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Changes in Unperturbed Dimensions Accompanying Helix–Coil Transitions in Cross-Linked Homopolypeptides, with Special Reference to Poly(hydroxybutyl-L-glutamine)

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ABSTRACT: Mean-square unperturbed radii of gyration, $\langle s^2\rangle_0$, have been computed as functions of degree of polymerization and helix content for cross-linked polypeptides of the poly(L-alanine) type. Zimm–Bragg statistical weights were assigned values appropriate for poly(hydroxybutyl-L-glutamine) in water. The g ($\equiv\langle s^2\rangle_{0,\text{ branched}}/\langle s^2\rangle_{0,\text{ linear}}$) for polypeptides with a finite degree of polymerization fall between the limits defined using random-flight statistics and rigid-rod behavior. For polypeptides of the molecular weight usually encountered, g varies strongly with helix content when helicity exceeds 20%. Partially helical polypeptides require substantially higher degrees of polymerization than do completely disordered polypeptides in order to attain the limiting g obtained from random-flight statistics.

Proteins frequently contain interchain cross-links. The most prevalent naturally occurring cross-link results from disulfide bond formation by two cysteinyl residues.[1] Covalent cross-links have also been chemically induced in protein complexes to determine which polypeptide chains are neighbors. This approach has been applied to chromatin,[2–5] ribosomes,[6] and membranes.[7] Treatment of mouse LA-9 cells with tetranitromethane, for example, produces a cross-link between the C-terminal half of histone H2B and the C-terminal half of histone H4.[5] Analysis of products of cross-linking reactions frequently includes gel permeation chromatography or polyacrylamide gel electrophoresis in either aqueous sodium dodecyl sulfate or aqueous acid–urea solution. Typical proteins are denatured under these conditions.[8] They may, however, still have a substantial fraction (up to ~50%) of their amino acid residues present in α helices.[9] Analysis of the transport properties of these proteins would be facilitated by knowledge of the effect of cross-linking on their unperturbed dimensions.

One approach to an estimate of the unperturbed dimensions would be to utilize random-flight statistics to compute parameters denoted by g[10] and f_i.[11]

$$g \equiv \frac{\langle s^2\rangle_0 \text{ for cross-linked molecule}}{\langle s^2\rangle_0 \text{ for analogous linear molecule}} \quad (1)$$

$$f_i \equiv \frac{\langle s^2\rangle_0^{1/2} \text{ for } i\text{th uncross-linked polypeptide chain}}{\langle s^2\rangle_0^{1/2} \text{ for cross-linked molecule}} \quad (2)$$

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The mean-square unperturbed radius of gyration is denoted by $\langle s^2 \rangle_0$: the "analogous linear molecule" in the definition for g has the same number of amino acid residues as the cross-linked polypeptide. Random-flight statistics yields easily handled expressions for g and f_i. Numerical results obtained from these expressions become exact for flexible molecules in the limit of infinite molecular weight. However, serious error may arise from application of random-flight statistics to molecules of low to moderate molecular weight. The size and direction of the error, as well as the number of chain bonds required to attain the high molecular weight limit, depend on the nature of the short-range interactions present.[11,12] While the asymptotic limit is attained quickly with random-coil polyglycine, it is approached rather slowly with disordered poly(L-alanine).[12] Application of eq 1 and 2 to disordered proteins will usually produce an underestimate for g, while f_i will be correct to within about 6% if the two cross-linked polypeptide chains have identical amino acid sequences.[13]

Incorporation of helical segments into cross-linked polypeptides reduces the number of flexible sites. It also causes certain rigid segments (the helices) to have lengths much greater than 3.8 Å, the distance between neighboring α-carbon atoms in a planar trans peptide unit. Appreciable consequences might be expected for the applicability of random-flight statistics to the estimation of g or f_i for partially helical proteins. We report here the results of rotational isomeric state theory calculations which address this point. These calculations utilize Zimm–Bragg[14] statistical weights appropriate for poly(hydroxybutyl-L-glutamine) in water.[15]

Calculations

Calculation of $\langle s^2 \rangle_0$ for cross-linked polypeptides can be achieved using rotational isomeric state theory in the form appropriate for branched molecules.[16,17] Adopted procedures differ in only two important aspects from those described previously[11] for disordered cross-linked polypeptides. First, the cross-link is represented by a single bond, 3.8 Å long, which is attached by a free joint to an appropriate α-carbom atom in each polypeptide chain. This approximation greatly simplifies the matrix expression for the configuration partition function and does so without any sacrifice in the ability to reproduce the experimentally obtained unperturbed dimensions of cross-linked disordered tropomyosin.[13] The second difference is that the statistical weight matrices for the amino acid residues must now allow for two states, helix and coil. The matrices are formulated from unity, σ, and s in the manner prescribed in ref 16 and 17. Here σs and s denote the equilibrium constants for the initiation and propagation, respectively, of a helical segment.[14] Poly(hydroxybutyl-L-glutamine) in water has $\sigma = 0.00068$, while s decreased from 1.04 to 0.95 as the temperature increases from 0 to 100 °C.[15] The conformational energy map for disordered residues is that obtained by Brant et al.[18] for the L-alanyl residue, and the α helix has $\phi, \psi = 133°, 122.8°$ and a value of 109.2° for the N–C$^\alpha$–C′ angle.[19] Unperturbed dimensions for linear polypeptides and the probability, p_h, that an amino acid residue will be in a helical segment were calculated in the manner appropriate for linear chain molecules.[20]

Results and Discussion

Molecular Weight Dependence When s is Unity. The value of s is unity for poly(hydroxybutyl-L-glutamine) in water at 35.8 °C.[15] This condition corresponds to the midpoint of the helix–coil transition for a polypeptide of infinite molecular weight.[14] The probability, $p_{h,l}$, that an amino acid residue in a linear polypeptide is in a helical state decreases at low molecular weight. Figure 1 depicts the relationship between $p_{h,l}$ and the number of virtual bonds, n_p.[21] For a linear molecule n_p is identical to the number of peptide bonds. The characteristic ratio, $C_l \equiv \langle s^2 \rangle_0 / n_p l_p^2$, is also shown. Here l_p represents the length, 3.8 Å, of one virtual bond.[21] The high molecular weight limit for C_l is slightly greater than the value of 1.54 attained when the helical content is zero.[18] The slope represented by $d(C_l/C_{l,\infty})/d(1/n_p)$ at $1/n_p = 0$ is −15 when the helical content is zero,[11] while it is −70 when p_h is 0.5. The helices present cause a substantial delay in the approach to the limiting characteristic ratio.

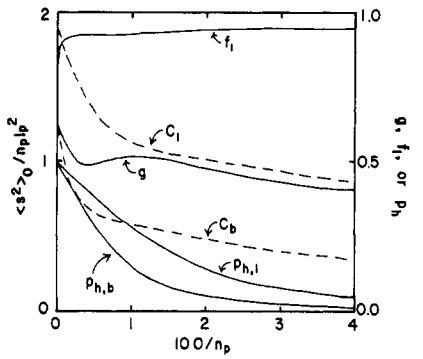

Figure 1. Unperturbed dimensions (C), helical contents (p_h), g, and f_1 for poly(hydroxybutyl-L-glutamine) in water at 35.8 °C ($\sigma = 0.00068$, $s = 1.00$) as a function of $1/n_p$. Subscripts l and b denote linear and cross-linked molecules, respectively. The cross-link occurs between the central amino acid residues in two identical polypeptide chains. Dashed lines, left ordinate; solid lines, right ordinate.

Figure 1 also presents results obtained when the central amino acid residues in two identical polypeptide chains are joined by a cross-link. Cross-linked molecules and linear molecules containing the same number of amino acid residues have identical values of n_p. The probability, $p_{h,b}$, that an amino acid residue in the branched molecule will be in a helical state is lower than $p_{h,l}$ at finite n_p because the polypeptide chain in the linear molecule contains twice as many amino acid residues as either of the two chains in the cross-linked polypeptide. In each case the asymptotic limit at infinite molecular weight is 0.5. Characteristic ratios, C_b, for the cross-linked molecules are always smaller than the C_l for the same n_p. A value of −140 is obtained for $d(C_b/C_{b,\infty})/d(1/n_p)$ at $1/n_p = 0$. Cross-link formation is accompanied by a delay in the approach to the limiting characteristic ratio. This result follows directly from the shorter chain length, and consequent slower development of the limiting helical content, in the cross-linked polypeptide.

Random-flight statistics yields $g = \frac{5}{8}$ for the cross-linked polypeptides currently under consideration.[11] Rotational isomeric state calculations yield this value only as the asymptotic limit, as shown in Figure 1. Lower g are obtained at finite n_p. The behavior depicted in Figure 1 is subject to rationalization as follows: Helical contents are low when n_p is less than 50. Under these circumstances g behaves in the manner found for completely disordered poly(L-alanine),[11] for which g attains a minimum of 0.39 at $1/n_p = 0.048$. For the completely disordered molecule, g rises continuously as $1/n_p$ decreases from 0.048. In contrast, this trend is reversed near $n_p = 100$ in Figure 1 due to the development of appreciable helical content. The longer chain length in the linear polypeptide causes it to develop helices more quickly than the cross-linked molecule. Hence C_l rises more rapidly than C_b, bringing about a depression in g. Helical contents of both molecules approach their common asymptotic limit at still higher n_p. Consequently g increases sharply when $1/n_p$ falls below 0.004. The value of $d(g/g_\infty)/d(1/n_p)$ at $1/n_p = 0$ is −100 in Figure 1, while it is only −24 when the helical content is zero.[11] A larger number of amino acid residues is required to

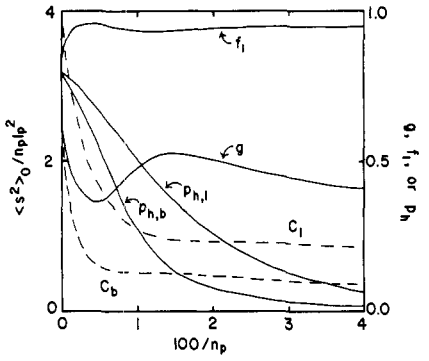

Figure 2. Same as Figure 1 except $s = 1.04$, corresponding to a temperature of 0 °C.

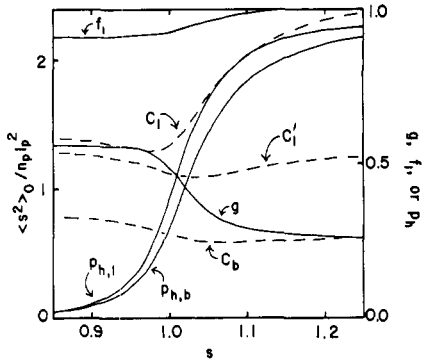

Figure 3. Helix–coil transitions for $\sigma = 0.00068$ and $n_p = 201$. The cross-link occurs between the central amino acid residues in two identical polypeptide chains. Dashed lines, left ordinate; solid lines, right ordinate.

approach the asymptotic limit specified by random-flight statistics when helices are present.

The behavior of f_1 is also depicted in Figure 1. Its high molecular weight limit is $\frac{4}{5}$, the value specified by the application of random-flight statistics.[11] Throughout most of the range depicted in Figure 1, however, f_1 is 0.92 ± 0.02. Its final sharp approach to the asymptotic limit commences only when n_p exceeds 1000. In contrast, the limiting f_1 is approached rather quickly if the polypeptide is completely disordered.[11]

Molecular Weight Dependence When $s = 1.04$. Poly(hydroxybutyl-L-glutamine) in water at 0 °C has $s = 1.04$. Molecules of infinite molecular weight now have 80% of their amino acid residues in the helical state. Behavior of $p_{h,l}$ and $p_{h,b}$ at finite n_p is depicted in Figure 2. The increase in s from unity to 1.04 produces higher helical contents at all n_p.[14] Limiting characteristic ratios are higher than those attained when s is unity. The increase results from the higher helical content and the doubling (from 39 to 79) of the average number of amino acid residues in a helical segment. Asymptotic values are approached more slowly than was the case when s was unity.

Behavior of g as a function of $1/n_p$ presents an exaggeration of the effects noted in Figure 1. In particular, the minimum near $n_p = 200$ is more pronounced. The asymptotic limit is still $\frac{5}{8}$, but the approach to the limit is delayed. A value of −200 is attained for $d(g/g_\infty)/d(1/n_p)$ at $1/n_p = 0$. The only important effect on the behavior of f_1 is the development of a shallow minimum near $n_p = 100$ and a maximum near $n_p = 200$.

Helix–Coil Transition at $n_p = 201$. The helix–coil transition for molecules with $n_p = 201$ is depicted in Figure 3. The anticipated cooperative[14] change in p_h is observed, with higher cooperativity being attained in the longer chain length of the linear polypeptide.

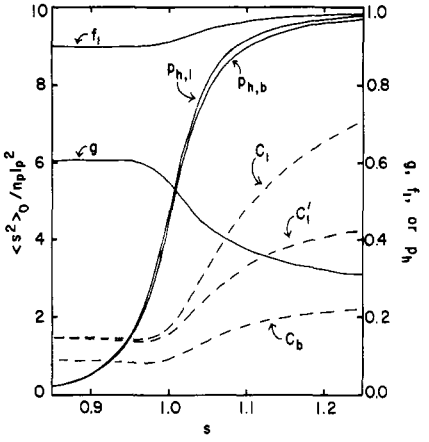

Figure 4. Helix–coil transition for $\sigma = 0.00068$ and $n_p = 801$. The cross-link occurs between the central amino acid residues in two identical polypeptide chains. Dashed lines, left ordinate; solid lines, right ordinate.

Three characteristic ratios are depicted. C_l and C_b denote the linear and cross-linked molecule, respectively, for which n_p is 201. Characteristic ratios for the linear polypeptides resulting from the rupture of the covalent cross-link are denoted by C_l'. Nearly identical unperturbed radii of gyration are obtained for the completely disordered polypeptide and rigid α helix when n_p is near 100. The shallow dip in C_l' near an s of unity arises because short helical segments then present are more compact than corresponding segments of random coil.[22] At the larger n_p appropriate for C_l the dip is less apparent and the rigid helix has a radius of gyration which is substantially larger than that of the random coil. Characteristic ratios for cross-linked molecules exhibit trends similar to those seen with C_l'. However, the result for C_b at high s is definitely lower than that attained at low s.

The largest g, 0.56, is attained at zero helical content. This result is less than the random-flight value of $\frac{5}{8}$ because the number of amino acid residues is not sufficient to justify use of a random-flight model for the disordered molecule.[11] Little variation in g occurs until s becomes 0.98, at which point C_l commences its increase in response to increasing helical content. Since C_b continues a slow decline, g decreases sharply from $s = 0.98$ to 1.1. The limiting g at maximal helix content is 0.25, the result anticipated for rigid rods cross-linked in the manner specified.[11] Poly(hydroxybutyl-L-glutamine) in water should exhibit a strong temperature dependence for g since s ranges from 1.04 to 0.95.[15]

The range for f_1 in Figure 3 is 0.91 to unity. Random-flight statistics yield a value of $\frac{4}{5}$, while cross-linked rigid rods have an f_1 of unity. Both f_1 and g experience their change over the same range of s.

Helix–Coil Transition at $n_p = 801$. Figure 4 depicts results for molecules which have $n_p = 801$. A more cooperative helix–coil transition is observed than was the case in Figure 3. Rigid α helices have larger radii of gyration than random coils for all molecules for which characteristic ratios are depicted in Figure 4. Extremely large s would be required to attain the maximal characteristic ratios, which are 10.6, 5.3, and 2.65 for C_l, C_l', and C_b, respectively. A few nonhelical residues will suffice to produce a significant drop in the unperturbed dimensions for polypeptides containing this number of amino acid residues. The range for g in Figure 4 is from 0.61 at $s = 0.85$ to 0.31 at $s = 1.25$. The former is close to the random-flight value of $\frac{5}{8}$. Values of s much larger than those depicted in Figure 4 would be require before g would fall to 0.25.

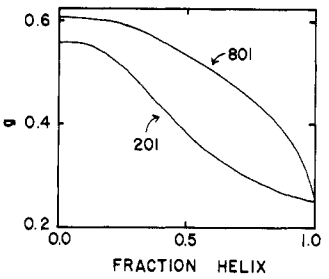

Figure 5. Comparison of the relationship between g and helical content when $\sigma = 0.00068$ and n_p is 201 or 801. The cross-link occurs between the central amino acid residues in two identical polypeptide chains.

The relationship between g and helical content for $n_p = 201$ and 801 is compared in Figure 5. If any disorder is present, g for $n_p = 801$ will exceed g for $n_p = 201$. The contrast is particularly striking when helical contents exceed 0.20. These effects have their origin in the increasing sensitivity of the unperturbed dimensions of predominately helical molecules to residual disorder as n_p increases and the fact that an n_p of 201 is insufficient to produce the asymptotic g for completely disordered molecules. In general, the true g and f_1 for partially helical polypeptides will lie between the limits defined by random-flight statistics and rigid rod behavior.

Implications for Cross-Linked Proteins. Proteins will differ from the homopolypeptides considered here in that 20 amino acid residues with different σ and s will be present. Homopolypeptides always have their highest helix-forming tendency in the middle of the polypeptide chain, while the chain ends tend to be disordered. In contrast, the amino acid sequence of a protein may yield maximal helix-forming tendencies at locations remote from the middle of a polypeptide chain. Those proteins which contain propyl residues will possess sites at which propagation of an α helix is extremely difficult, a feature not found in poly(hydroxybutyl-L-glutamine). Consequently the results presented here will not be expected to apply to any particular partially helical, cross-linked protein. The important conclusion to be drawn is that there is little reason to anticipate that random-flight statistics will be applicable to the estimation of the effect of cross-linking on the unperturbed dimensions of partially helical proteins. This problem must be approached using rotational isomeric state theory.

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Conformation of Hydrocarbon Chains Attached to a Planar Chromophore. A Monte-Carlo Study

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ABSTRACT: A diamond-lattice model and Monte-Carlo methods are used to simulate the conformational properties of hydrocarbon chains up to 30 carbons in length attached via an ester group to an aromatic chromophore. The chromophore was modeled in the lattice by specifying as occupied those lattice sites which best specify its geometry. Chains attached to the chromophore were examined for the case of second neighbor exclusion and with appropriate Boltzmann factors to simulate temperature effects on chain properties. Various properties of the chains were examined, including first, second, and fourth moments of the end-to-end vector **r**, and its projections onto the principal axes of a Cartesian coordinate system centered at C(1) of the chain. Values of the distribution of the end-to-end vector are reported for 10-, 20-, and 30-carbon chains as the projections of **r** onto the principal axes. Where comparison is possible with similar calculations on unsubstituted polymethylene chains, the effect of the chromophore on these properties of the chains is seen to be small.

When a hydrocarbon chain is attached to a bulky substituent, its conformational properties are affected. The steric effect of the substituent excludes a certain volume of space, and this in turn should perturb various conformational properties of the chains. The effect of the substituent on chain properties might vary with chain length, with temperature, with the shape of the substituent, and with the interaction potential between the substituent and the chain.

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