Notes

Theory of α-Helix-to-Random-Coil Transition of Two-Chain, Coiled Coils. Application of the Augmented Theory to Thermal Denaturation of ββ Tropomyosin

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In previous papers in this series, an equilibrium statistical mechanical theory has been developed and used to fit data on the thermal α-helix-to-random-coil transition of two-chain, coiled coil proteins.[1] To embody the "short-range" interactions, the theory employs independently determined experimental values of the parameters for helix initiation (σ) and propagation (s) for each amino acid in the sequence. Originally expressed in rather crude form, the theory has since been augmented to include the effects of loop entropy and out-of-register structures. Application of the theory to experimental data of helix content (from circular dichroism) vs. temperature allows evaluation of a quantity $-kT \ln w(T)$, which represents the free energy change when two, distinct, positionally fixed α-helical blocks (turns) are brought together, at temperature T, to form a positionally fixed segment of the coiled coil. In this way, the combination of theory and experiment allows one to dissect out the average "long-range"—i.e., helix–helix—interaction over the entire molecule.[1]

The augmented theory has thus far been applied in only one case, namely non-cross-linked rabbit αα tropomyosin, a coiled coil comprising two, identical 284-residue chains of known sequence. The resulting helix–helix interaction free energy, assumed to be uniform, shows a characteristic shape when plotted vs. T, and the theory not only provides a semiquantitative fit of the temperature dependence of the helix content over a 1000-fold range in protein concentration but is also consistent with light scattering data (which gives weight-average molecular vs. temperature) and with observations on the cross-linkability of the two chains.[1,2] No major discrepancy between theory and experiment has yet been found.

Some rabbit muscles contain tropomyosin molecules that include a chain (β-chain) of the same length as the α-chain, but somewhat different in sequence.[3-5] These β-chains differ from α-chains at 39 amino acid sites. The two chain types can be chromatographically separated under denaturing and reducing conditions and separately reconstituted to yield ββ as well as αα two-chain coiled coils.[3] The experimental thermal α-helix-to-random-coil transition of the non-cross-linked ββ species has already been reported.[6] This experimental thermal transition is similar in kind to that for the αα species but is shifted to somewhat lower temperature; i.e., the ββ double helix is less stable.[6,7]

The theory can be used to gain insight into, and to quantitate, this observed difference in stability between αα and ββ coiled coil species. The corresponding theory for single-chain polypeptides, i.e., the present theory not including helix–helix interaction, can be used to assess the short-range interactions in α- vs. β-chains. The result is already known:[6] although site-specific differences exist along the chains, the net, calculated helix content for the entire single chain is almost the same. Thus, the observed differences in helix content of αα vs. ββ species cannot arise from short-range interactions alone; a decisive role must be played by differences in the helix–helix interaction free energy. In the present work we quantitate those differences by fitting the theory to extant data on the thermal transition in ββ tropomyosin at near-neutral pH for comparison with the corresponding results for the αα species.

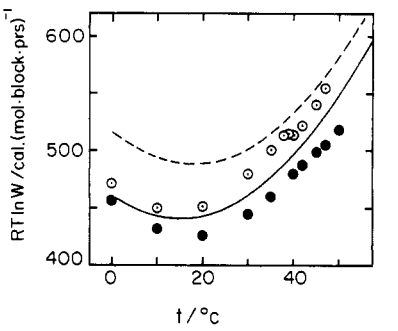

Figure 1. $RT \ln w(T)$ vs. temperature for non-cross-linked ββ tropomyosin at pH 7.4: open circles, 0.0100 mg·cm^{-3}; filled circles, 4.72 mg·cm^{-3}. Solid curve is the best fit algorithm. Dashed curve is the best fit algorithm obtained previously for αα tropomyosin (ref 1).

Methods

The methods employed were precisely those described earlier.[1] As before, algorithms giving $s(T)$ for each amino acid residue type were employed.[8,9] These were chosen to fit the experimental values determined in Scheraga's laboratory.[10] Values of σ were from the same source. The data base for helix content was obtained from spline curves through the data previously reported[6] for the ββ species in aqueous medium containing 0.500 M NaCl, 50 mM NaPi, and 1.0 mM DTT. Smoothed values were picked off the curves and employed as experimental values.

Values of $w(T)$ were obtained by trial, assuming uniform helix–helix interaction, for the ββ thermal curves at 0.01 and 4.72 mg/mL, a range only slightly smaller than that employed previously for the αα species.[1] Only data in the range of helix content from 15% to 93% were employed in the trials because of the extreme sensitivy of $w(T)$ to slight changes in helix content at very small and very large values of the latter. The values were fit to the equation

$$-\Delta G^{\infty} = RT \ln w(T) = BT \ln T + A_0 + A_1 T \quad (1)$$

wherein B, A_0, and A_1 are constants, and the resulting algorithm was used to generate theoretical values for comparison with experiment.

Results and Discussion

The results are shown in Figure 1 as $RT \ln w(T)$ vs. T. The open (filled) circles are the best fit to the smoothed experimental data for ββ tropomyosin near pH 7 at a protein concentration of 0.01 (4.72) mg·cm^{-3}. The solid curve is the algorithm (eq 1) that best fits the points and represents our best estimate of $RT \ln w_{\beta\beta}(T,7)$. The dashed curve shows the corresponding one previously determined for the αα species, i.e., $RT \ln w_{\alpha\alpha}(T,7)$.[1] The parameters appropriate to eq 1 for the ββ (αα) species are B =

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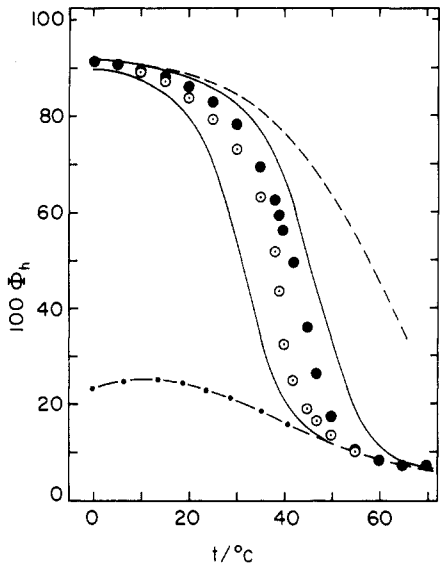

Figure 2. Percent helix vs. temperature for non-cross-linked $\beta\beta$ tropomyosin at pH 7.4: open circles, smoothed data for 0.0100 mg·cm^{-3}; filled circles, smoothed data for 4.72 mg·cm^{-3}. Solid curves are from theory using the best fit algorithm at the corresponding concentrations. Dashed curve is from theory for dimer species using the same algorithm. Dot–dashed curve is from theory for monomer species, i.e. single β-chains. The latter is indistinguishable from the result for single α-chains.

52.328 581 8 (52.627 425 9); A_0 = 15 522.9681 (15 793.4998); A_1 = −348.702 910 (−351.163 555).

It is immediately evident that the interspecies difference in helix–helix interaction free energy is subtle; the shapes of the curves are similar, and numerical values differ by no more than ~55 (and as little as ~30) cal·(mol of block pairs)$^{-1}$. These differences represent only ~10% of the total free energy of interaction. However, as noted previously, this translates to a difference in helix content that is measureable.

The success of this helix–helix interaction in fitting the data is assessed in Figure 2, where the smoothed data points of helix content for each concentration of the $\beta\beta$ species may be compared with the theoreticaly calculated values (solid curves). The fit is very similar to that found in the $\alpha\alpha$ case; i.e., it is semiquantitative.[1] As in the $\alpha\alpha$ case, the theory somewhat overestimates the concentration dependence.

Also shown in Figure 2 are theoretical curves for helix content of the monomer (single-chain) species (dot–dashed curve) and dimer species (dashed curve). The former is virtually indistinguishable from the corresponding curve for α single chains and the low helix content emphasizes once again how important helix–helix interactions are in producing the high values found in the native, two-chain structure.[1] This receives further emphasis from the (dashed) curve for the dimer species, which shows relatively elevated helix content up to rather high temperatures. As in $\alpha\alpha$ species, the loss of helix by $\beta\beta$ species is, in large part, due to chain dissociation.

It remains to be seen whether these subtle changes in interhelix interaction can be interpreted in terms of specific amino acid substitutions at the "a" and "d" positions that are responsible for the hydrophobic portion and the "e" and "g" positions responsible for the electrostatic portion of the interaction. The change from α to β chains entails a change in a total of 11 residues at "a" and "d" sites and a total of 5 at "e" and "g" sites.[4,5] It is unlikely, however, that much insight can be gained by simple inspection of the substitutions involved, because the local changes in helix–helix interaction necessarily interact in a complex manner with local variations in short-range interactions. The net effect on helix content is difficult to intuit.

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References and Notes

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(10) Scheraga, H. *Pure Appl. Chem.* **1978**, *50*, 315–324; see also the detailed list in ref 1.

Field Desorption Mass Spectrometry of Poly(olefin sulfones)

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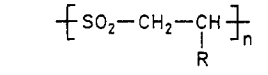
Poly(olefin sulfones) are copolymers of an alkene and sulfur dioxide, which usually have alternating structures (I). Poly(olefin sulfones) are thermally unstable, decom-



(I) R = ALKYL or H

posing to sulfur dioxide and the alkene on heating.[1] It has been reported[2] that the rates of thermal decomposition of several poly(olefin sulfones) show an approximate correlation with the number of hydrogen atoms on the carbon atoms β to the sulfone group. Bowmer and O'Donnell[3] have found that there is a better correlation when both the number of β-hydrogens and the ceiling temperature of the polymer are considered. Bowden et al.[4] have shown that poly(1-butene sulfone) undergoes a two-step degradation, with the initial degradation occurring in the temperature range 130–200 °C and the major degradation taking place at higher temperatures. Bowden et al.[4] suggested that the initial degradation takes place at weak links within the chain. Using a specialized mass spectroscopic technique, we have studied the degradation of poly(1-butene sulfone) and poly(propene sulfone) with a view to elucidating the mechanism of their degradation.

Poly(1-butene sulfone) and poly(propene sulfone) were prepared by the radical polymerization at −78 °C of equimolar amounts of the alkene and sulfur dioxide in the presence of a chain-transfer agent (bromotrichloromethane). The initiator was *tert*-butyl hydroperoxide

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