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On the Realization of the Helix–Coil Theory for Protein Chains in Solutions Containing Dodecyl Sulfate. Experiments on α-Tropomyosin and Bovine Serum Albumin

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ABSTRACT: Measurements are reported of α-helix content vs. temperature for reduced rabbit α-tropomyosin (α-Tm) and for reduced bovine serum albumin (BSA) in near-neutral aqueous solutions containing dodecyl sulfate anions (DS^-). The results for the two proteins are similar, α-Tm being somewhat more helical than BSA. The helix content for both is independent of substitution of Li^+ for Na^+, protein concentration (0.1–12 mg mL^{-1}), added salt concentration (33–500 mM), and DS^- concentration (18–30 mM). Increasing temperature reduces helix content, but in a rather noncooperative manner. In α-Tm, the results are almost the same whether or not each 284-residue protein chain is cross-linked to another at Cys 190. A recent prescription for the calculation of the helix content of proteins in DS^--added systems from the statistical mechanical theory of the helix–random coil transition is examined and found to be in serious disagreement with experiment, even when certain parameters are fixed by the experiments themselves. A detailed analysis of the prescription suggests the following: (1) The underlying idea, that only helix and random coil conformations need be considered for proteins in DS^-, may be correct; thus, the formal theory, which only accounts for short-range interactions (through parameters σ and $s(T)$), may be conceptually adequate. (2) The difficulties probably arise from flaws in the method for obtaining the input parameters appropriate to the DS^--added system from those presently available for the DS^--free system. The desirability of independent measurements of the input parameters for the DS^--added case is stressed.

I. Introduction

Over a period of some years, a statistical mechanical theory has been developed for treating the equilibrium between α-helical and randomly coiled conformations of single polypeptide chains in solution.[1] The theory assumes the equilibrium is dominated by "short-range" interactions, which are embodied in two types of parameters, the helix-initiation parameter (σ) and the helix-propagation parameter [$s(T)$], the latter being temperature dependent.

Each type of amino acid residue (e.g., alanine is expected to have its own characteristic σ and $s(T)$, and the values appropriate to aqueous medium have been determined and tabulated for virtually all the types of residues found in proteins, thus allowing the theory to be realized.[2-17] However, the theory's exclusion of long-range interactions makes it inapplicable to native protein molecules in aqueous media, since these display prominent tertiary and quaternary structural features. A beginning has been made in extending the theory to encompass those long-range interactions peculiar to two-chain, α-helical, coiled coils,[18-21] but these represent a very limited class of proteins, and, moreover, it is a bit early to judge the success of the endeavor.

Mattice et al. pointed out that the theory, even without such extension, might be applicable to single protein chains if all disulfide bonds are reduced and the chains dissolved in sodium dodecyl sulfate (SDS[22]) solutions, since there is evidence that the dodecyl sulfate anion (DS^-) in sufficient concentration disrupts tertiary and quaternary interactions, leaving only the short-range interactions to dictate the conformation.[23,24] In that work, Mattice et al. not only present this idea as a series of explicit assumptions bearing on the applicability of the formal theory but also give the further assumptions needed to realize the theory in DS^--added media (for which no experimental values of σ and $s(T)$ are available). Furthermore, they present enough calculations and experimental results for proteins near room temperature to demonstrate the promise of the approach.[23-25]

This is potentially a very germinal proposal not only because it bears on the question of limits of validity of the developing theory of polypeptide conformations but also because of the important role played by detergent solutions in the biochemical study of proteins. It is very common to study proteins in such media (for gel electrophoresis, for example), and, indeed, many important proteins (e.g., some membrane-bound proteins) have solubility properties that disallow solution studies in the absence of detergent. It would thus be highly desirable to have a theory that would allow calculation of the chain conformation in the DS^--added regime. Indeed, if such a reliable theory were available, it would allow one to calculate such things as local stabilities at specific chain sites[23]—information difficult or impossible to obtain by experiment.

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Specifically, Mattice et al. propose as applicable to single, reduced protein chains in aqueous solutions containing sufficient DS^- the following assumptions:[23,24]

(a) The native tertiary and quaternary structure is totally disrupted so that those long-range interactions that are important in the native structure may be ignored.

(b) The conformation is dominated by short-range interactions; i.e., no nonnative long-range interactions are important.

(c) The conformational free energies for the disordered residues are unaffected by the detergent.

(d) Helix-forming tendencies, i.e., values of σ and $s(T)$ for the individual types of amino acid residues, are unaffected by detergent except for the cationic residues arginine, histidine, and lysine for which there is an increase in σ and $s(T)$.

(e) The detergent-modified values of $s(T)$ are the same for arginine, histidine, and lysine. We refer to this as s_{RHK}.

(f) The detergent-modified parameter s_{RHK} is essentially independent of temperature, there being essentially no change in the range 17–30 °C and perhaps a modest decrease in the range 30–50 °C.

(g) The detergent-modified values of σ are the same for arginine, histidine, and lysine. We refer to this as σ_{RHK}.

(h) The value of σ_{RHK} may be calculated from s_{RHK} from the relationship determined by a correlation analysis of the DS^--free values of σ and s for the various amino acid residues near room temperature.

$$\ln \sigma = -7.58 + 8.63 \ln s \quad (1)$$

This bold set of assumptions, gleaned from a careful reading of the entire text of ref 23 and 24, is almost (but not quite) sufficient to allow realization of the theory (for reduced proteins in DS^-) from the existing set of measured values of σ and $s(T)$. Assumptions a and b proclaim the applicability of the formal theory to the problem in question, c is in a special category if calculation of helix content is the only objective (see below), and d–h provide a program for implementation of the theory using available σ and $s(T)$ values. The only missing link is the choice of the actual numerical value of s_{RHK} at one temperature. That done, assumptions e and f provide s_{RHK} at other relevant temperatures; g and h then provide σ_{RHK} near room temperature, and, since any σ is generally conceded to be independent of temperature, we have σ_{RHK} at any temperature. Assumption d, of course, provides σ and $s(T)$ for all the other residues from extant data. The missing value of s_{RHK} is easy to obtain. One simply has to choose it so as to give the correct value for the known helix content of a single reduced protein in DS^-. Thence, the helix content of any other reduced protein whose sequence is known should be calculable from the now fully realized theory.

Because of the importance of the question of protein conformation in DS^-, the power of these proposals of Mattice et al. in making an answer to this question accessible to theory, and the potential insight the success or failure of the assumptions might give to our understanding of the physics underlying these conformational problems, we present here the results of an experimental test of the prescription. This test involves the determination of equilibrium thermal denaturation curves of a protein that is highly α-helical in its native state, rabbit α-tropomyosin (α-Tm), and of a rather typical globular protein, bovine serum albumin (BSA), in a variety of aqueous media containing the detergent anion DS^-. These two proteins were selected because they are readily available to us and had previously been considered in detail by Mattice et al.[23,24], and because each has a relatively long chain which tends to reduce ambiguities inherent in consideration of chains that contain helical stretches of only a very few turns. Moreover, we believe α-Tm is particularly apt; it has no proline, whose presence complicates interpretation,[23] and even its native structure is remarkably similar to that of the synthetic helical polypeptides for which the theory was originally developed.

To cover as wide a temperature range as possible we employed lithium as well as sodium cations because of the limited solubility of SDS below room temperature. The bulk of earlier experiments were near room temperature.[23,24,26] We have also made relevant theoretical calculations on the basis of the assumptions, and the results are examined for their implications for the future development of the theory.

II. Methods

Proteins. For rabbit α-tropomyosin (α-Tm), preparation, reduction, and cross-linking were as previously described (except for the presence of 20 mM DS^- in the reduction in the present work).[20] In the absence of DS^-, protein concentration was determined as before by absorbance at 277 nm (ϵ_{277} = 0.314 cm^2 mg^{-1}).[20] Protein concentrations for DS^--added solutions were also obtained from absorbance at 277 nm; the extinction coefficient (ϵ_{277} = 0.308 cm^2 mg^{-1}) was determined by quantitative addition of an LDS or SDS solution to a known volume of DS^--free solution of known protein concentration and measurement of the absorbance.

Bovine serum albumin (BSA) was obtained from Sigma Chemical Co. and manipulated in a way similar to α-Tm. Occasionally, BSA precipitated when reduced in DS^- at the higher concentration of NaCl (500 mM); these solutions were discarded. Solutions with lower Na^+ concentration or with Li^+ of any concentration never did so. Both reduced proteins showed a single band when run in SDS–polyacrylamide gel electrophoresis. The adequacy of the reduction procedures has been demonstrated.[20,27] The extinction coefficient used for BSA concentration determination in benign media was 0.667 cm^2 mg^{-1} and refers to 279 nm.[28] By the same procedure described above for α-Tm, we found for BSA in DS^--added solutions ϵ_{279} = 0.673 cm^2 mg^{-1}.

Circular Dichroism (CD). CD measuements were made in thermostated quartz cells with a Jasco J-20 spectropolarimeter calibrated as described previously.[20] Temperature control and measurement were also as before.[20] Mean residue molecular weights used were 115 (α-Tm) and 113.9 (BSA).

Calculation of the fraction of residues in helical form Φ_h from CD measurements of partially helical polypeptides is generally performed through the equation[27]

$$\Phi_h = \frac{[\theta] - [\theta_c]}{[\theta_h^n] - [\theta_c]} \quad (2)$$

wherein all square-bracketed θ designate mean residue ellipticity at 222 nm, $[\theta]$ being for the entire solution, $[\theta_c]$ for a randomly coiled residue, and $[\theta_h^n]$ for a helical residue located within a helical stretch of average length in the molecule. A problem arises because $[\theta_h^n]$ depends on the average length of a helical stretch in the molecule and therefore on Φ_h.[20,29] As indicated previously, this dependence can be written in terms of the average number of helical stretches in the molecule I:[20]

$$[\theta_h^n] = [\theta_h^\infty]\left[1 - \frac{2.55I}{n\Phi_h}\right] \quad (3)$$

Table I
Conditions of Thermal Denaturations at pH 7.4

protein	C_p, mg mL^{-1}	$salt_{mM}$	$buffer_{mM}$	$detergent_{mM}$	DTT, mM	data symbol
α-Tm	0.100	$LiCl_{500}$	$Hepes_{20}$	LDS_{18}	0.5	open octagon
α-Tm	0.290	$LiCl_{500}$	$Hepes_{20}$	LDS_{18}	0.5	open delta
α-Tm	0.208	$LiCl_{500}$	$Hepes_{20}$	LDS_{18}	0.7	open diamond
α-Tm	0.208	$LiCl_{500}$	$Hepes_{20}$	LDS_{18}	0.7	open del
α-Tm	0.190	$LiCl_{500}$	Hepes	LDS_{30}	0.7	open square
α-Tm	0.198	$NaCl_{500}$	$NaPi_{50}$	SDS_{20}	0.63	open circle
α-Tm	10.1	$LiCl_{500}$	$Hepes_{20}$	LDS_{18}	5.0	open square
α-Tm	0.301	$LiCl_{100}$	$Hepes_{20}$	LDS_{18}	0.80	filled circle
$(α\text{-Tm})_2$ [a]	7.40	$LiCl_{500}$	$Hepes_{20}$	LDS_{18}	0.0	filled square
BSA	0.100	$LiCl_{500}$	$Hepes_{20}$	LDS_{18}	0.5	plus
BSA	0.111	$NaCl_{500}$	$NaPi_{50}$	SDS_{18}	0.5	cross
BSA	0.094	$LiCl_{500}$	$Hepes_{20}$	LDS_{30}	0.5	cross on minus
BSA	11.6	$NaCl_{33}$	$NaPi_{24}$	SDS_{18}	20.0	minus in diamond

[a] Cross-linked α-Tm.

in which $[\theta_h^\infty]$ is the mean residue ellipticity for an infinite helix and n is the total number of residues in the molecule. Inserting (3) into (2) and solving for Φ_h give

$$\Phi_h = \frac{[\theta] - [\theta_c] + (2.55I[\theta_h^\infty]/n)}{[\theta_h^\infty] - [\theta_c]} \tag{4}$$

and this equation was used along with estimated I values, $[\theta_c] = -10.0$, and $[\theta_h^\infty] = -386$ deg cm^2 mmol^{-1} as before to calculate fraction helix from CD for α-Tm.[20].

Unfortunately, eq 4 cannot be implemented quite so easily for BSA as for α-Tm. As Mattice et al. have pointed out,[23] in the regime where only α-helix and random coil are important, the presence of 28 prolines, through which α-helices cannot propagate, divides the BSA molecule into a series of conformationally independent peptides of various lengths.[30] Because none of the 28 prolines is terminal, but two of them are contiguous, the BSA chain has 28 such proline-free subchains varying in length from 1 to 60 (non-proline) residues. Thus, although a reasonable estimate can be made of I for the 284-residue α-Tm chain (which has no proline) from simple homopolymer theory,[20] such an estimate would be rather complex in execution and questionable in result for BSA.

For this reason, we adopted for BSA a somewhat coarser procedure similar to that routinely used by most workers. Most investigators employ eq 2, using for $[\theta_h^n]$ the constant value appropriate to the maximally helical polypeptide chain. This procedure ignores the dependence of $[\theta_h^n]$ on Φ_h but has the advantage of simplicity. Strictly speaking, it is correct only when the molecule is maximally helical, but the error is probably small.

To implement this, we calculate $[\theta_h^n]$ from eq 3 for BSA when it is as helical as it can get. The 28 prolines cannot be helical. Of the 28 subchains, four subchains have less than four residues, i.e., less than one helical turn. Counting the nine residues that appear in those subchains as necessarily random results in a maximum helix content of 582 − 28 − 9 = 545 helical residues (i.e., $\Phi_h = 545/582$) appearing in 28 − 4 = 24 = I helical stretches. Inserting this information into (3) we obtain $[\theta_h^n] = -342.6$ deg cm^2 mmol^{-1}. This was used in eq 2 along with the compromise value $[\theta_c] = -10.0$[20] to obtain Φ_h for BSA. Thus, Φ_h for BSA was obtained from

$$\Phi_h = \frac{|[\theta]| - 10.0}{332.6} \tag{5}$$

It is made clear below that it is important to establish a greatest lower bound for the fraction helix of BSA. We

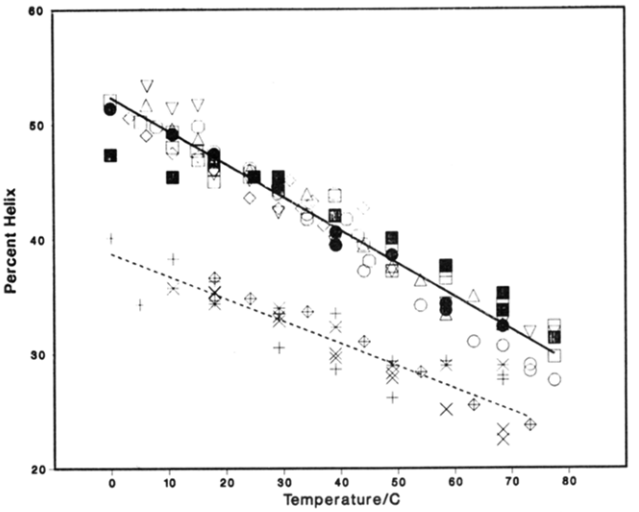

Figure 1. Experimental fraction helix vs. temperature for α-Tm and BSA. See Table I for exact conditions and individual sample documentation. Solid (dotted) curve, spline curve to fit all α-Tm (BSA) data.

can see from eq 4 that since I, the number of helical stretches in a molecule, is necessarily zero or positive:

$$\Phi_h > \frac{[\theta] - [\theta_c]}{[\theta_h^\infty] - [\theta_c]} = \frac{|[\theta]| - 10.0}{376} \tag{6}$$

Thus, the right-hand side of relationship 6 can be used with the measured CD to provide a greatest lower bound for the fraction helix. Comparison shows that this lower limit lies only ~13% below the value calculated from eq 5. This point bears importantly on the arguments presented below. It is noteworthy that the values obtained near room temperature in this investigation do not differ significantly from those obtained in other laboratories for either α-Tm[26] or BSA[23,24] even though different methods are employed to relate CD to helix content.

Theoretical Calculations. Fraction helix was calculated for single chains with the 2 × 2 statistical weight matrix method.[23] No coarse graining was employed.[18] Known sequences were obtained from standard sources for both α-Tm[31] and BSA.[30] Except as otherwise noted, values of σ and $s(T)$ were those obtained from algorithms[19,20] chosen to mimic the measured values obtained by Scheraga et al.[2-17] Unusual residues or those in doubt were treated as described by Mattice et al.[23,24]

III. Results and Discussion

The results of thermal denaturation experiments on both proteins for a wide variety of conditions are displayed in

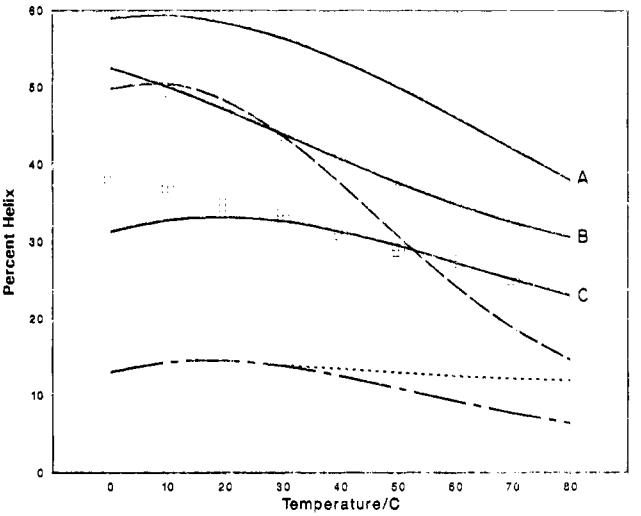

Figure 2. Experimental and theoretical fraction helix vs. temperature for α-Tm and BSA. Open circles, averaged data for α-Tm; squared plusses, averaged data for BSA. solid curves A (α-Tm) and C (BSA): theoretical results from $s_{RHK} = 1.7$ and $\sigma_{RHK} = 0.05$, as recommended by Mattice et al. Dashed curve (α-Tm) and dot–dash curve (BSA): theoretical results from constant s_{RHK} and corresponding σ_{RHK} (eq 1) chosen to match α-Tm data at 30 °C. Full curve B (α-Tm) and dotted curve (BSA): theoretical results from $s_{RHK}(T)$ chosen to match α-Tm data at each temperature and σ_{RHK} from eq 1 with s_{RHK} (30 °C).

Figure 1. Complete documentation of the conditions is given in Table I. Careful examination of these results leads to the following conclusions: (1) The two proteins, so very different in the native state, are rather similar in the reduced state in DS^- solutions, both quantitatively and qualitatively. BSA is about ten percentage points less helical at 0.0 °C, but its helix content declines slightly less sharply with temperature so that the difference is only about five percentage points near 80 °C. (2) Substitution of Li^+ for Na^+ produces no demonstrable change in helix content, where overlapping data are accessible, so that studies in LDS, which can be made over a much wider temperature range, are applicable to SDS as well. (3) Varying the protein concentration over the range 0.1–12 mg mL^{-1} has no effect, as expected if protein molecules are acting independently. (4) Varying the neutral salt concentration over the range 33–500 mM has no influence. (5) Varying DS^- concentration over the range 18–30 mM has no influence. (6) The conformation does not depend upon whether Hepes or phosphate buffer is used. (7) Cross-linking the α-Tm at Cys 190 has no influence in the temperature range 10–80 °C and results, perhaps, in a very slight reduction in helix content in the range 0–10 °C. Thus, the two chains are almost conformationally independent in DS^- even when they are "stapled" at one point. (8) Neither protein displays a curve that has the appearance of a highly cooperative transition. If there is any plateau region at either temperature extreme, it is not unequivocally evident, and there is no region of sharp change.

Since it is clear from Figure 1 that temperature and the nature of the protein employed are the only really significant variables, comparisons with theory are facilitated by displaying these data as average values for each protein at a given temperature. The data of Figure 1 are displayed in this fashion in Figure 2 at 10-deg intervals; circles represent the data for α-Tm, squared plusses for BSA.

We next examine how well the prescription of Mattice et al., summarized in Section I as points a–h, serves to fit the data. We see that assumptions a and b allow us to employ the helix–coil theory in question, since the theory only considers those two conformations. Assumption c seems unnecessary, in the present context, because only the free energy of the random form of a residue *relative* to its helical form can affect the helix content. Since it has no bearing on the result, we will defer commentary on this point until more important issues have been dealt with. Assumption d allows us to accept the full table of σ and $s(T)$ values, measured for the DS^--free case, for all residue types except arginine, histidine, and lysine. Assumptions e and f place upon us the burden of choosing an appropriate single value of s_{RHK} for these three residues, whence assumptions g and h allow calculation of the appropriate single value of σ_{RHK}.

As our first attempt at implementation, we accept not only the assumptions but the actual numerical values recommended by Mattice et al.; i.e., $s_{RHK} = 1.7$; $\sigma_{RHK} = 0.05$.[25] Theoretical curves calculated accordingly are shown on Figure 2 for α-Tm (solid curve A) and for BSA (solid curve C). The curve calculated for BSA is in remarkable agreement with experiment from near room temperature to the highest accessible temperature (~70 °C). Below room temperature, agreement is less satisfactory, becoming serious at 0 °C. Unfortunately, the curve for α-Tm obtained with these same parameters is quite unsatisfactory, being well above the experimental results for the entire range. One should keep in mind in making such judgments not only that α-Tm is probably the protein that ought to conform most closely to the model but also that the effect of DS^- is to reduce the helix content for highly helical proteins and increase it for relatively nonhelical ones. This latter leveling effect has as its practical result that the vast majority of proteins fall in the range 20–45% helix near room temperature. Of course, one must exclude a protein like collagen whose amino acid composition makes the α-helix an essentially irrelevant structure. Thus, a method that cannot do better than plus or minus ten percentage points is useless, since the universal guess of 32% helix, made instantaneously and with no computational effort whatever, does just as well in the vast bulk of cases.

We conclude, therefore, that the recommended[25] values of s_{RHK} and σ_{RHK} do not produce satisfactory agreement with our experiments. Evidently, any additional attempt to adjust these values to improve the BSA agreement at low temperature necessarily worsens that for α-Tm. Furthermore, in our opinion, this disagreement is already manifest in extant results for other proteins in DS^- even though the helix contents[25] were determined from a correlation curve of CD vs. theoretically calculated helix content for a large number of proteins[24] rather than from a CD analysis that, right or wrong, is essentially independent of the helix–coil theory. Recent CD experiments at room temperature on glucagon, secretin, and vasoactive intestinal peptide gave correlation-curve helix contents of 28%, 43%, and 47%, respectively; the respective theoretical values are 20%, 28%, and 37%.[25] In contrast to Robinson et al.,[25] we would judge the agreement to be unsatisfactory in at least two cases out of the three.

We next inquire whether a better pair of values of s_{RHK} and σ_{RHK} might be selected. We prefer a procedure in which α-Tm is used as a standard, since we have more data, the CD is slightly higher and therefore better determined, the calculation of helix content from CD is more reliable (see above), and the applicability of the helix–coil theory is more certain. Since, furthermore, the relationship proposed between s and σ (eq 1) refers to near room temperature and most of the calculations of Mattice et al. refer to 30 °C, we choose α-Tm at 30 °C as our standard. Using the theory, then, we find by trial that $s_{RHK} = 1.26$ and the

corresponding (eq 1) value of $\sigma_{RHK} = 0.00375$ provide a theoretically calculated helix content indistinguishable from the experimental value for α-Tm at 30 °C. Furthermore, keeping these values for σ_{RHK} and s_{RHK} constant, we can calculate the fraction helix at any other temperature for α-Tm and for BSA as well. Theoretical curves calculted on this basis are shown on Figure 2 for α-Tm (dashed curve) and for BSA (dot–dash curve).

Figure 2 shows that the theoretical result is in fair agreement with the α-Tm data below 30 °C (although the shapes of the theoretical and experimental curves differ), but above 30 °C the curves part company and differences are pronounced above 50 °C. Thus, the prescription does not work very well for a given protein over the accessible temperature range even when force fit near the center of the range.

Turning to the BSA results, we find an even less satisfactory situation. The theoretical prediction is far lower, by as much as a factor of 3, than the experimental results over the entire temperature range. Clearly, literal application of the prescription e–h cannot succeed.

We next inquire whether some readily effected modification of the prescription might be made that would improve the results. The most obvious such point of intervention is at assumption f, which, indeed, seems unlikely anyway, since all s values that have ever been measured have been temperature dependent. This assumption is actually rather readily eliminated. Having established a satisfactory s_{RHK} for α-Tm at 30 °C and its corresponding σ_{RHK}, we have only to maintain that value of σ_{RHK} (σ values are generally assumed to be temperature independent) and obtain values of s at other temperatures to fit the rest of the data for α-Tm. Implementing this program, we find values of $s_{RHK}(T)$ that are very well fit by the algorithm:

$$\ln s_{RHK} = 11.0406272 - (6306.0227/T) + (918524.569/T^2) \quad (7)$$

Using eq 7 to obtain s_{RHK} along with a constant $\sigma_{RHK} = 0.00375$ in the theory provides the solid curve B of Figure 2, which of course fits the data for α-Tm extremely well, since it has been forced to do so.

Application of eq 7 and the same σ_{RHK} to BSA produces the theoretical curve shown dotted in Figure 2. Comparison with the BSA experimental data is still very unsatisfactory. Furthermore, the severe discrepancies cannot conceivably be ascribed to the technical difficulties discussed above in calculating fraction helix from CD for BSA. We established in that discussion that although an exact method is not at hand, no defensible method could violate the inequality of relation 6, which provides a greatest lower bound for the fraction helix. Thus, although our experimental value of the helix content for BSA at, say, 30 °C is calculated here to be near 35% and this may be somewhat in error, that error cannot possibly change our conclusions: if the correct value is greater than the 35% helix we report, the agreement with theory is even worse than Figure 2 presently shows. If the correct value is *lower* than 35%, it will improve agreement, but since that correct value cannot be lower than the 29% obtained from (6), we still confront an experimental value that is factor of 2 larger than that obtained from theory. Since the same set of σ and $s(T)$ values that provides a satisfactory fit for α-Tm does not do so for BSA, clearly even this modified prescription fails. It is fundamental to the theory that the values of σ and $s(T)$ for each amino acid type be independent of the protein chain in which it appears.

We have been unable to devise any other readily effected modification of prescription a–h that would save the situation. Nevertheless, as already emphasized, it would be extremely useful from both the physical and biochemical points of view to be able to calculate such things as the helix content and helix probability profile along the chain. Consequently, an examination of prescription a–h is in order to shed light on how one might proceed in the future development of such a theory.

Assumptions a and b are more fundamental than the rest. These first two require that proteins in DS^--added solutions conform to the conceptual regime (short-range interactions only) that forms the basis for the theory itself. Of the remaining assumptions c is special (see below), and d–h provide a prescription for calculating the input parameters for the DS^--added case from those already available for the DS^--free case. Clearly then, if assumptions a and b fail, the theory itself is inapplicable, whereas failure of only d–h might simply alter the implementation procedure.

It seems to us, as it did to the original proponents of these ideas, that assumptions a and b are sufficiently plausible physically and their implications sufficiently powerful to deserve thorough testing. It is well documented that DS^- has a drastically destructive effect on quaternary and tertiary structure in proteins. It is but a short step from that fact to assumptions a and b, and these lead immediately to an extant theory, very successful in synthetic polypeptides, from which it is possible (with appropriate input parameters) to compute not only the overall helix content but the local secondary structure at any point in a protein chain of known sequence. It is axiomatic that anyone studying a protein in DS^-, and many do by choice or by necessity, would be interested in having such information. The only serious physical doubts about a and b involve, it seems to us, electrostatic interactions and the posible survival of appreciable β-structure in DS^--added media. If either are important, the theory would be inadequate. However, the lack of dependence on ionic strength seen here argues against the importance of the former as does the recent suggestion[21] that the short-range theory may suffice to explain the observed enhancement of helix content in acidic DS^--added media.[26] Furthermore, we have seen no clear evidence for the β-structure in our experimental spectra, but these are so far limited to two proteins. In our view, therefore, assumptions a and b are worthy of attention, even if it means the determination of the full panoply of σ and $s(T)$ values for each amino acid type in the DS^--added system at both neutral and low pH, a rather heroic undertaking.

Assumption c seems to us rather implausible and perhaps inconsistent with a and b but, in any case, superfluous if helix content alone is sought. It is unlikely that a randomly coiled leucine residue, for example, would be indifferent to the presence or absence of DA^-. Indeed, assumptions a and b would appear to depend heavily on the ability of DS^- to lower the free energy of such a residue relative to aqueous media without DS^-. However, if DS^- does lower the free energy of a randomly coiled leucine, it probably also does so for a helical leucine, which, in the absence of tertiary and quaternary structures, also has its hydrophobic side chain exposed. Whether the two cancel out or not is at present very much an open question. Only measurement of σ and $s(T)$ for leucine in the DS^--added system and comparison with already available values for the DS^--free system can give us an answer.

Assumption d seems to us rather doubtful, but not impossible. Certainly, one would expect changes, probably increases, in arginine, histidine, and lysine. Above, we have argued that hydrophobic residues might also be affected. Indeed, some evidence exists that they are affected. The

helix content of a certain tryptic fragment of glucagon changes from near 0% in DS^--free solution to 45–50% in DS^--added solutions.[32] Yet this fragment possesses no arginine, histidine, or lysine whatever. This would seem to be rather direct evidence that DS^- enhances helix-forming tendencies for side chains other than cationic ones. The magnitude of this helix-enhancing effect is large; in DS^- the number of helical residues in the fragment is 75% of that in glucogon itself although the fragment is only 38% as long. In our view, this is prima facie evidence that other than cationic side chains are influenced by DS^-. Since the effect in the fragment is even larger, relatively speaking, than in the intact glucagon chain, the conclusion drawn in ref 25 that this is a small effect in intact glucagon that only becomes dominant in the fragment because of the absence of cationic groups seems unwarranted. However, in our view it is somewhat hazardous to base conclusions on polypeptide chains, intact or excised, under conditions such that they contain only very short helical stretches. Even in intact glucagon in DS^-, the estimated number of helical residues is very near eight,[25] which is only a trifle more than two full turns. Such helices are likely to be distorted.

As for residues other than hydrophobic or cationic ones, the results are rather difficult to predict. We would point out, however, that even a small change in the values for a residue such as glutamic, because its s is near unity and its appearance so frequent, can appreciably alter the results. Only measurement of the σ and $s(T)$ values can give the answer.

Assumptions e–g seem unlikely. The table of values for the DS^--free system does not encourage the idea that residues of the same charge type have the same σ or $s(T)$ or that *any* s can be expected to be temperature independent. In the DS^--free system, arginine and lysine not only have different values of s at room temperature but the temperature coefficients are of opposite sign and the values of σ are very different. In DS^-, cancellation of effects could conceivably lead to such a result as is stated in assumption e–g, but this would be remarkably fortuitous. Finally, assumption h is a very useful result of an empirical correlation in the DS^--free system near room temperature. Whether it will hold up in the DS^--added case is impossible to guess.

There seems to be no way to avoid determination of σ and $s(T)$ in DS^- for, at least, arginine, lysine, alanine, leucine, and glutamic residues as probes of the issues raised by assumptions d–h. These are very common residues of different type and have a powerful influence on the results. If these lead to improvements in d–h and to a better picture than Figure 2 displays, then perhaps a limited number of host–guest measurements will be adequate. If not, then it will take nothing short of a full-blown effort to determine σ and $s(T)$ for each type of residue in DS^-. This is a somewhat daunting prospect, but the game would seem to be worth the candle. Proteins in DS^- are of great interest. Yet at present we have a rather complete set of measured input parameters for proteins in a regime (DS^--free) in which the theory is inapplicable and a powerful theory that is perhaps applicable for a regime (DS^--added) in which we have no measured input parameters.

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References and Notes

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(22) Abbreviations: SDS, sodium dodecyl sulfate; LDS, lithium dodecyl sulfate; DS^-, dodecyl sulfate anion; DTT, dithiothreitol; CD, circular dichroism; α-Tm, rabbit α-tropomyosin; BSA, bovine serum albumin; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; P_i, the usual mixture of phosphates near neutral pH. We describe complex aqueous media by giving the formula (or abbreviated name) of each solute with its millimolarity as subscript.
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