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ROLE OF TOPOLOGICAL CONSTRAINTS IN THE ALL-OR-NONE TRANSITION OF A GLOBULAR PROTEIN MODEL: THEORY OF THE HELIX-COIL TRANSITION IN DOUBLY CROSSLINKED, COILED COILS

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SUMMARY. Employing a recently developed statistical mechanical theory, the α-helix-to-random-coil transition in two-chain, coiled coils is shown to possess many of the essential qualitative features of the equilibrium folding process in globular proteins. The role of short vs. long range interactions in stabilizing the native structure is examined. We demonstrate in doubly crosslinked coiled coils how, due to the role of loop entropy, an intrinsically continuous conformational transition evolves into one well approximated by an all-or-none transition. Thus the present work points out the crucial role played by loop entropy in the conformational transition in coiled coils in particular and perhaps in globular proteins in general.

INTRODUCTION. The inherent structural complexity of globular proteins makes it extremely difficult to formulate a statistical mechanical theory of the native → denatured transition. Any successful theory must incorporate the short range interactions characterizing the intrinsic stability of the secondary structure in the absence of interchain contacts[1] and the long range interactions[2] that may be conceptually decomposed into two types. There are the presumably highly specific interactions between the segments of the polypeptide chain that lead to the unique native tertiary structure, and there are the less specific topological constraints, due to loop entropy (the reduction in configurational entropy when loops are constrained to a specific configuration relative to the case where the end of the loops are free[1,3]), associated with loops that must be present in a compact structure containing β-sheets and/or α-helices formed from a linear chain. Moreover, it must be able to produce the highly successful two-state model of protein folding[2-7] with

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interactions favoring the native state that are relatively weak. Unfortunately at present, the formulation of such a theory is beyond existing capabilities. Hence, the study of simplified model systems possessing many of the essential features of globular protein folding becomes important. As pointed out by Hodges et al,[8-9] α-helical, two-chain, coiled coils form such a system and in this communication, we report results from a theoretical treatment of the helix-coil transition of coiled coils[10-12] which suggests conditions under which an intrinsically continuous transition can evolve into an all-or-none transition having the essential features outlined above.

Two-chain, coiled coils of which a salient member is the muscle regulatory protein tropomyosin consist of two parallel, α-helical chains wound around each other with a slight supertwist.[13] Analysis of the amino acid sequence of tropomyosin reveals the presence of a quasirepeating heptet in which essentially every first and fourth residue is hydrophobic, the fifth residue is acidic and the seventh residue is basic. In the coiled coil structure, the 4,3 heptet leads to juxtaposed hydrophobic faces along with the possibility of salt bridge formation. Recognizing the possibility of such interhelical interactions and to account for the fact that single chain, helix-coil theory predicts isolated single chains of tropomyosin have a low helix content,[14] and yet the native coiled coil (dimer) is highly helical,[13] we introduced the helix-helix interaction parameter $\underline{w}$.[10] Application of the theory which includes loop entropy and mismatched chain association to noncrosslinked coiled coils predicts a continuous helix-coil transition of the following type.[11] In the dimer, due to the effect of loop entropy, there is essentially one interacting helical stretch per molecule (the presence of two or more interacting helical stretches requires constrained loops; such loops pay a heavy entropic price), but the helical stretch can occur anywhere and may be perhaps preceded or followed by alternating sequences of non-interacting helices and random coils. Furthermore, the dimer is in equilibrium with single chains of appreciably lower helix content. The theory described above has been successfully applied to non-crosslinked rabbit α-tropomoyosin.[15] In what follows, we

report on the theoretical predictions of the character of the helix-coil transition in a hypothetical, doubly crosslinked, homopolymeric analog of rabbit β-tropomyosin.

RESULTS

Consider a two-chain, coiled coil divided into N_B blocks (α-helical turns), each block of which contains m_i residues.[10] The presence of two crosslinks in the coiled coil has profound effects on the helix-coil transition as compared to the noncrosslinked case. On introducing crosslinks at blocks N_{C1} and N_{C2}, either blocks N_{C1} to N_{C2} inclusive must be fully helical and interacting or constrained random coil loops must be present. In the "all-or-none" model of the helix-coil transition, either the molecule is completely helical between crosslinks or it entirely lacks any interacting helices (it may however contain non-interacting helices). The former state is identified as the "native" conformation and the latter the "denatured" state. Figure 1 gives a schematic representation of the accessible states. We point out that in the "native" molecule, the helix-coil transition is still continuous for those residues not contained between the crosslinked blocks. We have also developed a more general model which allows for constrained random coil loops and interacting helices between crosslinks, the doubly crosslinked interior eyelet model, DCIEM. In both models the perfect matching limit of

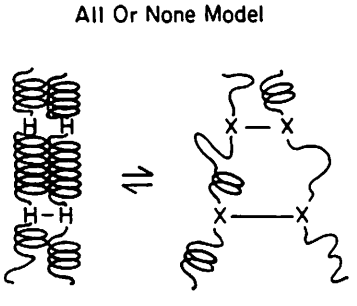

Figure 1. Schematic representation of the accessible states in the "all-or-none" model of the helix-coil transition in doubly crosslinked two-chain, coiled coils. H-H indicates the fully helical conformation of the crosslinked blocks and X indicates that any conformational state is accessible.

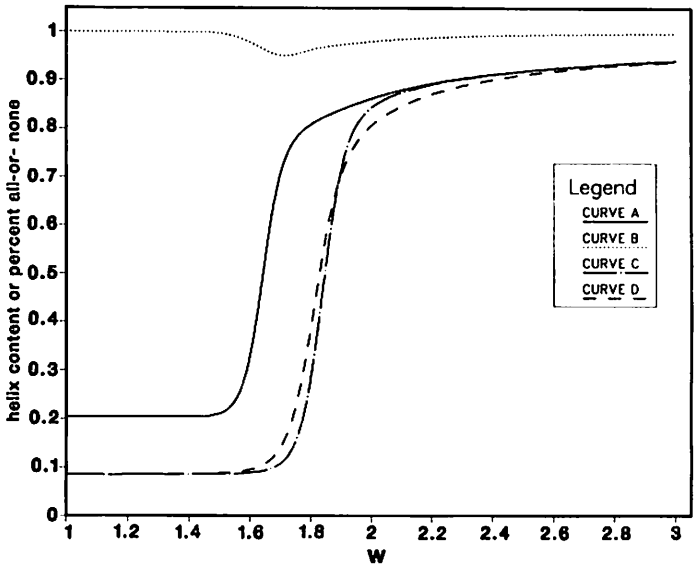

Figure 2. Plot of the overall helix content of a doubly (singly) crosslinked homopolymeric, two-chain, coiled coil with N_{C1} = 9 (or) N_{C2} = 48 calculated in the all-or-none model (loops excluded model) in curve A (curves C and D). Curve B is the plot of the fraction of doubly crosslinked molecules that satisfy the all-or-none criterion vs. $\underline{w}$. (See text for other parameters.)

the two chains is assumed. The details of the formalism are presented elsewhere and are rather involved,[12] here we present the salient results.

In Figure 2, we plot in curve A, employing the all-or-none model, the helix content vs. $\underline{w}$ of the doubly crosslinked, homopolymeric analogue of rabbit β-tropomyosin having $\underline{N}_B$ = 71, $\underline{m}$ = 4, $\underline{s}$ = 0.94, $\sigma = 5 \times 10^{-4}$, $\underline{N}_{C1}$ = 9, and $\underline{N}_{C2}$ = 48. Throughout the entire transition, the DCEIM model gives helix contents that lie within 2% of curve A. A more stringent test is to calculate the fraction of molecules for which the all-or-none model is valid; such a plot is shown in curve B. Even by this more sensitive criterion, the helix-coil transition is essentially all-on-none in the sense described above. By way of comparison, we also plot in curves C and D the helix content vs. $\underline{w}$ for coiled coils singly crosslinked at blocks $\underline{N}_C$ = 9 and 48 respectively. The procedure for calculating the helix content may be found in reference 16.

At low values of $\underline{w}$, where "denatured" states dominate, the higher residual helix content in doubly crosslinked chains vis `a vis singly crosslinked chains is due to loop entropy which destabilizes random coil states relative

to non-interacting helical states in the closed loop between the pair of crosslinked blocks. Similarly, the higher helix content at larger values of $\underline{w}$ is due to the destabilization of the "denatured" form (which of necessity contain large constrained loops) relative to the "native" form. This translates into the prediction that doubly crosslinked chains should melt at a higher temperature than singly crosslinked chains. This apparent stabilization of the native state on introduction of crosslinks seems to be a general consequence of loop entropy not tied to the models described here. A similar effect has been invoked by Scheraga et al to rationalize the enhanced stability of RNase A on introduction of an extrinsic crosslink.[17]

We would further expect that mismatched states of the two-chains can be ignored (they require the presence of two constrained loops). Moreover, the inclusion of site specific interactions acts to make the transition more cooperative, as mismatched states become even more disallowed. Thus, the qualitative conclusion that the helix-coil transition in doubly crosslinked molecules is all-or-none should be even more valid in the realistic case of a heteropolymeric, coiled coil.

To the best of our knowledge, this is the first time an all-or-none transition has emerged for a protein system from a statistical mechanical treatment in which a continuous distribution of states is allowed, and graphically points out the crucial role of loop entropy in determining the character of the conformational transition in doubly crosslinked, coiled coils. Thus, there seems to be a strong isomorphism between this system and globular proteins and if so, it seems reasonable to conjecture that an underlying origin of the all-or-none transition in globular proteins is also due to loop entropy. Much work, however, will be required before this insight can be translated into a realistic model of the native ⇄ denatured transition in globular proteins.

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