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A Theoretical Model Simulating the Anomalous Concentration Dependence of the Equilibrium Thermal Unfolding Curve of Noncrosslinked Tropomyosin

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Thermal unfolding curves of tropomyosin have so far been fit only semi-quantitatively by the statistical-mechanical theory of the helix-coil transition. The calculated values of helix content are a bit too small for the most dilute solutions and a bit too large for the most concentrated ones. The theory, as hitherto used, assumes a uniform helix-helix interaction, whereas evidence from studies on molecular segments suggests otherwise. A theoretical model incorporating such non-uniformity in helix-helix interaction is used to produce simulated thermal unfolding curves. These simulated curves, when fit to the theory using the assumption of uniformity, reveal precisely the same discrepancies seen with the experimental data. We conclude that non-uniformity in helix-helix interaction along the tropomyosin molecule is responsible for the small discrepancy between experimental data and the uniform-model theory previously employed.

In the last few years, a statistical mechanical theory has been developed to describe the equilibrium, thermal unfolding curves of two-chain, α-helical, coiled-coil proteins (1-4). The theory, in either its earlier crude form or its later, more complete, one, has been directly applied to several varieties of noncrosslinked, coiled-coil molecules (5-8). The theory not only fits the data of percent helix (from CD) vs. temperature semi-quantitatively over a 1000-fold range of protein concentration (6), but is also consistent with light scattering data (9), and with the observed high degree of crosslinkability of αα molecules at the position of their only cysteine, C-190 (6). The theory also explains successfully the substantially larger conformational stability at low pH (6). Finally, a theoretical calculation on a somewhat simplified model of the crosslinked molecule gives results that mimic the most prominent feature of the experimental unfolding curve, namely a characteristic "pre-transition" that is not seen in the noncrosslinked case (10).

Although these successes are encouraging, some difficulties remain. For example, it is always found that the theoretical values for helix content of

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noncrosslinked protein near neutral pH are a bit too small at the lowest protein concentration and a bit too large at the highest protein concentration (6). The discrepancy is not large, but it is systematic. In the present work, we inquire as to the cause of this anomaly.

It seems natural first to hypothesize that the fault lies in the assumption that the helix-helix interaction is the same at every turn along the molecule, because independent evidence exists that such an assumption cannot be correct (8,11). Hence, we explore the possibility that the discrepancy is caused, not by any fundamental deficiency in the theory, but simply by the use of a uniform inter-helix interaction for a molecule in which this interaction is not uniform.

The formal ability of the theory to handle non-uniformity suggests the following test of our hypothesis. From previous studies of tropomyosin segments (8,10,11), we can estimate regional values of the helix-helix interaction for, broadly speaking, the amino- and carboxy-terminal halves of the tropomyosin molecule (i.e. values for $-RT\ln\underline{w}_N$ and $-RT\ln\underline{w}_C$, respectively). We can then use these values to calculate thermal curves for a non-uniform model tropomyosin molecule. If our hypothesis is correct, the resulting "data" should simulate the behavior of the real experimental data. That is, our theoretically generated "data" should show the same discrepancies as real data do when they are fit by a theory that assumes uniformity.

Methods and Models

Calculations performed with the uniform model and the non-uniform model employ the same values for the intra-helix (short range) interactions. These are the effective (geometric mean) values found in α-tropomyosin chains. Specifically, the effective value of the helix initiation parameter is $\sigma_{eff} = 5.0 \times 10^{-4}$. For the helix propagation parameter, the effective values were obtained from: $\ln s_{eff}(T) = B_0 + B_1T^{-1} + B_2T^{-2}$, where T is in Kelvin and the coefficients are: $B_0 = -3.91450627$; $B_1 = 2207.20386$; $B_2 = -314{,}920.425$.

Calculations for the non-uniform model require two distinct algorithms for the free energy, RTlnw, of helix-helix interaction per pair of helical turns. For residues in the range of 1-133 of the 284-residue chain, we used: $RT\ln w_N = BT\ln T + A_0 + A_1T$, with $RT\ln w_N$ in cal/(mol of turn-pairs) and $B = 52.6274259$; $A_0 = 15{,}993.4998$; $A_1 = -351.163555$. On the other hand, for turns involving residues in the range 134-284, we used: $RT\ln w_C = RT\ln w_N - 250.0000$. These values are identical to those used previously in the context of crosslinked molecules (10). For non-registered structures, which are important in

the noncrosslinked case, a turn comprising amino-terminal-segment residues may be paired with a turn comprising carboxy-terminal-segment residues. For such mismatched pairs, we employ, as in the previous work (10); $w = (w_N w_C)^{1/2}$.

All parameters and procedures were thus identical to those employed in our earlier model calculations (10). The "data" theoretically generated from this non-uniform model were fit to the theory for the uniform model by the usual trial and error method. As usual, points used in the fit were confined to those yielding helix content in the range 15% to 93%. Outside that range, the helix-helix interaction becomes too sensitive to the precise value of helix content to yield meaningful results.

Results and Discussion

The thermal-unfolding "data" generated by the theory from the model with non-uniform helix-helix interaction are shown in Figure 1 as discrete, circled points. Results for two extreme concentrations are shown: 0.0044 mg•mL^{-1} and 5.2 mg•mL^{-1}. These values span the full range usually accessible in determination of helix content from circular dichroism (5). The "data" simulate real data quite well in general trend and magnitude. The solid curves in Figure 1 are discussed below.

When the "data" points of Figure 1 are fit to the theory employing a uniform helix-helix interaction, the values of $RT\ln\underline{w}(T)$ plotted on Figure 2 are

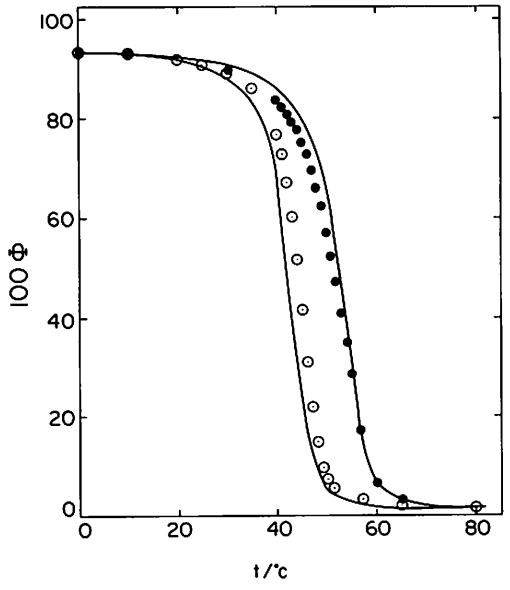

Figure 1. Simulated unfolding data for tropomyosin model with non-uniform helix-helix interaction. "Data" for 0.0044 mg•mL^{-1}: ○ . "Data" for 5.2 mg•mL^{-1}: ● . Solid curves are theoretical curves for the same, respective concentrations using the algorithm for the solid curve of Figure 2 as the uniform helix-helix interaction.

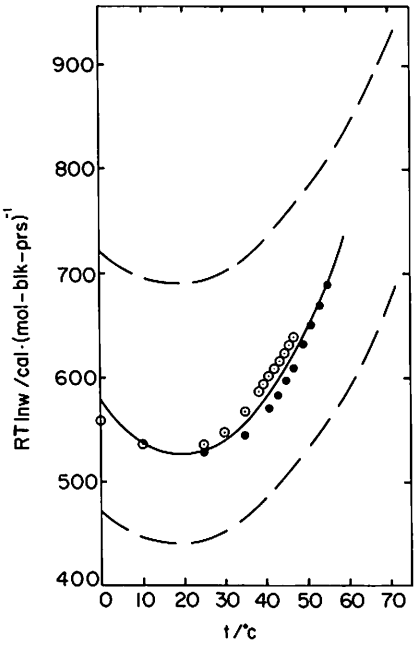

Figure 2. Helix-helix interaction free energy. Points give the values required by the uniform model to fit the simulated data (Figure 1) produced with the non-uniform model. Values for 0.0044 mg•mL^{-1}: ○ . Values for 5.2 mg•mL^{-1}: ● . Solid curve is fit to all points shown. Upper dashed curve shows RTln$\underline{w}_N$; lower dashed curve shows RTln$\underline{w}_C$.

obtained. Each circled point on Figure 2 represents the value of helix-helix interaction required to force the uniform model to yield the helix content given by the corresponding "data" point on Figure 1.

It is immediately evident from Figure 2 that the anomaly encountered in fitting real data is also present in the simulation. The values of RTln$\underline{w}$(T) required to fit the simulated thermal curve at the low concentration (open circles) lie systematically above those for the high concentration (filled circles). In the normal course, a compromise curve is drawn to best fit all the points. Such a curve is shown as the solid curve on Figure 2. Its algorithm is of the form given above with B = 79.4878173; A_0 = 23,790.92202; and A_1 = -530.907979. For comparison's sake, the corresponding curves for the stronger (amino-terminal segment) and weaker (carboxy-terminal segment) regional interactions are also shown (dashed curves) on Figure 2.

If the compromise (solid) curve of Figure 2 is used as the uniform interaction, the theory yields the thermal curves (solid curves) given on

Figure 1. Clearly, the systematic differences seen on Figure 2 are manifest in Figure 1 as a discrepancy between calculated and "observed" helix content. Specifically, the calculated values are always too small for the lowest concentration and too large for the highest. The simulated data of Figure 1 thus mimic precisely the slight mis-fit shown by real data.

Since data simulated with a non-uniform model, but fit to a uniform model, reproduce the anomaly seen in the real data, it seems likely that the cause is the same, i.e. that non-uniformity in helix-helix interaction also exists in the real molecule, as the data on segments suggest. Thus, far from revealing any fundamental flaw in the extant theory, this analysis suggests that the slight mis-fit of the concentration dependence of the thermal curve is a result of the relatively crude, preliminary form in which the theory has so far been used.

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