ADVANCES IN PROTEIN CHEMISTRY

Volume 24

C.B. Anfinsen, Jr.

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ADVANCES IN PROTEIN CHEMISTRY

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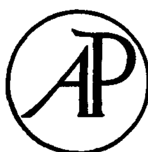

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Mortimer Louis Anson

MORTIMER LOUIS ANSON

1901–1968

By JOHN T. EDSALL

Here I set down a few personal recollections of the colleague and friend who was the founder of this series of *Advances,* and remained constantly active and concerned with its progress until his death. For me, at least, his death ends an era in the history of protein chemistry.

Nobody ever called him Mortimer, so far as I know, but his first name furnished the basis for the nicknames by which he was known to all his friends. In the early years we called him "Morty," but by the time the *Advances* began he preferred the middle syllable of his first name, and was "Tim" thenceforth.

He was an undergraduate at Harvard and received his bachelor's degree in 1922, one year ahead of me; but I first met him in Cambridge, England, in the fall of 1924. Jeffries Wyman and I had come over together that year to work in the Biochemistry Department, headed by Sir Frederick Hopkins. Alfred Mirsky and Tim Anson had arrived in Cambridge a year earlier and were working, in Barcroft's department, on hemoglobin and the interrelations of heme and globin. It was an exciting time for biochemists, and the study of heme proteins was just entering a new era, most dramatically illustrated by Keilin's great work on cytochrome with the rediscovery, and vast extension, of the long-forgotten studies of Mac Munn. Keilin's work showed that heme proteins played a far more central role throughout the living world than anyone had yet realized. Otto Warburg in Berlin, whom Tim came to know well and who influenced him greatly, was doing striking experiments on the role of iron in biological oxidations; but Warburg's greatest work was still to come. The study of muscle was making great progress, although with much controversy, in the laboratories of Meyerhof, Embden, and A. V. Hill; phosphocreatine and ATP were still unknown. Protein chemistry was progressing; Adair had just shown that the hemoglobin molecule was four times as large as most people had previously believed, and Svedberg was making his first studies in proteins in the ultracentrifuge. Anson and Mirsky were already beginning to think deeply about the problems of protein denaturation and proposed the then heretical idea that the process was reversible, and their experiments on hemoglobin provided strong evidence for their ideas.

They returned to the United States in 1925, spending a year in E. J. Cohn's laboratory at Harvard Medical School and then going on to the Rockefeller Institute. Here the paths of Anson and Mirsky began to diverge, although their collaboration continued for several years. Mirsky remained in New York while Anson moved to the Princeton branch of the Institute, where he was closely associated with John H. Northrop. Here he continued his studies on protein denaturation, and with Northrop developed the porous disc technique for measuring diffusion coefficients which has been so widely used since. His methods for assay of proteolytic enzymes, with denatured hemoglobin as a substrate, have been used since by numerous other workers and are still of value. In 1937 he achieved the first purification and crystallization of carboxypeptidase A. From this pioneer study has grown a vast series of researches on this enzyme, culminating within the last year in the determination of its complete amino acid sequence, in Neurath's laboratory at the University of Washington, and in the detailed three-dimensional structure worked out by Lipscomb and his associates.

Advances in Protein Chemistry was conceived in 1942 in the midst of the turmoil of the Second World War. Kurt Jacoby, of the Akademische Verlagsgesellschaft in Leipzig, had escaped from Nazi Germany and after many adventures had arrived in the United States, determined to start from scratch and build up a new publishing house. He already knew Tim Anson, who had traveled widely in Europe in the days before the war, and together they evolved the idea of a serial publication dealing with protein chemistry. At that point, in the spring of 1942, Tim sought me out and urged me to join him as a fellow editor. I find, from a letter from Mr. Jacoby dated August 12, 1942, that I had already accepted by that time.

It was a tumultuous and disturbed period in the midst of war. I was deeply involved in work on blood plasma fractionation, in the big wartime project headed by E. J. Cohn, and the demands upon us were so urgent that there was little time for anything else. Tim was about to end his 15 years of research activity at the Rockefeller Institute. On November 10 he wrote to me that he was leaving the Institute in the following week, to take up work with Continental Foods, Inc. in Hoboken, where he believed he could serve more effectively in working on the nutritional problems that had become so urgent because of the war. However, in our spare time we concocted a list of possible authors for our first volume and started to send out invitations. We wanted to make the *Advances* a truly international publication, but under the conditions of the war years, we were mostly restricted to American authors. We did however keep closely in touch with Kenneth Bailey, who had worked with me in the Department of Physical Chemistry at Harvard

Medical School during the months just preceding the war, and had returned to Cambridge, England, a few months after the war had begun. Not all our letters crossed the Atlantic safely. One that I sent to Bailey fell into the ocean in transit, due to enemy action, but was fished out and delivered to him safely, still in legible condition. He did manage to write a review on muscle proteins, which appeared in Volume I of the *Advances*. A few years later, by the time that Volume 5 was published, Bailey had joined us as a third editor, whose advice became indispensable to us for many years, and whose friendship meant so much to us. (See the personal tribute to Kenneth Bailey by S. V. Perry in Volume 20 of the *Advances*.)

The gestation period of Volume I was very long; one might call it elephantine. About two years elapsed from our initial planning to its actual appearance late in 1944. Considering the preoccupation of editors and authors alike with the urgent problems arising from the war, there was nothing surprising about this. As the war drew to a close, however, our editorial activities gathered momentum and Volume II, with eleven different contributions, appeared in 1945. This volume included Anson's sole published review in the *Advances*, on "Protein Denaturation and the Properties of Protein Groups." Here he summed up his work and thought of nearly 20 years, on the reactivity of specific groups in native and denatured proteins, on the reversibility of denaturation, and on what he called its all-or-none character. On this last point he championed explicitly the view that denaturation was what would now be called a two-state process—a concept which today appears to fit the facts for many though not all denaturation processes, as Tanford's articles in this and the preceding volume indicate.

Tim's interests were wide; he keenly appreciated literature, the theater, and music. Among other friends whose artistic and musical interests he shared was Bela Bartok, especially after Bartok had left his native Hungary to settle in the United States during his last years. Tim's marriage in 1945 to Nina Anton, who was active in the theatre, broadened his relationships outside of science still more.

During all the years from 1942 on he poured forth to his fellow editors of the *Advances* a constant stream of suggestions for authors and topics—sometimes in the talks we had together at intervals, and constantly in notes, short or long, generally handwritten but sometimes typed. He cast his net widely; besides the more orthodox topics, clearly identifiable as protein chemistry, he was concerned with the applications of proteins in industry, and especially with the role of proteins in nutrition and metabolism. The tragedy of protein deficiency disease for countless millions of people in so many parts of the world haunted him and was central to his concerns in his later years. Espe-

cially after he had retired from Continental Foods, Inc. in 1955, and had become an independent consultant, he devoted himself increasingly to the problems of food for the underdeveloped world, and the use abroad of American food supplies provided under Public Law 480. This brief mention of these activities must suffice here; several of Tim's colleagues in food science and nutrition have paid tribute to him at the Anson Memorial Dinner at the Amino Acid Fortification Conference in Boston and Cambridge on September 17, 1969, and what they have said concerning him will be published by the MIT press in the proceedings of the conference.

In 1955–56 the Ansons spent a year in Cambridge, Massachusetts. Thereafter for seven years they lived mostly abroad, chiefly in London, where they occupied a beautiful apartment at 100 Eaton Square. These latter years were clouded by Nina Anson's increasing suffering from arthritis. They had already decided to return to New York to live, when she died suddenly of a heart attack in October 1963. Tim returned to New York alone, to be near his daughter Jill (Mrs. John Szarkowski). His work on protein nutrition continued, including a conference in Japan in 1964, in which he played an active part as organizer and contributor. Early in 1966 he suffered a severe heart attack, from which recovery took many months.

His interest in the *Advances* never left him. When he and his fellow editors got together, after his recovery, he was as interested as ever in suggesting plans for future reviews. Sometimes he looked back yearningly to the days before 1942, when he had been active in research. He could recall all sorts of interesting experimental observations from those earlier days, and often could shrewdly appraise the work of more recent investigators, who picked up problems where he had left off. Some of the manuscripts we received in later years for the *Advances* he did not attempt to examine, if he felt they were beyond his scope. When he did tackle a manuscript, however, he invariably improved it. He strove to make authors write simply, clearly, effectively, to make the major points stand out while the minor ones fell into place. His criticisms of authors—sometimes very eminent authors—were often pungent, but they were fair, helpful, and much to the point, and they were tempered by his genial wit and by the essential kindness of his nature.

In the summer of 1968, he again suffered a severe heart attack, and was in hospital for many weeks; but he returned to his apartment, still active. Early in October I arranged to meet him for dinner on a visit to New York; but three days before the appointed time he returned to hospital, and two days after that he died. All of us involved in the *Advances* will miss him as the initiator of this series and will remember him as our unfailing friend.

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* Parts A and B were published in Volume 23 of *Advances in Protein Chemistry* (1968), starting on p. 121.

I. Introduction

This review of the subject of protein denaturation consists of three parts. Part A, dealing with the characterization of the denatured state, and Part B, dealing with the phenomenological aspects of the transition from the native to the denatured state, were published in the preceding volume of this series (Tanford, 1968).[1] This final portion of the review will consider theoretical models that may be constructed, and equations that may be derived from them, to account for the experimental observations reported in Parts A and B.

It should be emphasized that the objective of the theoretical models to be discussed is to understand the process of denaturation *per se*, and not to use denaturation as a tool for understanding the native state and the forces responsible for maintaining it. With respect to the latter problem, little has occurred in the last ten years to alter earlier conclusions by Kauzmann (1954, 1959) and by the present author (Tanford, 1958, 1962a).

[1] A number of interesting papers dealing with the topics of Parts A and B have been published during the last year. Of particular importance is a new calculation of the dimensions of randomly coiled polypeptide chains, which allows for the presence of both glycine and proline (Miller and Goebel, 1968). As predicted, the presence of proline reduces the random coil dimensions: the new values are in excellent agreement with the values observed in 6 *M* GuHCl, as given by Eq. (20) of Part A. Miller and Goebel also show, however, that the dimensions are relatively insensitive to the presence of knots of associated residues, and conclude that the agreement between calculated and observed dimensions cannot of itself be used to rule out the presence of such knots. The optical rotation and titration data cited in Part A, for proteins in GuHCl solution, provide additional evidence, of course, and make the presence of sizable regions of associated residues very improbable. It should be noted in this connection that Reisner and Rowe (1969) have isolated from *Paramecium* what appears to be the longest naturally occurring polypeptide chain reported so far: it consists of 2930 residues, with a total molecular weight near 300,000. When dissolved in 6 *M* GuHCl, in the presence of reducing agent, it had an intrinsic viscosity of 133 cc/gm, in good agreement with an extension of the data of Fig. 6 of Part A to longer chain lengths.

Other Additions and Corrections for Part A. An error was made in Part A in the discussion of denaturation of proteins by detergents. This error is corrected in footnote 3 on p. 46. Several substances related to GuHCl have been shown to have greater potency as denaturants: this work is referred to in Section IV,D. Information on the denaturation of proteins by 2-chloroethanol has been considerably augmented in a paper by Ikai and Noda (1968), and much new information on the action of alcohols in general and some other organic compounds is provided by Herskovits and Jaillet (1969).

Additions to Part B. Steiner and Clark (1968) have shown that proinsulin spontaneously refolds to its native conformation after denaturation and reduction, whereas insulin does not. Unpublished work by R. W. Roxby in the author's laboratory shows, however, that the denaturation of insulin by GuHCl, without rupture of disulfide bonds, is a reversible process. Polet and Steinhardt (1969) have succeeded in identifying sequential stages in the acid denaturation of ferrihemoglobins. The thermal denaturation of chymotrypsin has been found to have a ΔCp value much larger than any listed in Table XVI of Part B. Reference to this work is made in Table XV of the present paper.

The problems that come within the scope of this portion of the review may this be summarized as follows:

(1) The native and some of the denatured states having been characterized, and the forces responsible for maintaining a given conformation being known in a general way, can we account for the fact that the native state is thermodynamically stable under physiological conditions?

(2) Given that the native state is stable under physiological conditions, can we predict the effects of environmental changes (temperature, pH, addition of denaturants) on the equilibrium between native and denatured states so as to account for the loss of stability of the native state and the appearance of different denatured states under specified conditions? If the answer is in the affirmative, can we further account quantitatively for the detailed course of the transition from native to denatured state?

(3) Denaturants presumably do not act at long distance from the protein molecule. Can we identify the specific sites at which they act?

The answers to these questions prove to be somewhat disappointing, and it is evident that the overall problem of protein denaturation is not yet solved. The situation is particularly bad with respect to the first question raised above: the models available at this time do *not* predict that the native state should represent the stable conformation of a protein molecule in dilute aqueous salt solutions at neutral pH. Part of the reason for this is that there are strong forces favoring the native state and other strong forces favoring the denatured state, and that the native state is in fact favored under physiological conditions by only a small difference between these opposing factors. However, it is also evident that there are contradictions between available estimates of the numerical values for some of these factors, such as the free energy of hydrogen bonds within the native protein. These contradictions need to be resolved before a calculation of the absolute free energy, enthalpy, and entropy of denaturation can be taken very seriously.

The problem of assigning sites of action to protein denaturants can also be considered as not completely solved. Predictions can be made on the basis of model compound studies, but the results of protein denaturation studies as such have not so far yielded direct confirmation of such predictions. The sites of action of H^+ ions, however, represent an exception, and the effect of pH on denaturation equilibria can be taken as completely understood within the limitations of our knowledge of the precise locations of acidic and basic groups on native proteins, and our ability to calculate pK differences on the basis of interactions between closely spaced groups.

The most successful portion of the paper is that dealing with the second question raised above. This question involves not the *absolute* values for the free energies and other thermodynamic parameters for denaturation processes, but the *changes* in these parameters with changes in environ-

mental variables. These changes can be predicted semiquantitatively. We can account both for the products formed under different conditions and for the character of the transitions from native to denatured state, at least for the simple proteins that have been studied in detail. Thermal denaturation represents a partial exception: it depends on a knowledge of the absolute values of ΔH for the various denaturation processes. If empirical values for ΔH at one temperature are assumed, however, the variation in ΔH with temperature is at least partly understood.

Thus one conclusion to be drawn from this paper is that much work remains to be done. However, the areas of uncertainty involve numerical values for factors that are important in denaturation. They do not suggest any error in presently accepted views on the fundamental principles that determine the relative stabilities of native and denatured states of proteins under different conditions.

Symbols to Be Employed

The symbols to be used in Part C differ somewhat from those used in Parts A and B, because the number of thermodynamic and kinetic parameters used in this Part is necessarily larger than those required to describe the experimental results without theoretical interpretation. The following will be used consistently throughout the paper:

Conformational States. N represents the native state, D any denatured state, RC the randomly coiled state (usually with disulfide cross-links intact), ID the incompletely disordered state obtained by thermal denaturation. The states RC and ID have been singled out because they are the only states for which we have enough experimental information for comparison with theoretical prediction.

Free Energy Changes. The symbol ΔG *without subscript* will be used to designate the free energy change in the transition $N \rightarrow D$. Subscripts will be used only when ΔG refers to a reaction or conformational transition other than the process $N \rightarrow D$, or when two distinct denatured states have to be distinguished in an equation. ΔG_0 will be used to designate the value of ΔG in an arbitrary reference medium, usually water or a dilute salt solution at 25°C.

The symbol δG_{tr} will represent the free energy of transfer of a protein molecule from one solvent medium to another, *without conformational change.* The symbol $\delta\Delta G$ represents the effect of solvent medium on ΔG.

The symbols Δg_i and δg_i represent contributions to the corresponding ΔG or δG that are assigned to a *small portion of a protein molecule,* when the method of localization of free energy contributions is used.

Other Thermodynamic Variables. Symbols to be used will be consistent

with those given for free energy, e.g., ΔH, δH_{tr}, Δh_i, δh_i will represent the enthalpy portions of ΔG, δG_{tr}, Δg_i, δg_i.

Equilibrium Constants. The symbol K *without subscript* will always refer to the equilibrium constant for a reaction N $\rightleftharpoons$ D. Subscripts will be used under the same conditions as apply to ΔG. The value of K in a reference medium (usually water) will be designated as K_0.

The symbol K_a will be used uniquely for acid dissociation constants.

II. General Equations for Equilibria between Native and Denatured States

Denaturation is a reversible process for many proteins. This means that the native and denatured states represent equilibrium states under the conditions where they exist. The native state must have a lower free energy than all accessible denatured states in the native environment; similarly each particular denatured state must become the state of lowest free energy in the particular environment where it is found experimentally to be the predominant state.

A major requirement for any theory of denaturation is that it must be able to account for the free energy differences between the known conformational states, and the effects of environmental conditions upon them. To achieve this goal, it is necessary to consider the various factors that affect the free energy and other thermodynamic parameters and to incorporate them into equations that can form the basis for calculations. The objective of this section is to present such equations in the most basic form, applicable to all equilibria between different conformational states, regardless of the actual characteristics of the states involved.

A. Localization of Free Energy Contributions

1. Isothermal Transitions in Water or Dilute Salt Solutions

It is logical to begin the discussion with the native environment, to consider the native and known denatured states in this environment, and to express the total free energy differences between the states as sums of contributions from all the physical and chemical factors that affect them. There is no unique way in which this must be done: the choice is dictated by convenience and by the ability to evaluate individual contributions either theoretically or from experimental studies of suitable model systems. The procedure used here is essentially that employed in an earlier paper (Tanford, 1962a); a very similar procedure has been used by Brandts (1964b). Both procedures are based to a large extent on two earlier papers by Kauzmann (1954, 1959) in which the various factors that should con-

tribute to the free energy difference between native and denatured states were enumerated, and their relative importance was evaluated.

a. The Order-Disorder Term (ΔG_{conf}). Most of the atoms of the typical native protein molecule occupy fixed positions. In most denatured states, some or all of the parts of the molecule are randomly disposed. This randomness results from the fact that rotation occurs about single bonds of the polypeptide backbone and side-chain groups (Ramachandran and Sasisekharan, 1968). If all allowed orientations about a single bond were to have equal energy, then ΔG_{conf} would contain only an entropy term. If there are $x_{z,D}$ bonds, each with z alternate orientations (for single bonds involving carbon atoms, usually $z = 3$), in the denatured state, and $x_{z,N}$ in the native state, the entropy change would be $R\Sigma\Delta x_z \ln z$, where $\Delta x_z = x_{z,D} - x_{z,N}$, and the sum extends over all values of z. The value of ΔG_{conf} would be given by

$$\Delta G_{conf} = -T\Delta S_{conf} = -RT\Sigma\Delta x_z \ln z \tag{1}$$

In reality, different rotation angles have unequal energies, even at the minima that correspond to stable rotational states, and the differences are particularly pronounced for the bonds of the polypeptide backbone (Ramachandran and Sasisekharan, 1968). As a result, one rotational orientation is generally favored over others at a given bond in a random conformation. The entropy is thus less than that given by Eq. (1). On the other hand, the free energy minima themselves are not particularly sharp, so that an additional contribution to the entropy arises from motility (or "free volume") in a given rotational state. In addition, there has to be an energy term, representing the difference between the average energy at each bond and the fixed energy at the same bond in the native state. The overall expression for ΔG_{conf} is thus

$$\Delta G_{conf} = \Delta H_{conf} - T\Delta S_{conf} \tag{2}$$

and numerical values cannot be estimated with any confidence at the present time. The reader is referred to Volkenstein (1963) for further general discussion of this topic, and to Brant *et al.* (1967) for specific discussion of the random polypeptide chain.

b. Short-Range Interactions in Random Regions of a Protein Molecule. Interactions between adjacent peptide groups in random regions of a protein molecule, and interactions with proximal portions of attached side chains, are included in the calculation of the torsional potential function for rotation about single bonds of the polypeptide backbone, and are therefore automatically included in the calculation of ΔG_{conf}. Interactions with solvent will be considered separately below.

c. Short-Range Interactions within Ordered Regions ($\Delta g_{i,int}$). Ordered

regions of a protein molecule may be stabilized by hydrogen bonds and van der Waals forces (nonbonded interactions) between moieties of the molecule that are brought into contact by the three-dimensional order, though they may be far apart in the linear sequence of amino acids. To include the free energy of such interactions in ΔG for the denaturation process, we arbitrarily divide the protein molecule into convenient portions: each peptide backbone unit, for example, may be considered as a single portion, some side chains may be considered as single portions, others may be considered as consisting of two separate portions, as, for example, the lysyl side chain, for which the NH_3^+ group may be taken as a separate entity from the hydrocarbon chain that joins it to the peptide group. The total free energy of all short-range interactions within the ordered regions of a protein molecule is then divided among these separate portions in a logical manner, e.g., the free energy of forming a hydrogen bond between two groups is divided evenly between the two groups. We shall use $g_{i,\mathrm{int}}$ to designate the free energy assigned to the ith part of the molecule, and $\Delta g_{i,\mathrm{int}}$ the difference between this quantity for denatured and native states. If, in the course of denaturation, a part of the protein molecule is transferred from the inside of the native structure to a position in a random portion, $\Delta g_{i,\mathrm{int}}$ will simply be a measure of the loss of interaction free energy in the native state, i.e., $\Delta g_{i,\mathrm{int}} = -g_{i,\mathrm{int,N}}$.

Since only contacts between one part of a protein molecule and other parts of the same molecule are included in the $\Delta g_{i,\mathrm{int}}$ terms, these terms are necessarily independent of the solvent in which a denaturation process may occur. In general, each $\Delta g_{i,\mathrm{int}}$ will include both energy and entropy contributions.

If we imagine the reaction $N \rightarrow D$ as occurring in a vacuum, we can write the total free energy change as

$$\Delta G = \Delta G_{\mathrm{conf}} + \sum_i \Delta g_{i,\mathrm{int}} \tag{3}$$

the summation extending over all portions of the protein molecule.

d. Short-Range Interactions with the Solvent ($\Delta g_{i,\mathrm{s}}$). When the reaction $N \rightarrow D$ occurs in solution, contacts with the solvent will make important additional contributions to the free energy. Free energy changes resulting from the hydration of ions, and from hydrophobic interactions between water and nonpolar parts of the protein molecule are included in this category. We shall use $g_{i,\mathrm{s}}$ to indicate the free energy associated with the solvent contacts for each portion i of the protein molecule, and $\Delta g_{i,\mathrm{s}}$ for the change in this quantity that accompanies the denaturation process. In a native protein there are many portions that have no contacts with solvent at all. If such portions are exposed to the solvent in a denatured state,

$\Delta g_{i,s}$ will represent the free energy of solvent contacts in the denatured state only. If a portion of a protein molecule (e.g., the ionic terminus of a lysine or arginine residue) is freely exposed to solvent in both native and denatured states, the $\Delta g_{i,s}$ term for that portion will be taken to be essentially zero.

e. Long-Range Electrostatic Interactions (ΔW_{el}). The only long-range interactions that contribute to the free energy change for protein denaturation are Coulombic interactions between charged groups. The contribution (W_{el}) of these interactions to a protein molecule in any given conformation can be calculated as described elsewhere (Tanford, 1961). The difference between this calculation for the denatured and native states is ΔW_{el}. There will usually be a pH range near the isoelectric pH of the protein where $\Delta W_{el} \simeq 0$ for any denaturation process. In the subsequent discussion we shall generally assume ΔW_{el} to be zero where denaturation processes are considered without reference to a particular pH value.

f. The Total Free Energy Change. The total free energy difference between the native state and any denatured state, by summing the contributions discussed above, becomes

$$\Delta G = \Delta G_{conf} + \sum_i \Delta g_{i,int} + \sum_i \Delta g_{i,s} + \Delta W_{el} \qquad (4)$$

It should be noted that this quantity refers to the difference in free energy between two conformational states of the protein; in the *same* solvent medium, without change in protein concentration, temperature, or other external variables. It should be independent of protein concentration over a wide range. It is automatically a "standard" free energy change and related to the equilibrium constant for denaturation, $K = (D)/(N)$, by the relation

$$\Delta G = -RT \ln K \qquad (5)$$

Both ΔG and K do not in any way depend on the units in which the concentrations of denatured and native forms are expressed.

2. The Effects of Temperature and Pressure

The effect of temperature on the equilibrium constant K for any denaturation process is given by the enthalpy change for the reaction,

$$\Delta H = -R[\partial \ln K/\partial(1/T)] \qquad (6)$$

It is simply the sum of the enthalpy components of the individual terms of Eq. (4), i.e.,

$$\Delta H = \Delta H_{conf} + \sum_i \Delta h_{i,int} + \sum_i \Delta h_{i,s} + \Delta H_{el} \qquad (7)$$

where Δh_i is the enthalpy component of each Δg_i, and ΔH_{el} is the enthalpy component of the electrostatic free energy. ΔH_{el} is probably negligibly small for all denaturation processes. In our previous paper (Tanford, 1962a), ΔH_{conf} was assumed to be zero. As was pointed out above, this assumption is an oversimplification.

From the difference between ΔG and ΔH one obtains the entropy change for the process. In terms of Eq. (4),

$$\Delta S = \Delta S_{\mathrm{conf}} + \sum_i \Delta s_{i,\mathrm{int}} + \sum_i \Delta s_{i,\mathrm{s}} + \Delta S_{\mathrm{el}} \tag{8}$$

where Δs_i is the entropy component of each Δg_i, and ΔS_{el} the entropy component of ΔW_{el}. With $\Delta H_{\mathrm{el}} \simeq 0$, $\Delta S_{\mathrm{el}} \simeq -\Delta W_{\mathrm{el}}/T$.

It is important to note that some of the interactions considered here have large effects on the partial molal heat capacity of the protein molecule. Thus neither ΔH nor ΔS can be considered as independent of temperature, i.e.,

$$\frac{\partial \Delta H}{\partial T} = T \frac{\partial \Delta S}{\partial T} = \Delta C_p, \tag{9}$$

ΔC_p can again be split into components in analogy to Eqs. (7) and (8).

The effect of pressure on K can be treated similarly to the effect of temperature, i.e.,

$$\frac{\partial \ln K}{\partial P} = -\frac{\Delta V}{RT} \tag{10}$$

and ΔV is again a sum of contributions from individual interactions as in Eqs. (7) and (8).

3. The Addition of Denaturants and Other Substances

A major simplification is possible in describing the effects of denaturants and other substances on the equilibrium between native and denatured states, at constant temperature and pH. Those contributions to ΔG that do not represent interactions with the solvent, i.e., those occurring in Eq. (3), are not altered. The major contribution to changes in ΔG must come from the terms $\Delta g_{i,s}$, with a possible minor contribution from changes in ΔW_{el}. The change $\delta \ln K$ or $\delta\Delta G$, that results from any changes in the composition of the solvent, can therefore be expressed as

$$-RT\delta \ln K = \delta\Delta G = \sum_i \delta\Delta g_{i,\mathrm{s}} + \delta\Delta W_{\mathrm{el}} \tag{11}$$

The quantity $\delta\Delta G$ may also be expressed in terms of the free energy of transfer (δG_{tr}) of a protein molecule in any given conformation from one solvent to another. From the following diagram,