Theory of α-Helix-to-Random Coil Transitions of Two-Chain, Coiled Coils. Application to a Synthetic Analogue of Tropomyosin

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ABSTRACT: A theory developed previously for the stability of the native structure in two-chain, α-helical coiled coils is applied to singly (disulfide) cross-linked tropomyosin and to a singly (disulfide) cross-linked, synthetic, 43-residue peptide analogue (called here XY_5) of tropomyosin. Algorithms are obtained from fits of extant data for the helix stability parameter (s) for each amino acid residue type as a function of temperature; these reproduce the data to high precision. Extant data for the helix initiation parameter (σ) are used as is. Employing these s and σ values to realize the theory and using extant thermal denaturation data for XY_5 and for tropomyosin, we have determined the temperature dependence of the helix–helix interaction parameter $w(T)$ of the theory for the two substances. These $w(T)$ functions then allow smoothed theoretical fits of thermal denaturation data and calculation of helix probability profiles along the polypeptide chains for XY_5 and for tropomyosin. Theoretical predictions are generated concerning the thermal denaturation and helix probability profiles of yet unsynthesized, longer chain homologues (XY_k) of the synthetic material XY_5, including XY_{39}, whose 281-residue chain would more closely model the 284-residue chain of tropomyosin. The importance of degree of polymerization in dictating helix content and profile and the difficulties in choosing models to match both the σ and $s(T)$ values that govern short-range interactions and the $w(T)$ that governs interhelix interactions are clearly demonstrated. Thermodynamic implications of the $w(T)$ functions obtained are discussed and shown to imply algebraic signs for standard enthalpy, entropy, and heat capacity changes that are difficult to reconcile with the expected hydrophobic and salt-bridge nature of the interactions. Predictions are generated concerning the behavior of non-cross-linked XY_5, for which very few data are available. Some possible future directions of theory and experiment are outlined.

I. Introduction

The native tropomyosin molecule consists of two polypeptide chains. Each chain comprises 284 amino acid residues and is essentially completely α helical. The two α-helical chains that constitute a single native molecule are set side by side, in parallel and in register, with a slight supertwist.[1-15] Studies of the amino acid sequence reveal that there is a quasi-repeating heptet of amino acids (designated by letters a–g) along each chain.[9,13-15] Within each heptet, positions a and d are almost invariably occupied by hydrophobic residues, e by an anionic residue, and g by a cationic residue. When such a chain is coiled into an α helix, the a and d residues appear on the same surface, allowing a favorable, hydrophobic interaction with an adjacent helical chain of the same type. Furthermore, this juxtaposition brings e and g residues close to opposite charges on the adjacent chain, providing salt-like, favorable interactions.[9,13] Because of its relatively simple structure, tropomyosin is not just interesting as an important muscle protein, but represents a natural model for the study of α helices and of helix–helix interactions, both of which exist in abundance in globular proteins as well.

For some time, a statistical theory has existed that treats the helix–random coil equilibrium in single-chain polypeptides.[16-20] In this theory the helix content is assumed to depend upon so-called "short-range" interactions characterized by a helix stability parameter (s) and a helix initiation parameter (σ). The magnitude of s and of σ are in turn assumed to depend only upon the nature of the amino acid residue and upon temperature but are difficult to calculate from first principles. In a massive effort extending over many years, Scheraga and co-workers collected data on synthetic polypeptides and employed a host–guest technique to determine s and σ values appropriate to each of the various amino acids found in proteins.[21-34]

These experimental values have been used and the resulting "realized" theory developed to the point where α-helix content and other properties have been rather successfully calculated for globular proteins of known sequence under conditions such that tertiary and quaternary structure does not exist, i.e., where the short-range interactions dominate and where α helix and random coil are essentially the only conformations of interest.[35] Although this modest success does not justify casting the theory and its attendant table of experimental input parameters in bronze just yet, it clearly encourages further study, for only by pursuing this realized theory to its logical conclusions can we assess its power to explain existing experiments and to suggest new ones and predict their outcome.

In that spirit we have begun to investigate the extension of this approach to include native protein structures. Tropomyosin, because of its relative structural simplicity, suggested itself as a first step and the realized theory immediately has something provacative to say about this molecule. It predicts that a single tropomyosin chain would have a rather low helix content (15–20%) in benign media near room temperature,[35] whereas the measured helix content of the two-chain species is over 90% under those conditions. Taking this at face value forces the view that interhelix interactions make a major contribution to stabilization of the helix in each chain.

For these reasons, we very recently extended the theory to include interhelical interactions in cross-linked or non-cross-linked, two-chain α helices of the tropomyosin type.[36] Cognizance is taken of the quasi-repeating heptet structure by a coarse-graining procedure which requires a block of four successive helical residues (a–d) or of three successive helical residues (e–g) to interact with the corresponding sequence on the adjacent helical chain in order to produce the decrease in free energy that represents the interaction. This standard free energy change when two such blocks of helical residues (one on each chain of a singly cross-linked, two-chain dimer) are allowed to interact is written as $-kT \ln w$. Thus, the parameter w embodies

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this interchain interaction, and the earlier paper[36] shows how to graft this additional interaction onto the theory that already contains the array of short-range (s,σ) interactions appropriate to the amino acid sequence.

Strictly speaking, just as each type of amino acid has its own value of s and of σ, each pair of interacting side chains should have its own characteristic value of w. At the very least, a distinction perhaps ought to be made between (hydrophobic) interactions of the four-residue blocks and (salt link) interactions of the three-residue blocks. The theory is cast in such a form that such distinctions could be made, but in the absence of a priori information on the relevant values of w (which is analogous to the table of values of s and σ), it is difficult to do so in practice. Hence, in the earlier work,[36] a single, average value of w was assumed to hold along the entire length of the chain.

In this connection, a recent remarkable series of experiments on the thermal stability of the α-helical, coiled coil formed by a synthetic analogue of tropomyosin should prove very informative.[37] This synthetic analogue has, in the single-letter code, the sequence Ac-K-C-A-E-L-E-G-K-(L-E-A-L-E-G-K-)$_5$OH, and thus has a 43-residue chain. We will refer to this chain as XY_5, thus viewing it as an (acetylated) N-terminal octapeptide followed by five identical (except for the terminal carboxyl) heptapeptides.[38] This polymer is extremely carefully designed as a significant model for tropomyosin.[37] The cysteine has an immediate environment that mimics that of the lone cysteine (Cys-190) in α-tropomyosin and also allows cross-linking to a second chain; and the repeating heptet has apolar residues (leucines) in core a and d positions, anionic residues (glutamates) in e positions, and cationic residues (lysines) in g positions, as required. Furthermore, the uniformity of this model makes it an excellent subject for the theory, since one would expect the use of a single value of w for each block to be a rather good representation of the truth in such a case.

We report here calculations made on this synthetic analogue XY_5 using the theory described earlier[36] in conjunction with tabulated values of s and σ.[21–34] This then allows calculation of the fraction helix in the cross-linked dimer as a function of w at any given temperature. Use of the thermal denaturation data on XY_5 in conjunction with the theory then allows evaluation of w for XY_5 as a function of temperature, thus providing a measure of the interchain contribution to the stability. Helix probability profiles along each chain in the dimer are also calculated, and predictions are made concerning the comparative behavior of non-cross-linked XY_5 molecules.

It must be recognized, however, that in spite of the care with which the model is designed, its degree of polymerization (43 residues/chain) is very far from tropomyosin (284 residues/chain) and that this does affect helix content. We therefore calculate similarly the stability properties of longer chains of sequence XY_k whose degree of polymerization (n) is therefore $n = 7k + 8$. These calculations can be viewed as predictions of the behavior of yet-unsynthesized models that are homologous to XY_5 and analogous to tropomyosin, and the calculations begin to allow analysis of which differences between XY_5 and tropomyosin stem from specific chemical composition differences and which simply from chain length.

In a manner similar to that employed for XY_5, we used the thermal denaturation data for cross-linked tropomyosin to estimate $w(T)$ for it. This allows comparison of the effect of the leucine hydrophobic core in XY_5 with that of the more varied hydophobic interactions in tropomyosin. In this manner, the realized theory makes possible the dissection from the observations of the relative influence of chain length, short-range interactions, and interhelix interactions.

II. Methods

Calculations of the sort described here can be no better than the input parameters employed to realize the underlying theory. In previous theoretical studies of the same ilk,[35] numerical values of these input parameters were mostly required at only a singe temperature (30 °C). In the present case, we wish to generate calculations over a rather wide temperature range (10–80 °C). We require, therefore, s and σ for each residue type as functions of temperature. It is generally conceded that σ is only slightly dependent on temperature, but the temperature dependence of s for each amino acid is still required.

The extensive researches of Scheraga et al.[21–34] do indeed provide tables of s vs. temperature (usually from 0–70 °C) for each amino acid. However, it is inconvenient in performing the numerical computations to insert the actual data. Instead, we have used an algorithm to generate values of s for each amino acid at any temperature within the range of the data. Because s is an equilibrium constant, one's first guess would be that ln s might be linear in T^{-1}, as would be the case if the corresponding standard entropy and enthalpy were temperature independent. In fact, less than half of the residue types show s data that are well fit to that approximation. Consequently, we fit the data for each residue over the range 10–70 °C to the equation

$$\ln s = B_0 + B_1T^{-1} + B_2T^{-2} \tag{1}$$

wherein the B_i are constants and T is the Kelvin temperature. The best fit was determined by unweighted least squares or, in a few cases, by the method of average points, but in every case it was verified that the s values obtained algorithmically are essentially indistinguishable from the actual experimental values. The numerical coefficients appropriate for generation of s vs. T are presented in Table I along with the σ values used. In performing the calculations, we employed the same algorithm to extend the temperature region to 80 °C, 10 °C beyond the data themselves.

All the theoretical equations used are as presented earlier.[36] The coarse-graining technique selected was of the $m = 4,3$ type for all of the tropomyosin chain and for most of the chain in its XY_k analogues. To account for the N-terminal octet in the analogues, the first five residues were treated as a block, and then the next three; thereafter, strict 4,3 coarse-graining was used. Thus, in the repeating heptets, the -L-E-A-L- blocks, which contain the core hydrophobes, were treated as a block of four, and the -E-G-K- blocks, which contain salt-bridging residues, as a block of three. This leaves open the possibility of employing different w values for the two different types of interaction, but for the present calculations the same w was used for every block.

Data on XY_5 are all taken from Hodges et al.[37] or from a detailed supplement graciously provided to us by Professor Hodges. Data on tropomyosin are taken from the study by Lehrer.[39] To convert reported circular dichroism (CD) to fraction helix, we employ the usual relation

$$f_h^n = \frac{[\theta^n] - [\theta_c]}{[\theta_H^n] - [\theta_c]} \tag{2}$$

wherein f_h^n is the fraction helix of an n-residue polypeptide, $[\theta^n]$ is its mean residue ellipticity (deg cm^2 dmol^{-1}), $[\theta_H^n]$ is the mean residue ellipticity of a complete, n-residue α

Table I
Coefficients for $s(T)$ Algorithm

residue	B_0	B_1	B_2	$10^4\sigma$
Ala (A)	−0.354	121.8	0.0	8.4
Arg (R)	−4.489	2551.6	−359 086	0.1
Asn (N)	0.443	−201.3	0.0	0.1
Asp (D)	−7.538	3616.4	−445 782	70.0
Cys (C)[a]	−11.072	6628.4	−1 013 995	0.1
Gln (Q)[b]	−0.362	95.6	0.0	6.0
Glu (E)	−0.362	95.6	0.0	6.0
Gly (G)	−6.573	3964.0	−643 300	0.1
His (H)[c]	−3.246	1864.2	−261 172	18.0
Ile (I)[d]	1.030	−322.1	0.0	1.0
Leu (L)	−4.076	2516.0	−377 500	30.0
Lys (K)	−6.069	3678.0	−562 800	1.0
Met (M)	−3.143	1618.8	−190 048	51.0
Phe (F)	−3.246	1864.2	−261 172	18.0
Ser (S)	−11.072	6628.4	−1 013 995	0.1
Thr (T)	0.574	−227.0	0.0	0.1
Tyr (Y)	−21.124	11983.0	−1 694 691	66.0
Val (V)	1.030	−322.1	0.0	1.0

[a] Assumed same as Ser. [b] Assumed same as Glu. [c] Assumed same as Phe. [d] Assumed same as Val. After this work was completed, new values for Ile were reported (Fredrickson, R.; Chang, M.; Powers, S.; Scheraga, H. *Macromolecules* **1981**, *14*, 625-632). These are fit by $B_0 = -0.846$, $B_1 = 287.4$, $B_2 = 0.0$, $10^4\sigma = 55.0$. Use of the new values yields no significant change in the results reported here for tropomyosin (which has little Ile) and no change whatever for XY_5 and its homologues (which have no Ile).

helix, and $[\theta_c]$ is the mean residue ellipticity of a complete, n-residue random coil, all ellipticities being at 222 nm. At the latter wavelength, the ellipticity for a complete, n-residue helix is given by[40,41]

$$10^{-4}[\theta_H{}^n] = -3.86[1 - 2.55n^{-1}] \quad (3)$$

which we employed to obtain $[\theta_H{}^n]$. The value of $[\theta_c]$ is uncertain, so we calculate $f_h{}^n$ using both $[\theta_c] = 0$ and $[\theta_c] = -2000$ deg cm^2 dmol^{-1},[42] plot the average, and indicate the two values obtained as limits of error.

To obtain the temperature dependence of w for a given cross-linked polypeptide, s values appropriate to a given temperature were generated and the helix content was calculated from theory for a succession of values of w at that temperature. The experimental helix content at the temperature was then used to obtain the value of w appropriate to that cross-linked polypeptide at that temperature by graphical or linear-numerical interpolation. The result for each polypeptide, then, is a series of w values, one for each temperature for which CD data are available. To obtain smoothed values of w for that polypeptide at arbitrary temperature, these discrete values were fit by least squares to the equation

$$RT \ln w = A_0 + A_1T + A_2T^2 \quad (4)$$

wherein the A_i are constants, T is Kelvin temperature, the units used for $RT \ln w$ are cal (mol of block pairs)$^{-1}$. The smoothed values of $w(T)$ generated by eq 4 are then recycled through the now "fully realized" theory to produce what we take as the best theoretical curve of fraction helix vs. temperature for that cross-linked, polypeptide dimer. This method can be applied with confidence to XY_5. For various, mostly technical, reasons, the results obtained for tropomyosin are less secure. This will be discussed below.

III. Results

Dependence of s on T. The numerical values of s and σ dictate the helix content of an isolated ("monomeric") polypeptide chain or of noninteracting chains in a cross-linked dimer. The inter-helix interactions are superimposed (through w) upon these tendencies to produce the observed percent helix of the cross-linked dimers. Thus, it is always important to keep in mind, as a kind of base line, the helix content of the noninteracting chains. Since we are primarily concerned with thermal denaturation, i.e., the change in helix content with temperature, our base line is clearly a consequence of the magnitude of s and σ and particularly of the way in which s changes with temperature.

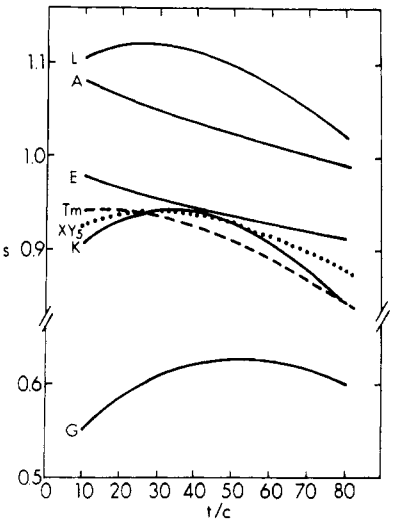

Figure 1. Temperature (Celsius) dependence of s. Solid curves are for amino acid residues L, A, E, K, and G, as marked. Dashed curve, s_{eff} for α-tropomyosin. Dotted curve, s_{eff} for XY_5. Note break in ordinate axis.

Figure 1 displays these changes (solid curves) for those residues particularly significant in the analogue polypeptides XY_k, i.e., for A, L, E, K, and G. The curves shown are obtained from our eq 1 and Table I but are indistinguishable from the original data.[21-34] Clearly, the experimental s values not only vary over a wide range from residue to residue but differ also in the way in which they depend on temperature. This dependence has, as will be seen, serious consequences for the thermal stability of the polypeptide in question.

To gain insight into the effect this might have for particular chains, it must be recognized that s is an equilibrium constant so that the "effective" s for a given chain can be defined as $s_{eff} = [\prod_{i=1}^{n} s_i]^{1/n}$, i.e., the geometric mean s of the residues in the chain. This effective s is also shown in Figure 1 for α-tropomyosin (dashed) and for XY_5 (dotted). If $k > 5$, the curve for XY_k is essentially the same as for XY_5, so the same curve holds for all the analogue polypeptides considered here.

It is at once evident from Figure 1 that the mean intrinsic helix-favoring tendency of the amino acid residues in XY_5 (or XY_k) differs significantly from that in tropomyosin. The short-range interactions are simply not the same in the two and they do not depend upon temperature in the same way.

A Check on Coarse Graining. If the two chains do not interact (i.e., if $w = 1$), then the helix content and helix probability profile will be the same as if the chains were separated. In that case, no coarse graining is necessary, the earlier theory[35] applies, and the "exact" helix probability profile (i.e., with $m = 1$) can be calculated. The result of such a calculation for XY_5 using s values appropriate to 30 °C is shown in Figure 2 (open circles, dashed line), wherein i, the residue number, is assigned with unity at the N terminal. The pattern seen is clearly a result of two effects: periodic small-scale variations caused by variation of s and σ for the individual residues within the repeating

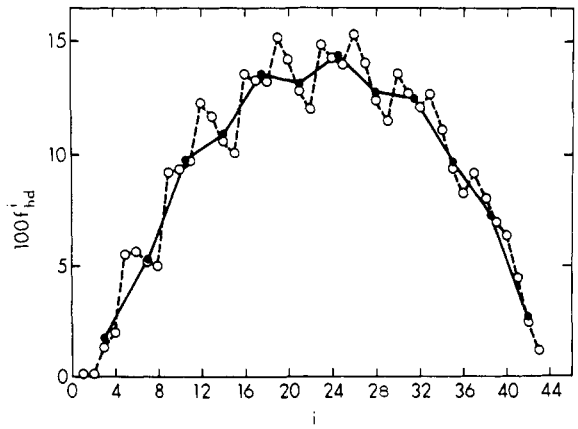

Figure 2. Helix probability profiles for noninteracting ($w = 1$) XY_5 helices at 30 °C. Open circles with dashed line: $m = 1$; 9.46% helix. Filled circles with full line: $m = 4,3$; 9.34% helix.

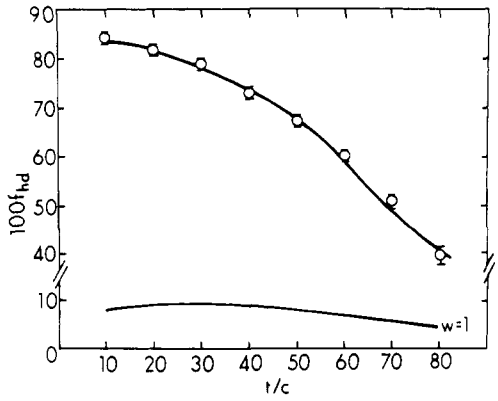

Figure 3. Percent helix ($100f_{hd}$) of cross-linked XY_5 vs. Celsius temperature. Open circles are data points from ref 37. Error bars represent range provided by use of −2000 deg cm^2 dmol^{-1} or zero for $[\theta_c]$ in eq 2. Full curve through data is from theory with $w(T)$ as described in text. Lower full curve is from theory for noninteracting ($w = 1$) chains. Note break in ordinate axis.

heptet, superposed upon the normal statistical tendency for decreasing helix content near the chain ends. The overall helix content is calculated to be 9.46%. This is small compared even with noninteracting tropomyosin chains at the same temperature (17.2%), simply because of the lower chain length of XY_5 (43 residues/chain compared with tropomyosin's 284). The s and σ values for the residues of XY_5, in fact, intrinsically favor helix slightly *more* than those in tropomyosin at this temperature (see Figure 1 and results below).

The helix probability profile for noninteracting ($w = 1$) XY_5 chains given by the $m = 4,3$ coarse-graining calculation is also shown on Figure 2 (filled circles, full line). Although much of the fine structure is removed by coarse graining, the distinction between blocks of 4 and of 3, which stems from different effective s and σ, shows up clearly and the general trend agrees well with the "exact" ($m = 1$) calculation. Furthermore, the calculated overall helix content given by coarse graining is 9.34%, in excellent agreement with the 9.46% cited above the exact case. We believe this justifies the 4,3 coarse-graining calculations, and, inasmuch as similar agreement was obtained for all cases reported below, this mode will be used without further apology in the calculations that follow.

Cross-Linked XY_5. The thermal denaturation data from Hodges et al.[37] for this substance are shown in Figure 3 as experimental points whose error bars convey the unavoidable uncertainty in calculating fraction helix from mean residue ellipticity. The lower solid curve is our theoretical base-line curve for noninteracting chains with

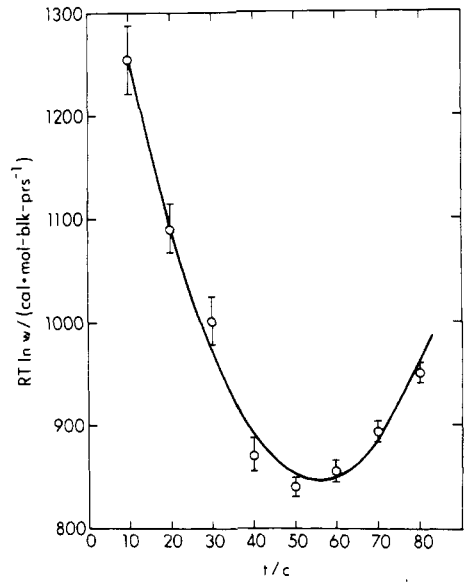

Figure 4. $RT \ln w$ (cal (mol of block pairs)$^{-1}$) vs. Celsius temperature for XY_5. Open circles are values required by realized theory to give experimentally observed helix content. Error bars stem from uncertainty in calculating percent helix from CD. Solid curve is unweighted least-squares fit to second-order polynomial. Coefficients are $A_0 = 22150$, $A_1 = -129.55$, and $A_2 = 0.1969$.

the XY_5 sequence. Note that the latter curve follows the corresponding one for s_{eff} (Figure 1), rising to a shallow maximum near 30 °C and thence falling to predict very small helix content (<5%) at the highest accessible temperature (~80 °C). Note also the vast discrepancy between the helix content experimentally observed at any temperature and that calculated with $w = 1$. The realized theory clearly requires that single chains of XY_5 be only very slightly (<10%) helical over the entire temperature range.

The individual values of w required at each temperature to bring the low helix content calculated for the isolated chain up to that observed experimentally are shown in Figure 4 as $RT \ln w$ vs. t. The points clearly go through a minimum and are well fit by the solid curve, which is the unweighted least-squares curve given by eq 4 with $A_0 = 22150$, $A_1 = -129.55$, and $A_2 = 0.1969$. Recycling the smoothed values of w back into the theory provides smoothed values of percent helix vs. temperature for XY_5 dimers with interacting helices. These are shown in Figure 3 as the upper solid curve. Clearly, this provides a satisfactory fit to the experimental data, but this in itself is no virtue since $w(T)$ was essentially chosen to force such a fit.

Once in possession of $w(T)$ for XY_5, we can calculate helix probability profiles for it at any temperature. Some examples are presented in Figure 5, in particular for 30 °C, where it is 78.4% helix, and 70 °C, where it is 49.0% helix. For comparison, profiles are also shown for the noninteracting ($w = 1$) helices at the same temperatures. The dominant feature of these profiles at this low degree of polymerization ($n = 43$) is the pronounced helix-depressive effect of the chain ends, which persists virtually to the center of the chain. That is, in spite of the exactly repetitive nature of the chain, the helix probability does not shown an essentially constant region except in the very center at very high overall helix content. As will be seen, this is in marked contrast to the profiles shown by analogues (XY_k) of higher degree of polymerization.

Cross-Linked XY_k. Having a fully realized theory suitable for XY_5 should be nearly equivalent to having one for its higher homologues XY_k (with $k > 5$), since all such homologues perforce must have chain–chain interactions

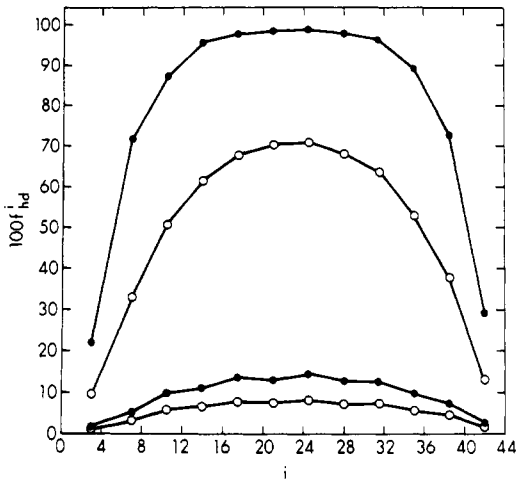

Figure 5. Helix probability profiles for cross-linked XY_5. Filled circles, 30 °C: upper curve, $w = 5.04$, 78.4% helix; lower curve, $w = 1$, 9.34% helix. Open circles, 70 °C: upper curve, $w = 3.65$, 49.0% helix; lower curve, $w = 1$, 5.42% helix.

essentially equal to those in XY_5. That is, the $w(T)$ obtained from the fit to experiments on the latter polypeptide should suit higher XY_k polymers as well since the same chemical groupings are involved in the interactions. It is thus easy at this point to generate predictions about higher homologues of XY_5, none of which have as yet been synthesized. Moreover, it is vital to do so since the strong randomizing effect of the chain ends (see Figure 5) makes a 43-residue chain such as XY_5 an inherently imperfect model for a 284-residue chain such as tropomyosin, however carefully they have been otherwise matched. For this reason we have used the same $s(T)$, σ, and $w(T)$ values employed for XY_5 to calculate fraction helix for cross-linked dimers of XY_k polymers with $k = 7$, 13, and 39, i.e., for 57-, 99-, and 281-residue chains. The latter, of course, is highly significant in that it has essentially the same degree of polymerization as tropomyosin and is therefore the analogue of greatest interest, although it probably will never be made. Unfortunately, it would require, at present, nothing less than heroic effort to produce XY_{39}, but XY_7 might not be out of reach, and, moreover, it is important for theory to extrapolate experiments (on a 43-residue polymer) into the range of greatest interest (~280 residues).

We therefore display in Figure 6 some of the results of our calculations for these cross-linked analogues of higher degree of polymerization (n), both as percent helix vs. t (Figure 6A) and as percent helix vs. n^{-1} (Figure 6B) for several temperatures. It is immediately clear from the figure that degree of polymerization is a very important variable in dictating helix content for these substances. All the XY_k peptides necessarily have almost identical $s(T)$, σ, and $w(T)$; yet, the fully realized theory predicts that the helix content would vary at, for example, 80 °C from near 40% for the dimer of the 43-residue chain studied by Hodges et al. to over 90% for the dimer of a homologous 281-residue chain. It is also clear that a theory is needed to perform such analysis, because the dependence of percent helix on degree of polymerization is not simple (Figure 6B).

Helix probability profiles for XY_5 have already been presented (Figure 5). In Figure 7 we display them for the dimer of the 281-residue polymer XY_{39} at 30 °C as the upper solid curve; the lower solid curve is the corresponding one for noninteracting ($w = 1$) dimer chains, shown for comparison. Unlike the homologous shorter peptide (XY_5, Figure 5), wherein "end effects" persist to

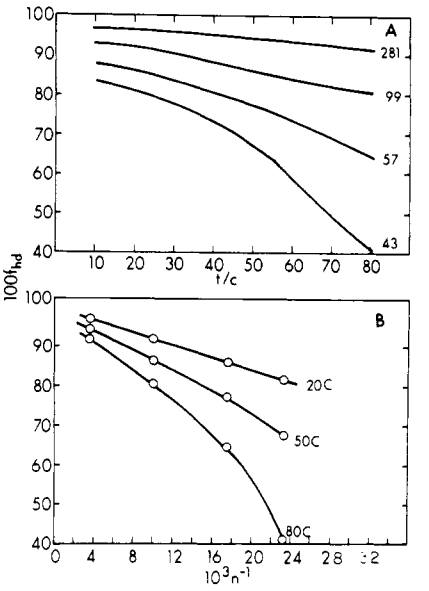

Figure 6. Dependence of percent helix on degree of polymerization and temperature. (A) Percent helix vs. Celsius temperature. All curves from theory at degree of polymerization (n) as marked. (B) Percent helix vs. reciprocal of n. Open circles are from theory for same n values as in (A) and at temperatures as marked.

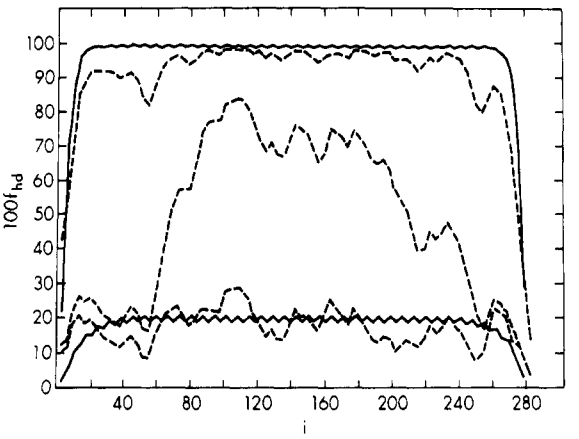

Figure 7. Helix probability profiles for cross-linked XY_{39} (solid curves) and for cross-linked α-tropomyosin (dashed curves). Upper solid curve, XY_{39} at 30 °C ($w = 5.04$, 96.1% helix); lower solid curve, noninteracting XY_{39} at 30 °C ($w = 1$, 18.3% helix). Top dashed curve, α-tropomyosin at 30 °C ($w = 2.56$, 89.8% helix); middle dashed curve, α-tropomyosin at 50 °C ($w = 1.93$, 48.3% helix); bottom dashed curve, noninteracting α-tropomyosin at 30 °C ($w = 1$, 17.2% helix).

the middle of the chain, the longer peptide of the same regular structure shows a long central region (comprising all residues interior to ~35 residues from either end) of essentially constant helix probability, there being only a slight "pinking shears" effect due to differences in effective s and σ between 4-residue and 3-residue blocks. This constancy is not simply a result of the very high overall helix content (96.1%) coupled with the imposition of a ceiling at 100%, since it is just as characteristic of the noninteracting ($w = 1$) case, which has rather low (18.3%) helix content. Indeed, it is characteristic of all profiles of XY_{39} we have calculated at whatever helix content. These are not shown because they reveal nothing new. These profiles for the 281-residue XY_{39} are thus in marked contrast to those displayed by XY_5 (Figure 5) and, as will be described below, also contrast with those for tropomyosin, albeit for a different reason.

Cross-Linked Tropomyosin. The effort to realize the theory fully in the case of tropomyosin is not as

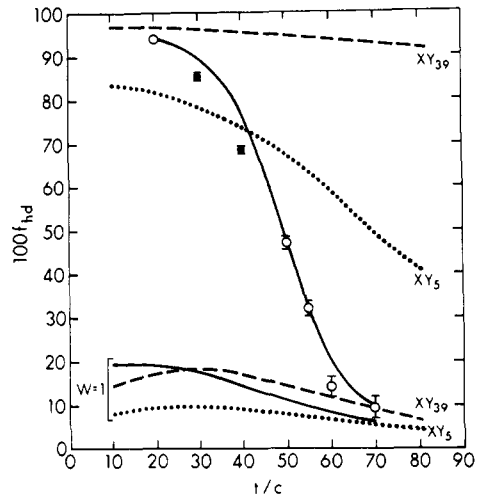

Figure 8. Percent helix ($100f_{hd}$) of cross-linked tropomyosin vs. Celsius temperature. Open circles, experimental points (ref 39). Filled circles, lower limit to experiment (see text). Error bars give limits for calculation of percent helix from mean residue ellipticity. Solid curve, theory for tropomyosin using $w(T)$ (upper) and $w = 1$ (lower). Dashed curves, theory for XY_{39}. Dotted curves, theory for XY_5.

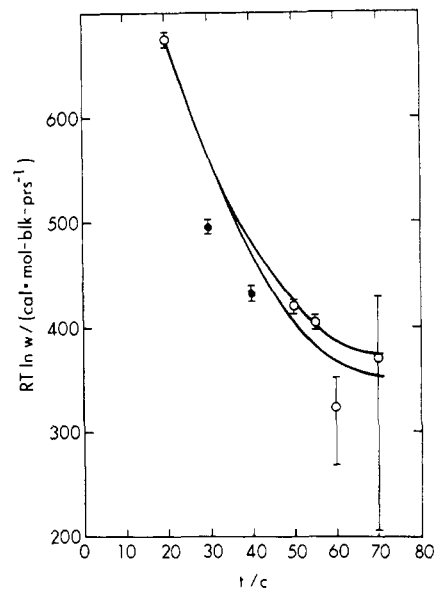

Figure 9. $RT \ln w$ (cal (mol of block pairs)$^{-1}$) vs. Celsius temperature for tropomyosin. Open circles, values required by realized theory to give experimentally observed helix content. Filled circles, same for lower limit as described in text. Error bars stem from uncertainty in calculating percent helix from CD. Upper solid curve, weighted least-squares fit to second-order polynomial. Coefficients are $A_0 = 14554$, $A_1 = -82.67$, and $A_2 = 0.1205$. Lower solid curve, unweighted least-squares fit to second-order polynomial.

straightforward as for its XY_k analogues. There are several difficulties, some fundamental and some technical, that cannot be reliably addressed at present. Our results, therefore, have to be presented somewhat tentatively. It is appropriate here to summarize these difficulties and our rationale for the adopted procedure.

First, we have thus far used the theory to treat homohelical molecules only, i.e., dimer molecules in which both helices are identical in sequence. Although the formalism itself can easily be extended to heterohelical dimers, it seems unwise to do so at this stage because of the complexities this introduces into the interhelix interaction. Unfortunately, the thorough thermal denaturation data on quantitatively cross-linked homohelical tropomyosin that are needed to realize the theory fully only exist for rabbit skeletal tropomyosins, which is known to comprise two slightly different polypeptide chains (α and β), which form a mixture of $\alpha\alpha$- and $\alpha\beta$-dimeric species in a ratio of about 3:2.[39] However, such data as do exist for the isolated $\alpha\alpha$-dimeric species[43] suggest that its thermal denaturation curves are very similar to those obtained on the native mixture. Consequently, we employed the more complete data set from studies of the native mixture.[39]

Second, the experimental denaturation curves for cross-linked tropomyosin in benign medium show an "anomaly" in the region ~25–40 °C that has been referred to as a "pretransition".[39] Cogent arguments have been used to ascribe this anomaly to alterations in short-range conformational stability near cysteine-190 that attend formation of the interchain disulfide bond. In our terms, the s and σ values near a Cys-190 that is disulfide linked are no longer as they were in non-cross-linked chains. Since we have no data available on how to modify our table of s and σ appropriately, the theory cannot be realized in the temperature region where the segments near Cys-190 are in transition. Of course, it is possible to *choose* a value of w at any temperature to fit any helix content, but then w loses its physical significance as an interhelix interaction. For this reason, in treating cross-linked tropomyosin, we have used only data points outside the anomalous temperature region. These data are shown as the open circles in Figure 8. The error bars, as before, reflect the uncertainty in obtaining percent helix from mean residue ellipticity.

Since this hiatus in the data is uncomfortably large, it is desirable to have some guide in that region. We, therefore, show also on Figure 8 the experimental points (filled circles) in that temperature region for non-cross-linked tropomyosin.[39] The non-cross-linked dimer species suffers no such distortion in s and σ values, and its helix content is really what we would like to have. Unfortunately, this is not necessarily identical with the observed helix content in the non-cross-linked system because of the unknown equilibrium concentration of monomers at the protein concentration of the experiment. However, since the monomer's helix content is much less than the dimer's, these experimental points serve as a lower limit to what we would like to have. They are shown for guidance but were not used in obtaining smoothed values of w (see below) for tropomyosin. This entire problem is immaterial in the analogues XY_k, since there the cross-link is very near the end of the chain which is almost never helical anyway.

Third, as error bars indicate, once one reaches helix content below ~25%, the uncertainty in deducing helix content from CD becomes quite large. This, as will be seen, has serious consequences in estimating $w(T)$ for tropomyosin.

Fourth, tropomyosin has varied types of hydrophobes in the core, which must lead to local heterogeneities in the value of w. We have no way at present of evaluating these.

Putting these objections aside for the moment, we proceed to use the usual realization of the theory (i.e., use the same $s(T)$ and σ as before) along with the experimental points to obtain discrete values of w for tropomyosin. These are shown in Figure 9 as $RT \ln w$ vs. t. Evidently the hiatus and the uncertainties discussed above make it difficult to fit a smooth curve to these results with any great deal of confidence. We proceed as best we can. Because the filled circles merely represent lower limits on the helix content, they were not included in the fit to eq 4. Furthermore, the vast difference in error of the points at the two highest temperatures compared with the others strongly argues for a fit by weighted least squares. Such

a fit is shown as the upper solid curve in Figure 9. This curve is given (units of cal (mol of block pairs)$^{-1}$) by A_0 = 14 554, A_1 = −82.67, and A_2 = 0.1205. The unweighted least-squares curve is shown as the lower solid curve and actually does not differ very much. Each fits the data acceptably, considering the error bars, and it would be difficult to justify any choice of curve appreciably different from these.

It is thus abundantly clear that the $w(T)$ required by the realized theory to fit the thermal denaturation data for tropomyosin is quite different from that required by the XY_k polypeptide analogues. For one, the overall magnitude of w is appreciably larger in XY_k (compare Figures 4 and 9), and although the $s(T)$ and σ values being used for the stretch of chain near Cys-190 in cross-linked tropomyosin overstimate its helix potential and therefore lead to underestimation of w, this effect cannot possibly be large enough to alter the qualitative conclusion. For another, the temperature dependence of w is different, showing a pronounced minimum near 62.25 °C for XY_k, whereas for tropomyosin, although it is hard to say, it appears that if a minimum exists at all, it is likely to be above 70 °C, where there is very little helix anyway. It appears, then, that the all-leucine hydrophobic core of XY_k involves interactions appreciably different from those in the more heterogenous tropomyosin.

The weighted-fit curve, when used to generate smoothed values of $w(T)$ for use in the theory, yields calculated values of percent helix vs. temperature for tropomyosin as shown in the upper solid curve in Figure 8. This curve fits the data rather well and is consistent with the view that the filled circles are lower limits. For comparison, the curve for noninteracting (w = 1) tropomyosin helices is also shown as the lower solid curve, and the corresponding theoretical curves for XY_{39} (dashed) and XY_5 (dotted) are also given.

Figure 7 shows calculated helix probability profiles for tropomyosin (dashed curve) allowing comparison with XY_{39}, its 281-residue analogue. Here, chain length can play no role since the two are almost identical in that regard. In all the tropomyosin curves the inhomogeneity of the amino acid sequence (and therefore of s and σ values) produces wide fluctuations in helix stability along the chain. The lowest pair of curves shows each substance at 30 °C with noninteracting (w = 1) chains; XY_{39} has the slightly higher helix content (18.3% vs. 17.2%) because of slightly greater effective s and σ of its constituent residues. The uppermost dashed curve shows tropomyosin with interacting chains at 30 °C, where it is highly (89.8%) helical. In spite of the high helix content, substantial variation in helix probability exists in tropomyosin, compared with XY_{39}, even as many as 80 residues from the ends. The vast fluctuations possible near the tropomyosin transition midpoint are shown by the middle dashed curve, which is for 50 °C (48.3% helix). These enormous excursions in helix probability are results of local variation in $s(T)$ and σ characteristic of a natural protein. When the theory can begin to insert local variations in w as well, even greater excursions will likely be seen. Indeed, the differences described above between all-leucine core interactions and the average of those in tropomyosin suggest that they may be very large indeed. No polypeptide as uniform in structure as XY_k can mimic that aspect of the tropomyosin molecular structure.

IV. Discussion

In this section we set forth some of the ways in which the tropomyosin analogue XY_5 differs from tropomyosin, examine some of the physical consequences of the helix–helix interaction parameters (w) that emerge from the realized theory, generate some further predictions concerning the behavior of tropomyosin and its analogues, and sketch some possible future directions that the development of the realized theory and of supporting experiments might take.

The most obvious difference between model and protein lies in the degree of polymerization. The statistical theory strongly emphasizes the important role of this variable in determining helix content and since no subtle chemical differences are involved, comparative predictions generated, such as those of Figure 6, probably have a high degree of certainty. Figure 6A makes a rather emphatic statement concerning the inappropriateness of direct comparison of thermal denaturation of XY_5 with tropomyosin. The theory states plainly that even a 281-residue polymer virtually completely homologous in sequence to XY_5 would display a thermal denaturation profile quite different from it. The more appropriate model for tropomyosin is the 281-residue peptide XY_{39}, and the prohibitive difficulty in synthesizing it forces one to rely on a theory from which its properties can be deduced from those of its smaller homologues, such as XY_5. We wish to stress that the need for such a theory will remain whether the present one stands or falls.

This is further underscored by comparison of the helix probability profiles of XY_5 (Figure 5) with those of XY_{39} (Figure 7). The two are quite dissimilar because of the strongly randomizing effect of the chain ends, an effect which dominates the profile in the case of the 43-residue chain but plays a rather minor role in the 281-residue chain. In making further comparison, therefore, we will attempt to relate the properties of tropomyosin to those theoretically deduced for XY_{39}, rather than those actually observed for XY_5.

Before we leave this subject of statistical end effects, one more remark is perhaps not out of place. Chou–Fasman parameters are sometimes used to estimate helix content or to obtain helix probability profiles.[42,44–47] There is no doubt that, in the absence of a physical theory such as the one we are attempting to adumbrate, these parameters can be useful as a rough guide. However, their use is not without serious pitfalls. First, that approach can never reveal anything about the effects of a physical variable such as temperature. Second, the parameters are characteristic of the globular proteins forming the statistical data base from which they were derived—proteins in which α helices exist in environments and are even packed at angles, quite different from those in coiled coils.[48] Third, most authors have employed only the helix parameter ($P_{\alpha i}$), whereas presumably (in tropomyosin, anyway), it is the *relative* stability of helix and random coil that is at issue. Fourth, such usage may be particularly hazardous when applied to very short helical stretches within protein chains dissolved in detergent solutions,[42] wherein long-range interactions (which are included in $P_{\alpha i}$) are absent and statistical end effects may be very severe.

Another significant difference between the homologous models and their protein prototype lies in the effective value of the helix stability parameter. Although the s_{eff} values of the two (see Figure 1) are almost the same at room temperature, the temperature dependences differ. The s_{eff} for tropomyosin is almost constant from 10–20 °C and then falls quite markedly above that, while the s_{eff} of XY_k is maximal at 30 °C, so that at low temperature short-range interactions disfavor XY_{39} helices relative to tropomyosin, while at high temperature the position is reversed. This result is explicitly spelled out in Figure 8,

wherein the predicted denaturation curves for isolated (noninteracting) chains of XY_{39} and of tropomyosin are shown ($w = 1$ case), and in Figure 7, where large differences in the helix probability profiles appear. This reemphasizes that even a model as carefully designed to mimic a real protein as XY_5 has been, and as carefully extrapolated to the appropriate degree of polymerization, must be used circumspectly. It might be possible, using the algorithms of Table I, to design an analogue that would possess an $s_{eff}(T)$ more closely resembling tropomyosin's and, at the same time, not violate any of the canons laid down by the heptet structure.

The realized theory demands also that the XY_k analogues differ strikingly from tropomyosin with respect to helix–helix interaction within the dimeric coiled coil. The values of w obtained for XY_5 (Figure 4) indicate that for a coiled coil with all-leucine hydrophobic core and all glutamate–lysine salt linkages, the helix–helix interaction results in a change in standard free energy (infinitely dilute reference state, units of cal (mol of block pairs)$^{-1}$) given by

$$-\Delta G^{\infty}_{XY_k} = RT \ln w_{XY_k} = 22150 - 129.55T + 0.1969T^2 \quad (5)$$

which amounts to a stabilization by some 1030 cal (mol of block pairs)$^{-1}$ near room temperature. Since each four-residue block (a–d) involves two pairs of leucine–leucine interactions and each three-residue block involves two pairs of glutamate–lysine salt bridges, this corresponds to 515 cal of stabilization per mole of hydrophobic residue pairs or salt-linked residue pairs.

The corresponding relationship for the standard free energy of helix–helix interaction, averaged over the α-tropomyosin molecule, is somewhat difficult to ascertain for reasons already sufficiently discussed above. Our best, if tentative, estimate at present is

$$-\Delta G^{\infty}_{Tm} = RT \ln w_{Tm} = 14554 - 82.67T + 0.1205T^2 \quad (6)$$

which amounts to some 616 cal per mol of block pairs or 308 cal per mol of hydrophobic residue pairs or salt-linked residue pairs. Thus, at room temperature, the XY_k molecules display almost twice the interhelix stabilization shown by native tropomyosin.

This difference is augmented at higher accessible temperatures (60–80 °C) where eq 5 yields a pronounced increase in stabilization for the analogues while eq 6 shows no such thing for tropomyosin. It is the combination of rapid deterioration of short-range (s) and long-range (w) stabilization in tropomyosin dimers about 50 °C that leads to the precipitous loss of helix content in that range that is seen in Figure 8. The XY_k analogues, on the other hand, suffer a milder drop in s_{eff} in this range, and it is mostly offset by an increase in stabilization by long-range (interhelix) interactions (w); hence XY_{39} suffers only modest losses of helix in the same temperature range (Figure 8). Apparently, the all-leucine core produces very pronounced stabilizing effects at any temperature and particularly at high temperatures where they are needed because of falling s_{eff}. It is noteworthy, however, that even the stabilization observed in the analogues (308 cal (mol of residue pairs)$^{-1}$ at room temperature) is small compared to the total expected from transfer of a pair of aqueous leucine side chains to an organic phase (3600 cal (mol of residue pairs)$^{-1}$).[49]

The free energies given by eq 5 and 6 are, molecularly speaking, composites. They include, at the very least, averaged contributions from hydrophobic interaction and from salt-link formation between positionally fixed blocks,

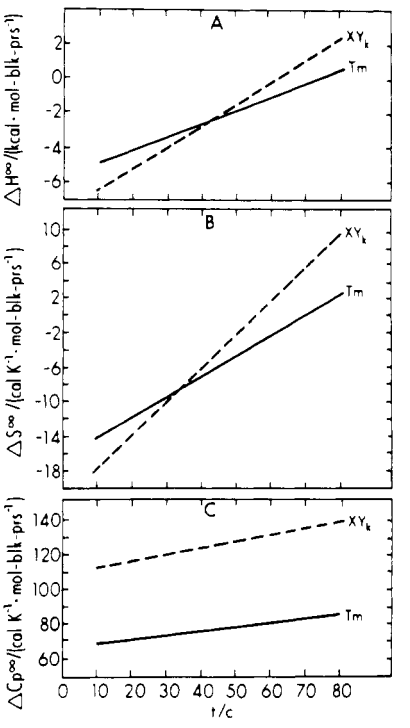

Figure 10. Temperature (Celsius) dependence of ΔH^{∞}, ΔS^{∞}, and ΔC_p^{∞} deduced from $\Delta G^{\infty} = -RT \ln w(T)$ for XY_k (dashed curves) and tropomyosin (solid curves): (A) ΔH^{∞} (kcal (mol of block pairs)$^{-1}$); (B) ΔS^{∞} (cal K^{-1} (mol of block pairs)$^{-1}$); (C) ΔC_p^{∞} (same units as (B)).

plus an entropic contribution from the decreases in rotational freedom that accompanies the turning on the block–block interaction. Because of this remaining complexity, a detailed dissection of $-RT \ln w$ would be premature. However, a brief examination may better reveal the magnitude of the task remaining.

The free energies above imply for the other relevent thermodynamic properties of the interaction:

$$\Delta H^{\infty}_{XY_k} = -22150 + 0.1969T^2 \quad (7a)$$

$$\Delta S^{\infty}_{XY_k} = -129.55 + 0.3938T \quad (7b)$$

$$\Delta C^{\infty}_{p,XY_k} = 0.3938T \quad (7c)$$

and

$$\Delta H^{\infty}_{Tm} = -14554 + 0.1205T^2 \quad (8a)$$

$$\Delta S^{\infty}_{Tm} = -82.67 + 0.2410T \quad (8b)$$

$$\Delta C^{\infty}_{p,Tm} = 0.2410T \quad (8c)$$

and these quantities are displayed in Figure 10 as functions of temperature, whence it is plain that all three increase with temperature, ΔH^{∞} and ΔS^{∞} being negative at room temperature and becoming positive at rather high temperatures (>60 °C), while ΔC_p^{∞} is positive throughout. It is a disturbing feature of these results that they are, for all three properties of both substances, precisely opposite to those expected for a positionally localized hydrophobic interaction.[50] Moreover, if we accept as a first approximation to the salt link a continuum model in which the charge–charge distance is temperature independent in both the interacting and noninteracting states and in which the ion atmosphere may be ignored, we find that the signs of the thermodynamic properties are dictated by the temperature dependence of the effective dielectric constant. If the latter were given by the relation for water, $\epsilon = 305.7e^{-T/219}$, we find that the electrostatic contributions

ΔH^{∞}_{es}, ΔS^{∞}_{es}, and $\Delta C^{\infty}_{p,es}$ ought all to be positive. Except in the case of ΔC^{∞}_{p}, these are, again, opposite to the results of Figure 10, except at high temperature.

Apparent paradoxes of a similar kind have been known for some time.[50] If hydrophobic bonds, whose strength increases with temperature, hold protein structures together, why do proteins insist, ordinarily, on unfolding when heated? The usual answer[50] is that hydrophobic interactions do not *dominate* the unfolding free energy. However, the same paradox equally characterizes salt linkages. The resolution might be thought to lie in the deterioration of the short-range (s and σ) stabilizations at higher temperature. Our results above seem to insist, however, that while short-range stabilizations do indeed fall at sufficiently high temperature, their decline is not nearly steep enough to resolve the paradox. The reader is reminded that this conclusion is based on acceptance of the table of s and σ values presently available to realize the theory.

Since the data of Hodges et al.[37] on XY_5 include only one measurement on reduced XY_5 in the absence of urea, it is difficult to say much about the non-cross-linked case. However, a few preliminary estimates can be made that may prove heuristic.

Hodges et al. find a mean residue ellipticity for the non-cross-linked molecule very near 10 °C of 23 300 deg cm^2 dmol^{-1}. This corresponds (eq 2) to a helix content of very near 63%. We have already seen that at this same temperature the cross-linked dimer is 84.2% helix and our calculations show (Figure 3) that the monomers ($w = 1$ case) are very close to 8.0% helix. The overall helix content θ_h in the non-cross-linked system is given by[36]

$$\theta_h = g_m f_{hm} + (1 - g_m) f_{hd} \tag{9}$$

in which g_m is the weight fraction (of total polypeptide) that is in monomeric form in the solution whose circular dichroism was measured, and f_{hm} (f_{hd}) is the fraction helix in the monomer (dimer). Thus $g_m = (0.842 - 0.63)/(0.842 - 0.08) = 0.278$, which states that there was appreciable dissociation into monomer in the solution measured. Since this solution was at a known total polypeptide concentration of 0.575 mg mL^{-1},[51] these results lead to an estimate of the equilibrium constant for the dimerization of XY_5. We have[36]

$$K = (1 - g_m)/(2C_0 g_m^2) \tag{10}$$

wherein C_0 is the total formal concentration of polypeptide in formula weights of chains per liter of solution. In this case, $C_0 = 0.575/4624 = 1.24 \times 10^{-4}$ M and since $g_m = 0.278$, we obtain $K = 3.8 \times 10^4$.

We can also obtain K by another route. Theory also says[36]

$$K = uZ_d/Z_m^2 = uZ_d(w \neq 1)/Z_d(w = 1) \tag{11}$$

wherein Z_d (Z_m) is the partition function for the non-cross-linked dimer (monomer) and u is estimated as the excluded volume of two helical blocks in contact. The latter has been roughly estimated as 0.216 L (mol of block pairs)$^{-1}$.[36] The results above indicate that at 10 °C, the XY_5 dimer is characterized by $w = 9.33$. The theory also allows calculation of the partition function,[36] for which we find $Z_d(w = 9.33) = 2.24 \times 10^5$. At the same temperature, we find $Z_d(w = 1) = 2.18$, whence eq 11 yields $K = (0.216)(2.24 \times 10^5)/2.18 = 2.2 \times 10^4$. Considering the sensitivity of $Z_d(w)$ to the precise value of w and the crudity of our approximation to u, this has to be considered very good agreement with the value 3.8×10^4 obtained above from very limited circular dichroism data on non-cross-linked XY_5. The theory then avers that the observed helix content of non-cross-linked XY_5 is, in part, a result of partial dissociation into monomer species that possess relatively little helix content, and it therefore follows from the mass action law (eq 10) that the percent helix observed for non-cross-linked XY_5 ought to fall quite appreciably with polypeptide dilution in the range usually employed for CD measurements. A theoretical analysis of the data on non-cross-linked tropomyosin along these same lines would also be possible.

A substantial amount of further theoretical work suggests itself immediately. Several fragments of tropomyosin have been carefully prepared and subjected to CD studies of helix content.[52] These could be examined in a manner analogous to the above treatment of XY_k and tropomyosin. The temperature dependence of w obtained would be of great interest.

Furthermore, we have already emphasized that values of w obtained in this manner are, molecularly speaking, composites. It might be feasible to develop a theory for the interchain salt-link interactions, which could then be subtracted from the total. Similarly, the configurational entropy contribution might yield to theoretical attack. This contribution is intimately tied up with the loop entropy problem and might best be handled either by the method of Zimm[53] (in treating DNA helix–coil transitions) or by Monte Carlo simulations. If these contributions could both be subtracted from the total, the remainder would lay bare the hydrophobic contribution and its temperature dependence.

From the fundamental point of view we believe the basic theory to be sound. If its results and predictions prove incorrect, the weak link may very well prove to be in the set of input parameters (s and σ) needed for its realization. These have been obtained by a long, arduous and indirect process;[21-34] success is by no means proven. Even accepting the present formalism and the numerical values of s and σ, however, there is one additional theoretical question that ought to be addressed. The only interactions *within a single helix* that are considered thus far are the short-range ones embodied in s and σ. Everything else has been included, willy-nilly, in w. There is one possible type of long-range interaction within a single XY_5 helix that may be both appreciable and theoretically treatable. Within a given heptet, the negative glutamates at b and e positions and the positive lysines at g will experience charge–charge interactions. The geometry is such that the lysine is on the opposite side of the helical rod from the glutamates. Thus, it is not only rather far from the glutamates, but at a position where the effective dielectric constant is very large;[54] furthermore, the other lysines nearest to our lysine are on the same side, but a full heptet away in the helix axial direction. For these reasons, it seems likely that intrahelix charge–charge interactions involving lysines are not very important. However, the same cannot be said of the b-glutamate–e-glutamate interactions. These, being on the same side (smaller effective dielectric constant) and rather close spatially, may interact appreciably even at the relatively high ionic strength of most experiments on these substances. This "helical wheel" effect[55] may be calculable from electrostatic theory. Being repulsive, the particular interaction in question would tend to lower the estimate of single-helix stability and thus demand a compensating increase in w over the values reported here.

The challenge remaining to experimentalists is equally immediate. Synthesis of higher homologues of XY_5 would be very important in light of the predictions made here concerning their helix content. These predictions are not

so trivial as to be a sure thing. It may be, for example, that the configurational loop entropy change makes a very strong contribution to $-RT \ln w$ and one that depends on the distance of the block from the cross-link. If so, then the existence of identical hydrophobic and salt-link interactions in all the XY_k polymers does not suffice to ensure that they have identical $w(T)$, as was assumed here. The point is worth investigating. Synthesis of Y_jXY_k polymers with a cysteine in various chain locations, thus allowing for a cross-link at various positions, would be very informative. Where possible, it would also be extremely desirable for both cross-linked and non-cross-linked polymers or fragments to be measured in CD studies, and particularly important that any non-cross-linked forms be studied as a function of concentration. The presence of monomers (of low helix content) in equilibrium with dimers is clearly suggested by the theory and is central to its conclusions but has never been seen, or looked for, experimentally. Needless to say, direct examination of the state of aggregation of the non-cross-linked polypeptides by, say, light scattering would also be invaluable.

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