic bonding).

The presence of chaotropic ions may alter the water structure to such an extent that the ordering of water about apolar groups is no longer necessary, thus permitting these groups to enter the water phase and allowing unfolding or dissociation processes to take place. The net effect of a chaotropic ion upon tertiary structure probably depends upon the relative magnitudes of the salting-out, the binding and the hydrophobic effect. At one extreme are ions such as sulfate, which salt out strongly and favor helical content and association, while at the other extreme are ions like thiocyanate, which salt out weakly and favor random-coil conformations and dissociation. As shown here, these differences in ionic properties can be exploited to study the dissociation of antigen–antibody complexes and to devise new procedures for the immunospecific purification of antibody.

Acknowledgments

The technical assistance of T. Grow and J. Kavanagh is gratefully acknowledged.

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Purification and Partial Characterization of Enterotoxin C Produced by *Staphylococcus aureus* Strain 137*

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ABSTRACT: Enterotoxin C elaborated by *Staphylococcus aureus* strain 137 was purified by chromatography on a CM-cellulose column and gel filtration through Sephadex G-75 and G-50. The purified enterotoxin exhibited a high degree of homogeneity as determined by synthetic boundary spreading and by electrophoresis. This was confirmed by estimation of purity by the double-diffusion tube technique. The highly purified toxin is a simple, colorless, and antigenic protein with a sedimentation coefficient of 3.0 S. A molecular weight of about 34,100 was obtained from sedimentation and diffusion measurements. The basic nature of the protein was revealed by its isoelectric point which is 8.6 in Veronal buffer of 0.1 ionic strength. It possesses a marked degree of toxicity; 5 μg produces emesis in rhesus monkeys (2–3 kg) within 2–5 hr after intragastric administration.

Data on intrinsic viscosity, frictional ratio, and axial ratio suggest compactness of the enterotoxin molecule.

Various strains of *Staphylococcus aureus* produce a group of toxic substances which are called enterotoxins because of their effects on the gastrointestinal tract. Vomiting and diarrhea occur 2–5 hr after ingestion of food contaminated with these substances. An amount as low as 5 μg causes typical food poisoning symptoms when administered intragastrically to rhesus monkeys.

In 1963, a systematic nomenclature using sequential lettering was established to classify these food poisoning agents as immunologically distinct entities (Casman *et al.*, 1963). The purification and physicochemical properties of enterotoxin A (Chu *et al.*, 1966) and exterotoxin B (Bergdoll *et al.*, 1959, 1961, 1965a; Frea *et al.*, 1963; Schantz *et al.*, 1965; Spero *et al.*, 1965; Wagman *et al.*, 1965) have been reported.

An intensive search for new immunological types of enterotoxin stemmed from the common occurrences of staphylococcal food poisoning. In 1965, the identification of a new enterotoxin as enterotoxin C was reported (Bergdoll *et al.*, 1965b) and *S. aureus* strain 137 was selected as the prototype strain. *S. aureus* strain 361 elaborates the same immunological type of enterotoxin as that produced by strain 137. Availability of the purified enterotoxins produced by strains 137 and 361 made it possible to study and compare the exterotoxins from the two strains. Dissimilarities in certain properties have been observed which are presented in the accompanying paper (Avena and Bergdoll, 1967).

This communication deals with the purification of exterotoxin C produced by *S. aureus* strain 137. It also describes some of the properties of the purified enterotoxin.

Experimental Section

Materials. The CM-cellulose used in this work is a Selectacel ion-exchange product, no. 77, type 20 (Carl Schleicher & Schuell Co.). Before use, it was treated with 0.1 N NaOH, washed with distilled water until the pH of the washings was 7.0–7.5, followed by treatment with 0.1 N HCl, and then washed thoroughly to remove excess acid. Before packing into the columns,

*From the Food Research Institute and Department of Foods and Nutrition, University of Wisconsin, Madison, Wisconsin. *Received December 7, 1966.* This investigation was supported by a contract with the U. S. Army Biological Center, Fort Detrick, Frederick, Md.