those of the other fractions, the solutions were combined and were concentrated to 20 ml. The addition of 150 ml. of distilled methanol resulted in the immediate crystallization of the product. After the mixture had stood overnight, the reddish-brown crystals mixed with a waxy-white, low-melting solid were filtered off. The impurity, which was soluble in chloroform, benzene, methanol and water, was removed by washing the mixture repeatedly with hot water and methanol. The yield of crystalline residue of pure platinum(II) tetraphenylporphine was 52 mg. or 10% of the theoretical amount.

Tetra-(*p*-methoxyphenyl)-porphinopalladium(II) (X = OCH_3, M = Pd(II)).—This palladium(II) chelate was prepared with the same procedure as the one described above for tetraphenylporphinopalladium(II). All the metal chlorin, chlorin ligand, unreacted porphine and other impurities were removed by chromatographing a chloroform solution of this chelate on a talc column. Chloroform was also used as the developing solvent. The lustrous light red crystals weighed 0.263 g., which represents 58% of the theoretical yield.

Tetra-(*p*-methoxyphenyl)-porphinoplatinum(II) (X = OCH_3, M = Pt(II)).—A solution of the ligand in 200 ml. of glacial acetic acid was heated to 100° and 1.45 g. of potassium chloroplatinite dissolved in 4 ml. of water was added. A yellow-green color developed and a fine green precipitate appeared. Five grams of sodium acetate was then added and the reaction mixture was stirred. A slight brown-green color developed, but after the reaction mixture was heated at 100° for 20 hours, the color changed to yellow-green. The cooled solution was filtered on a Büchner funnel and the filtrate was saved. The purple-black solid was washed with water, dried at room temperature, dissolved in trichloroethylene, and was chromatographed on a talc column, the upper section of which contained oven-dried talc. A mixture of chloroform and trichloroethylene, and finally chloroform alone was used as the developing solvent. The first 500 ml. of eluate contained pure platinum chelate with absorption maxima at 540, 513, 475 and 407 mμ in benzene. Succeeding fractions, however, contained impurities with increased absorption at 540 mμ. Adsorbed in ascending order of the column were a section of gray-brown material, some unreacted ligand, and some brown-black deposits of platinum. The impure chloroform fractions were rechromatographed on talc. All the orange-colored solutions obtained were combined, concentrated and crystallized by dilution with distilled methanol. Only 31 mg. or 6% of the theoretical yield of pure orange-red crystals was obtained. *Anal.* Calcd. for $C_{48}H_{36}O_4N_4Pt$: C, 62.12; H, 3.88; Pt, 21.05. Found: C, 62.10; H, 3.90; Pt, 20.60.

Concentration of the acetic acid filtrate from this main reaction with 0.1 g. of additional potassium chloroplatinite at 10–20 mm. pressure and 53° yielded an amorphous green material which solidified from this solution as it was concentrated. The blue-black solid which formed when this material was dissolved in hot water was purified in a chloroform solution on a talc column. A 54-mg. sample of silvery-red-violet crystals was obtained after crystallization of the concentrated eluate with methanol.

Tetra-(*p*-chlorophenyl)-porphinopalladium(II) (X = Cl, M = Pd(II)).—A chloroform solution of the ligand and a mixture of 0.25 g. of palladium(II) chloride in 75 ml. of hot methanol and 3 g. of sodium acetate was refluxed for 2 hours. Further refluxing in this solvent media resulted in the formation of more metal chlorin, but did not appreciably improve the yield of palladium porphyrin. After isolation of the solid in the usual manner, it was again heated for 2 hours with 250 ml. of glacial acetic acid, additional palladium chloride, and sodium acetate. The color change from green to red-brown in this acid medium indicated that additional chelate had formed. Three chromatograms in trichloroethylene were necessary in order to obtain the palladium porphyrin eluate in a pure form. The yield of lustrous, pink-orange crystals weighing 0.125 g. which represents 28% of the theoretical amount.

Tetra-(*p*-chlorophenyl)-porphinoplatinum(II) (X = Cl, M = Pt(II)).—To 0.200 g. of tetra-(*p*-chlorophenyl)-porphine in 250 ml. of hot glacial acetic was added 0.3 g. of potassium chloroplatinite and 5 g. of sodium acetate in 10 ml. of water. The resulting green-brown mixture was refluxed for 24 hours. The solution was then concentrated to 100 ml., the solid was filtered off, and the filtrate was saved. The residue was washed with water and air-dried. The green filtrate was further treated at 100° with excess platinum chloroplatinite and sodium acetate for 15 hours. This brown-green solution was concentrated, diluted with water and the product was filtered off. The solid material was dissolved in trichloroethylene together with the solid in the first step, and this filtered solution was chromatographed on a talc column, the upper section of which was oven-dried. The orange eluate was free from any unreacted ligand, metal chlorin or other impurity. The eluate was concentrated and was diluted to six times its volume with methanol. The lustrous, orange-brown crystals obtained weighed 10 mg., which represents 4% of the theoretical value.

The absorption spectra were employed to characterize the tetra-(*p*-chlorophenyl)-porphine platinum(II) chelate. The characterization and purity of all other chelates were determined from elemental analysis, and the quality of the visible and ultraviolet absorption spectra[14]; extinction coefficients listed in Table IV are given as criteria of purity.

Infrared Spectra.—Infrared absorption measurements were carried out with a Perkin–Elmer model 21 double beam spectrophotometer. Sodium chloride optics were used in the region from 4000 to 650 cm.$^{-1}$, and an interchangeable potassium bromide prism assembly was substituted in order to make measurements in the 650 to 400 cm.$^{-1}$ region. All spectra were determined on the solid form with potassium bromide as a diluent.

WORCESTER, MASS.

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF NORTHWESTERN UNIVERSITY]

The Binding of Organic Ions by Proteins. Effects of Changes in Solvent and their Implications as to the Nature of the Complexes

BY IRVING M. KLOTZ AND SAMUEL W. LUBORSKY[1]

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The binding of dyes by serum albumin has been found to increase in solutions to which glycine or β-alanine has been added. However, this effect is not due simply to the substantial increase in dielectric constant but reflects some specific interaction of the added amino acid. These observations, as well as many other aspects of the formation of protein complexes, can be interpreted in terms of a model which largely attributes the stability of such complexes to the cooperative formation of an ice-like hydration lattice between the complexing species.

Introduction

The stability of protein complexes reflects largely the character of the macromolecule and small ligand, but it is also affected by the nature of the environment. The energetics of binding, however, are not generally explicitly expressed in terms of these factors. Rather the free energy of binding is usually apportioned between two terms,

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an intrinsic affinity and an electrostatic contribution

$$\Delta F = \Delta F_{\text{intrinsic}} + \Delta F_{\text{electrostatic}} \quad (1)$$

The details of the electrostatic term depend upon geometric features and charges of the participants in the complex as well as on the solvent, but in any event, the term includes the dielectric constant D as a factor. Thus, to a reasonable approximation equation 1 can be restated as

$$\Delta F = \Delta F_{\text{intrinsic}} + \Phi/D \quad (2)$$

where Φ represents all remaining factors of $\Delta F_{\text{elect.}}$ (Actually the dielectric constant also appears twice in the ionic strength term in Φ but in such a way that its contributions to Φ tend to cancel out.)

It follows then that in an interaction where the electrostatic effect contributes a positive term to the free energy of combination, a decrease in dielectric constant should increase the repulsion between species and hence decrease the extent of complex formation. Such a decrease in dielectric constant can be obtained by the addition of an organic solvent such as dioxane to the aqueous solution. Binding experiments in dioxane-water solutions[2] have in fact been carried out. However, the results are not amenable to simple electrostatic interpretation, because uncharged organic molecules, such as dioxane, can compete with organic ions for attachment to the protein. This competition alone could be responsible for the observed large decreases in binding by the protein.

To avoid this difficulty we have used an amino acid at a *p*H at which it is essentially all in its isoionic form. Several investigations[3,4] indicate that glycine is not bound by serum albumin. A two molar aqueous glycine solution has a dielectric constant of over 120, compared to 80 for water. Consequently the electrostatic factor in equation 2 should be decreased appreciably by added glycine.

In this paper a variety of binding experiments in such solutions of high dielectric constant are described. They indicate that equation 2 is an inadequate description of factors affecting the stability of protein complexes and suggest instead a profound effect of the molecular state of the solvent. Consequently a general inquiry has been made into many puzzling aspects of protein binding and an interpretation has been developed based on the viewpoint that the solvent generally plays an important molecular role in these interactions.

Experimental

Dialysis Experiments.—The extent of binding of azo compounds by bovine serum albumin was measured by the equilibrium-dialysis technique described previously.[5] Unless otherwise noted, all solutions contained 0.2% protein, in 0.01 *M* NaCl to minimize any Donnan effects. Solutions were adjusted to the desired *p*H by addition of hydrochloric acid or sodium hydroxide. As has been customary, separate experiments were carried out to establish the time necessary for equilibration. Nine hours was found sufficient, the period being slightly longer than that in the absence of glycine. Tests of reversibility of binding showed that glycine did not produce any difficulties.

Binding by the cellophane bags was especially high in solutions containing glycine. It was essential in these experiments, therefore, to obtain complete curves of bag-binding as a function of dye concentration and to use these for corrections of binding in the presence of protein.

Reagents.—All salts were reagent grade. Glycine and β-alanine were C.P. grade, the former having been purchased from the H.M. Company, Ltd., Santa Monica, Cal., and the latter from Mann Laboratories, New York. Crystallized bovine serum albumin was a product of Armour and Co. Methyl orange was a commercial sample of reagent grade. *p*-Aminoazobenzene (Eastman Kodak Co. "white label") was recrystallized three times from a methanol–water mixture. Analysis by titanous chloride reduction of the azo group indicated a purity of 96.3%.

Results

To examine the applicability of equation 2, the binding of methyl orange by bovine serum albumin was studied in a series of solutions with glycine concentrations ranging (in half-molar increments) from 0–2.5 *M*. The results at one *p*H, 6.2, are summarized in Fig. 1, in terms of r, the moles of bound dye per mole protein, *versus* the logarithm of (A), the concentration of free dye in equilibrium with the bound dye. It is immediately apparent that the extent of binding increases with increasing concentration of glycine and thus with increasing dielectric constant. Such a trend is qualitatively in agreement with equation 2 insofar as the net charge on the protein at *p*H 6.2 is negative and anions are being bound.

For a more quantitative comparison, the binding constant k_1 for the uptake of the first anion by the protein was evaluated from

$$\lim_{(A)\to 0}\left[\frac{r}{(A)}\right] = k_1 \quad (3)$$

by graphical methods described previously.[3] Thereafter, the free energy change ΔF_1° for the binding of the first anion by bovine serum albumin was computed from

$$\Delta F_1^\circ = -RT \ln k_1 \quad (4)$$

As expected from equation 2, ΔF_1° 's for solutions of different glycine concentration vary approximately linearly with $1/D$ (Fig. 2).

Binding experiments at *p*H 9.1 (Fig. 3) also illustrate the ability of glycine to increase the extent of complex formation. Surprisingly, however, the effect is not appreciably greater than at *p*H 6.2. The slope of the graph of ΔF_1° *versus* $1/D$ (Fig. 2) is not much greater[6] at *p*H 9.1 despite the fact that the net negative charge on the protein is larger by perhaps a factor of 3.

Experiments were carried out, therefore, at *p*H's 5.2 and 4.2, to extend the measurements into the range where the net protein charge changes from negative to positive. At both *p*H's glycine *increased* the extent of binding of negative ions just as it did at higher *p*H's, where the net protein charge was negative. The slope of ΔF_1° *vs.* $1/D$ did not change sign at the more acid *p*H (Fig. 2). We have thus additional indication that the effect of glycine is not due simply to a change in dielectric constant of the medium.

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(6) However, some interference with this expected increase may arise from the presence of substantial amounts of competing $NH_2CH_2COO^-$ anions in glycine solutions at *p*H 9.1.

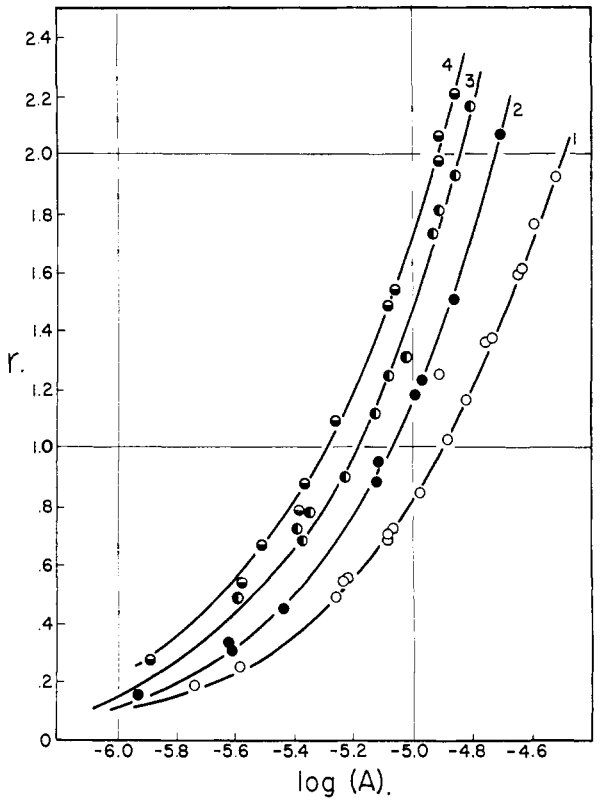

Fig. 1.—Effect of glycine at various concentrations on the binding of methyl orange by 0.2% bovine serum albumin in 0.01 *M* NaCl at *p*H 6.2 and 25°. Glycine concentrations: (1) ○, 0 *M*; (2) ●, 1.0 *M*; (3) ◐, 2.0 *M*; (4) , 2.5 *M*. Binding data for some glycine concentrations have been omitted for purposes of clarity.

A similar conclusion was reached from further experiments with still higher dielectric constants achieved by using β-alanine in place of glycine. Some of these are summarized in Fig. 4A. In 5 *M* aqueous β-alanine, the dielectric constant is approximately 250 as compared to 120 for 2 *M* glycine. Despite the much higher dielectric constant of the former solution, the effect on binding by serum albumin is almost identical with that of glycine solutions.

That the effect of glycine, and of β-alanine, is due to more than a change in dielectric constant is shown even more strikingly in experiments on the binding of an uncharged molecule, *p*-aminoazobenzene, by serum albumin. Here, too, the addition of 2 *M* glycine produces a distinct increase in uptake of the small (uncharged) molecule (Fig. 4B). Since the interaction is between protein and a molecule without charge, the increased binding cannot be attributed to an electrostatic effect

Discussion

The observed behavior of protein complexes in the presence of high concentrations of glycine can be interpreted in terms of some concepts of the nature of the hydration of proteins.[7] A discussion of binding in the presence of glycine can more readily be understood, however, after some remarks on the effects of solutes on the structure of

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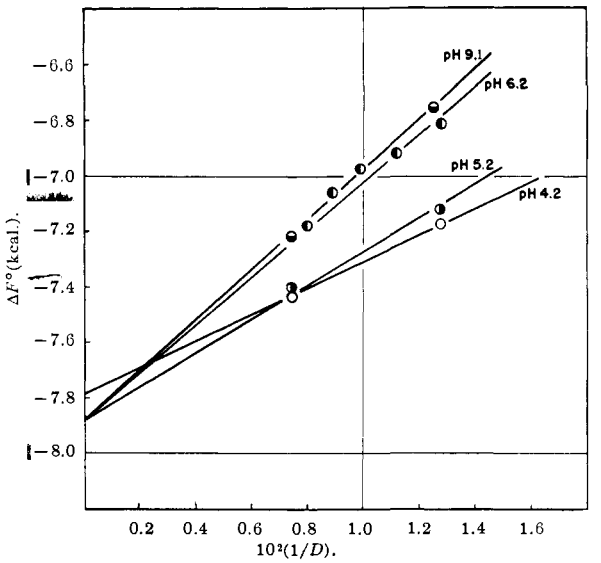

Fig. 2.—Dependence of free energy of binding on dielectric constant in glycine solutions at 25°. ◒, *p*H 9.1, 0.01 *M* NaCl; ◐, *p*H 6.2, 0.01 *M* NaCl; ◑, *p*H 5.2, 0.01 *M* NaCl; ○, *p*H 4.2, 0.11 *M* NaCl.

water and a description of protein binding in general in terms which take account explicitly of the role played by solvent molecules.

A solute can affect the structure of water in its neighborhood in various ways.[8-14] An ion (*e.g.*, K^+ or Cl^-) orients the water molecules of the first hydration shell centrosymmetrically. Further from the ion, however, the electric field is usually not strong enough to effect the same orientation but generally is sufficient to disrupt any structure organized by coöperative effects in the solvent itself. With a non-polar solute, on the other hand, any disorientation due to the electric field disappears and a lattice-ordering predominates, as seems evident from many physicochemical properties of non-polar solutes in water.[8-14] Non-polar side-chains in proteins might be expected, likewise, to induce a crystalline, cage-like, arrangement of hydration water, with the added possibilities of long-range coöperative effects due to the presence of many such side-chains bound to the frame of the protein molecule. It is in terms of the ice-like nature of this hydration water that we can account for much of the puzzling behavior of protein complexes.

It has been found previously[15] that the binding of a few detergent anions by serum albumin changes the ionization properties of this protein in a direction which can be explained by an increase in the extent of the "frozen" water of the protein. This can be visualized as the result of adding non-

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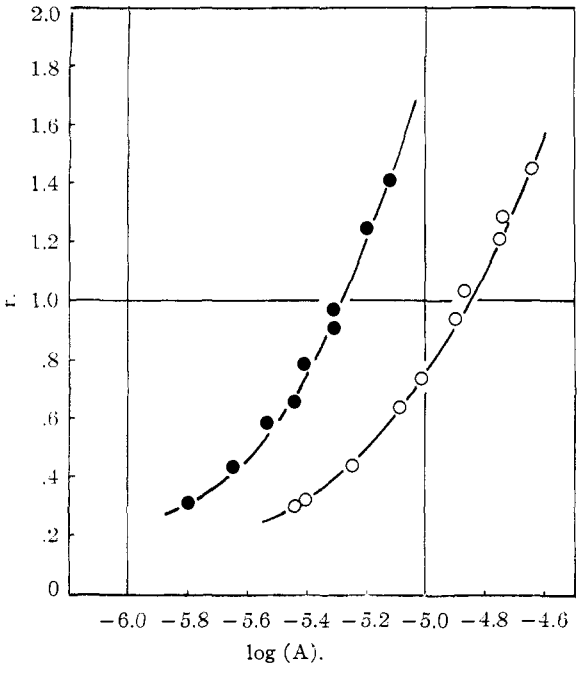

Fig. 3.—Effect of glycine on the binding of methyl orange by 0.2% bovine serum albumin in 0.01 *M* NaCl at *p*H 9.1 and 25°: ○, no glycine; ●, 3.15 *M* glycine.

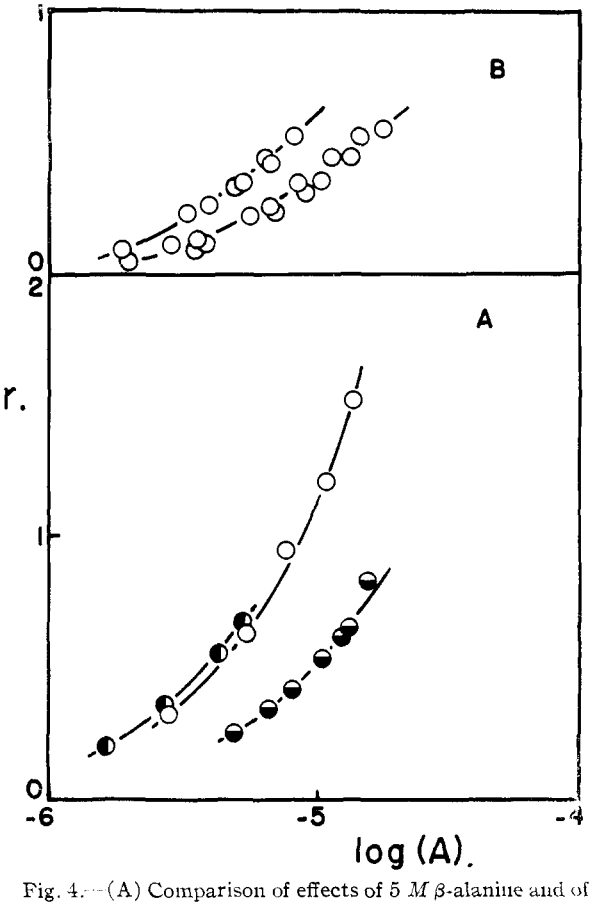

Fig. 4.—(A) Comparison of effects of 5 *M* β-alanine and of 2 *M* glycine on the binding of methyl orange by 0.2% bovine serum albumin in 0.06 *M* phosphate buffer, *p*H 6.7 ± 0.2, 25°. ◒, in buffer only; ○, buffer + 5 *M* β-alanine; ◐, buffer + 2 *M* glycine. (B) effect of 2 *M* glycine on the binding of *p*-aminoazobenzene by bovine serum albumin in 0.06 *M* phosphate buffer *p*H 6.7 ± 0.2; lower curve, without glycine, upper curve with.

polar side-chains when the organic anions come into juxtaposition with the macromolecule and the consequent extension of the framework for the building of a water lattice (Fig. 5). Some contribution to the stabilization of the ice lattice may also be made by the removal of the disturbing influence of cationic side-chains of the protein when compensating anionic charges from the small molecule are present.

In terms of these concepts we would view the binding of all types of organic molecules by serum albumin as a consequence of the potential ability of this protein to form much more extended ice lattices when holding small molecules. The bound molecule and the protein side-chains coöperatively build a more extended iceberg. From this viewpoint the small molecule becomes attached to the protein not because of any strong direct attraction by the macromolecule but rather because of the energetic stabilization accompanying the formation of a bigger hydration lattice.

Interpretation of the Effect of Glycine on Binding.—Thus the influence of glycine may be understood in terms of what this amino acid can do to the solvent rather than to the protein. Small organic molecules, charged or uncharged, in simple aqueous solution can be expected to have some frozen water of hydration. Upon the addition of zwitterionic glycine, a water-structure breaker,[16,17] a perturbing influence on this water is introduced. Consequently the organic molecule turns more readily to the more hospitable environment of the protein envelope, where with the assistance of water-structure-making side-chains a coöperative stabilization of the water lattice may ensue (Fig. 5). On this basis one can see that glycine should affect the binding of uncharged organic molecules as well as of anions. Likewise one can understand increased binding, in the presence of glycine, with serum albumin throughout the *p*H range of 4.2 to 9.1, for the net charge on the protein, or small molecule, is not a critical feature, insofar as the effect of glycine is concerned. Similarly one can understand why β-alanine is less effective than glycine in increasing binding, for β-alanine has much less of a structure-breaking effect on the solvent than has glycine.[16,17]

Interpretation of other Binding Phenomena.—In terms of the idea of an ice-like hydration lattice, we can also develop a plausible interpretation of numerous other puzzling aspects of protein binding. For example, although organic anions evidently combine at cationic loci such as lysine side-chains in serum albumin, free cationic amino acids have not been found to form corresponding complexes. In terms of our model, binding occurs in the protein because various side-chains, particularly uncharged ones, provide a basis for a coupling of hydration sheaths with the sheath of the small

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(17) W.-Y. Wen, Ph.D. Dissertation, University of Pittsburgh, 1957.

molecule and consequently a coöperative stabilization occurs. In the free amino acid, where only one side-chain is available, coöperative effects are much less likely. Furthermore, even after combination with an organic anion, the free amino acid would still contain nearby charged α-amino and carboxyl groups, influences which tend to disrupt the ice lattice.

Essentially the same explanation can be given for the ability of serum albumin to bind simple inorganic ions, such as chloride, even though simple amines, in their cationic form, give no evidence of ion-pair formation with inorganic anions. In the protein, combination of a cationic side-chain with an anion such as Cl^- removes the net charge of the side-chain and its disrupting influence on the water lattice of the macromolecule. Consequently the now uncharged side-chain can participate in a coupling of its ice lattice with those of other nonpolar groups of the protein and thereby produce an extension and stabilization of the water lattice. Such a coöperative interaction would be lacking in a solution of a simple cationic amine.

Other features of anion binding by bovine albumin are also more readily interpretable. It is known that very low concentrations of bound organic anions, such as sodium dodecyl sulfate, stabilize serum albumin. This effect is often attributed to increased van der Waals forces which then hold the macromolecule together more strongly. It has also been observed, however, that when the number of bound detergent ions exceeds 10–12, binding suddenly increases,[18,19] in a coöperative manner; this effect has been attributed to an unfolding of the protein by the bound anions. It may very well be that a stabilizing effect is converted into a disruptive effect over a small range of anion concentration. The following alternative interpretation, however, seems more reasonable. Binding of the initial 1–10 anions is associated with an extension and stabilization of the ice-like lattice, as pictured in Fig. 5. At a certain stage in binding when the number of bound anions becomes appreciable, coöperative stabilization of the water lattice can occur not only between anion and protein but also between neighboring anions on the protein. As a result the uptake of more detergent ions is favored.

In terms of the model being presented, the difference in binding abilities of serum albumin as contrasted to ovalbumin may be attributed to differences in potential for forming extended ice lattices. For molecular reasons outlined previously,[20] the cationic side-chains of serum albumin may be relatively unrestricted in orientation. This freedom, in addition to their charge, keeps them from coupling with the hydration lattice formed by nonpolar side-chains. In other words, in this protein there are areas, around lysine residues for example, where water is essentially non-frozen (except for those molecules in the first shell around the $-\overset{+}{N}H_3$ charge). Consequently the binding of an organic anion will create conditions, as described above,

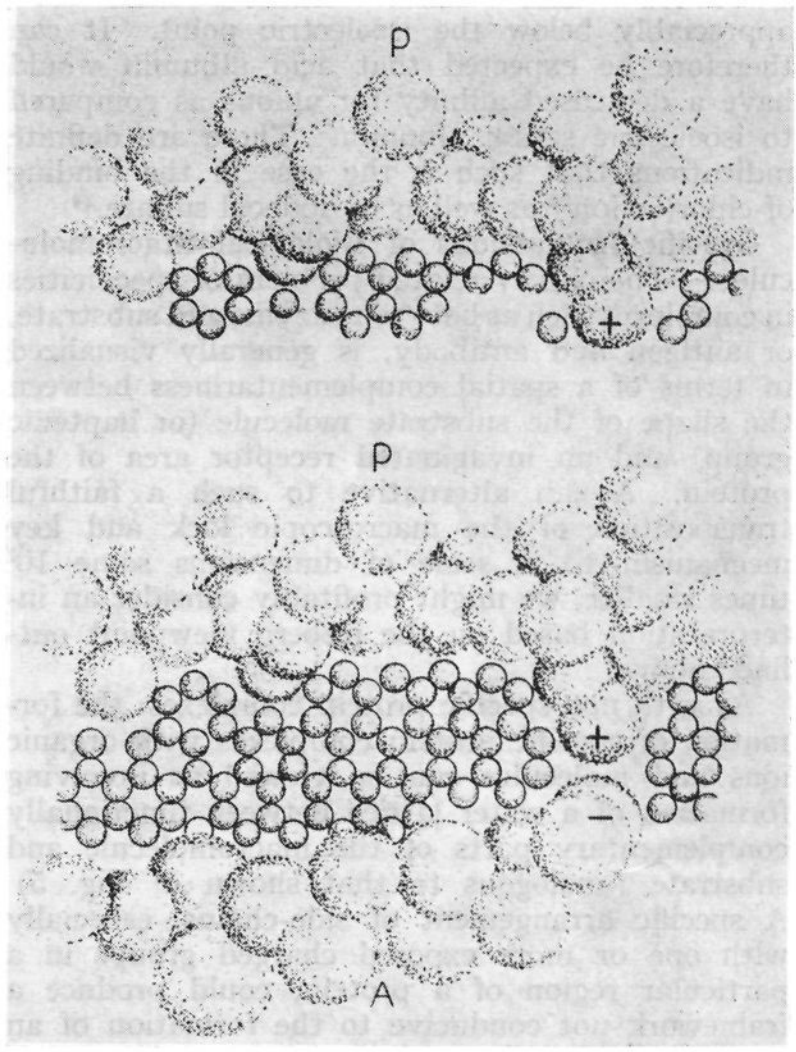

Fig. 5.—Schematic representation of coöperative hydration of protein, P (top), when it forms a complex PA (bottom).

favorable for the extension and stabilization of the water lattice. In contrast the cationic residues of ovalbumin may be restricted in orientation due to hydrogen-bonding to hydroxy amino acid residues. Also the disturbing influence of the charge may be compensated for by the stabilizing influence of the same hydrogen bonds. Thus in ovalbumin, hydration lattices could be essentially complete at the outset. Consequently, added small molecule ligands would have little to contribute toward further stabilization of the hydration sheath.

Such a view of the difference between serum albumin and ovalbumin is in line with observations on the ionization behavior of groups covalently attached to these two proteins.[7,15] In titrations from the isoelectric pH downward, serum albumin acts as if the ice lattice is incomplete until the pH drops below about 3.5, whereas ovalbumin behaves as a "masked" protein throughout the acid pH range.

This viewpoint can also account for the observations that after heating, ovalbumin[21] and γ-globulin[22] acquire the ability to bind organic ions; heat presumably would disrupt the hydration lattice and thus provide a situation in which an added organic molecule could contribute to a reestablishment and restabilization of the hydration sheath in certain regions of these perturbed protein molecules.

Finally, as mentioned above, titration curves of conjugates of serum albumin indicate that this protein acquires an extensive rigid lattice at pH's

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(21) G. Oster, *J. chim. phys.*, **48**, 217 (1951).
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appreciably below the isoelectric point. It can therefore be expected that acid albumin would have a decreased affinity for anions as compared to isoelectric serum albumin. There are definite indications that such is the case in the binding of chloride ion[23] as well as of dodecyl sulfate.[24]

Specific Interactions of Biological Macromolecules.—The closely related problem of specificities in complexes such as between enzyme and substrate, or antigen and antibody, is generally visualized in terms of a spatial complementariness between the shape of the substrate molecule (or haptenic group) and an invaginated receptor area of the protein. As an alternative to such a faithful transposition of the macroscopic lock and key mechanism to a scale of dimensions some 10^7 times smaller, we might profitably consider an interpretation based on the iceberg viewpoint outlined above.

As with non-specific protein complexes, the formation of specific protein complexes with organic ions and molecules can be viewed as involving formation of a water lattice between functionally complementary parts of the macromolecule and substrate (analogous to that shown in Fig. 5) A specific arrangement of side-chains, especially with one or more exposed charged groups in a particular region of a protein, could produce a framework not conducive to the formation of an ice-like lattice. The approach of a substrate of a suitable shape and particularly with a substituent of charge opposite to that of the protein side-chain could provide conditions for the coöperative formation of an ice lattice linking ligand to macromolecule. Thus again the stability of the enzyme–substrate complex may be viewed as not necessarily due to any strong attraction between macromolecule and small molecule but rather as the consequence of the energetic stabilization accompanying the formation of a more extended hydration lattice. An essentially similar picture would be drawn for hapten–antibody interactions.

The essential difference between proteins such as enzymes or antibodies capable of specific complex formation with a limited number of substrate or hapten molecules, and serum albumin, which is able to bind non-specifically a wide variety and large number of small molecules, is the magnitude of the open regions of the macromolecules. In serum albumin, which is exceptional, the composition of amino acids may be such that over wide regions of the macromolecule, water is not organized into an extended lattice structure. Consequently many organic ions can approach the surface and find many regions where coöperative stabilization and extension of the lattice can be effected; hence many of the small molecules may be bound. In contrast, the composition of amino acids of most proteins may be such that each macromolecule is almost entirely covered by an ice-like sheath and only one or a few limited regions are open. Only one or a few small molecules can thus combine with one of these biologically specific proteins, the specificity arising, as elaborated above, from the ability of the hydration layers of the small molecule and the macromolecule to mesh and form an extended lattice.

With this deviation from the conventional viewpoint as to the basis of configurational specificity, other aspects of enzyme behavior can also be readily interpreted. For example, competitive inhibition by molecules structurally analogous to the substrate would be attributed to the likelihood of the analog forming a coöperative lattice in the same region of the enzyme as that available to the substrate. Enzyme inactivation, particularly when reversible, by substances such as urea, may be viewed as a consequence of the dissociation of the ice-like structure bonding substrate and macromolecule together, due to hydrogen-bond formation between urea and the water molecules of this lattice. Removal of urea would permit re-formation of the lattice between enzyme and substrate, if the urea has not also perturbed the framework of the protein molecule itself. In this way one can understand reversibility of enzyme denaturation. Activation effects of substances such as metals or alcohol may be a consequence of their structure-making or structure-breaking influences on the hydration lattice. Through the medium of the hydration lattice one can also understand various long range intramolecular and intermolecular effects.[25]

It would seem worthwhile, therefore, to re-examine other features of specific interactions from the point of view that the solvent may play a central role in stabilizing protein complexes.

Acknowledgments.—This investigation was supported in part by a research grant (H-2910) from the National Institutes of Health, United States Public Health Service. We are also indebted to Mr. Richard E. Heiney and Miss Virginia H. Stryker for some of the binding measurements and to Dr. W.-Y. Wen for helpful comments.

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