the energies of the corresponding pairs of representative points of the two hydrocarbon chain conformations in Figure 4. Therefore, a pair of points in the diagram represents the stability of a given conformation of the complex and determines implicitly the distance between the terminal carbon atoms. In the case of polyornithine, these coincide with the C_β atoms (C_γ for polylysine); the distance between these atoms can assume only the fixed values pertinent to the backbone conformations of the poly(α-amino acid). This, together with the energy, strongly limits the possible conformations of the macrocomplexes.

The conformation represented by the pair of stars in Figure 4 and corresponding to the 1–5 bridge is by far the most favorable in the case of the α-helical polyornithine complex. Moreover, only in this case do the angles of rotations near the peptide backbone, $\chi_{\alpha\beta}$ and $\chi_{\beta\gamma}$, which satisfy the definition of the macrocomplex structure in terms of torsional angles, occur in the deepest energy minima of the diagram in Figure 5, top. Here, the conformational energy of a dipeptide residue having the peptide skeleton conformation fixed in the right-handed α helix is shown in terms of the two rotation angles $\chi_{\alpha\beta}$ and $\chi_{\beta\gamma}$ for the side-chain bonds C_α–C_β and C_β–C_γ, respectively.

In the case of the pyridoxaldimine bridge, the similarity of the energy diagram with that of the salicylaldimine bridge leads to the same conclusions (see Figure 4).

Figure 5, bottom, illustrates the case of the β conformation of the backbone relevant for Gramicidin S, where the presence of the dyad axis almost parallel to the two junctions to ornithine allows the two enantiomeric complexes with conformationally equivalent hydrocarbon chains to be bound to the polypeptide matrix. The resulting two diastereoisomeric complexes are represented by different figures on the energy diagrams in Figure 4 and Figure 5, bottom.

Discussion and Conclusions

Figure 6 illustrates the local structure of the polyornithine and polylysine complexes. The two diastereotopic faces of the square-planar copper complex are alternately anchored to the positions 1–5 and 1–4, respectively, for polyornithine and polylysine. In the latter case, the longer hydrocarbon side chain allows a higher degree of conformational flexibility.

Figure 7 illustrates the proposed structures of diastereoisomeric copper(II) salicylaldimine complexes of Gramicidin S. In contrast to helical macrocomplexes, the Gramicidin S molecule (or β-sheet polypeptide conformations in general) extend over the whole length of the square-planar complex so that side effects could arise. These effects are amplified by differential solvation of the macromolecular surface. In fact, solvents preferentially solvating the carbonyl groups (eclipsed by the benzene rings in the upper diastereoisomer), such as methanol and trifluoroethanol, stabilize the lower conformation in Figure 7, whereas solvents such as chloroform, DMF, and TMP stabilize the upper diastereoisomeric configuration. This effect is absent in the case of Gramicidin S complexes of pyridoxaldimine, where one of the two diastereoisomers is stabilized on account of possible hydrogen bonds between the pyridoxaldimine CH_2OH group and the amide C=O group of D-phenylalanine residues, as model building suggests.

As a general conclusion, ordered polypeptide matrices are capable of reacting stereospecifically with square-planar copper complexes because of the intrinsic chirality of their binding surfaces. Such chiral discrimination can be amplified by cooperative as well by differential solvation effects.

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Effect of Urea on the Intrinsic Viscosity of Randomly Coiled Poly(α-L-glutamate)[1a]

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Use of urea as a denaturant for proteins is widespread, and there is enormous interest in the precise mode by which it exerts this action. Since there is also a sizable literature on the use of water-soluble synthetic polypeptides as model substances for proteins, it would be expected that the effect of urea on the physical properties of the most thoroughly investigated polypeptide, poly(α-L-glutamate) [abbreviated $(Glu)_n$] would long since have been exhaustively delineated. This appears not to be the case; indeed, although the influence of urea on the helix–coil transition has been studied by the titration method and found, as expected, to favor the random coil form,[2] very few measurements of other physical properties came to light in our literature search.

Since one important hypothesis on the molecular basis for urea's denaturing action holds that it denatures by virtue of a strong, attractive interaction with the exposed peptide groups in the random coil form of a protein or polypeptide,[3] it seemed to us that the intrinsic viscosity (being notoriously sensitive to molecular dimensions of random coils) of $(Glu)_n$ random coils would be strongly influenced by urea. We report such measurements here. The results are surprising.

Materials and Methods

Unless otherwise indicated, all experimental details were as described earlier.[4,5] Baker reagent grade urea was recrystallized from ethanol. The sample of $(Glu)_n$ was purchased from Sigma Chemical Co. Measurement of its intrinsic viscosity at pH 7.1 in 0.1 M NaCl at 25.5 °C and use of the earlier calibration[5] showed it to have a weight-average molecular weight of 77 500. A thorough study of its intrinsic viscosity vs. salt (NaCl) concentration at pH 7.1 showed a dependence that is in quantitative agreement with the relationship found earlier.[5] Each individual intrinsic viscosity was determined by fitting measurements of η_{sp}/c at at least five concentrations (g dL^{-1}) to the usual equation $\eta_{sp}/c = [\eta] + k'[\eta]^2c$. Dialysate was always used as solvent. Media of two very different ionic strengths were used; both were buffered at pH 7.1 with a mixture of Na_2HPO_4 and NaH_2PO_4. In detail, these two media were (in addition to any urea present)[6] $(NaCl)_{0.1}(NaP_i)_{0.01}(7.1)$ and $(NaCl)_{3.0}(NaP_i)_{0.01}(7.1)$. The temperature was 25.50 ± 0.01 °C for all.

Results and Discussion

The experimental findings are summarized in Figure 1 as a plot of intrinsic viscosity (dL g^{-1}) vs. molarity of urea.

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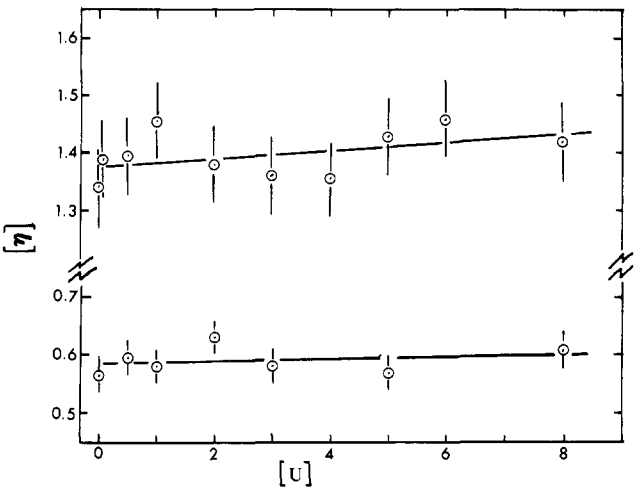

Figure 1. Intrinsic viscosity (dL g^{-1}) of $(Glu)_n$ vs. molarity of urea. Upper set of points is for $(Glu)_n$ in $(NaCl)_{0.1}(NaP_i)_{0.01}$-$(urea)_M$(7.1). Lower set is for $(Glu)_n$ in $(NaCl)_{3.0}(NaP_i)_{0.01}$-$(urea)_M$(7.1). The estimated range of experimental error is indicated on each point. The line through each set is determined by a simple least-squares fit. Note break in ordinate axis.

In all cases data are at pH 7.1, so that every glutamate residue bears a negative charge and only the random coil form is present. The upper set is at ionic strength 0.11 M; the lower is at 3.01 M.

As Figure 1 plainly shows, urea has almost no influence on the intrinsic viscosity of $(Glu)_n$ random coils at either modest or high ionic strength. The linear least-squares line shown for the data at ionic strength 0.11 gives a value of 1.38 dL/g for no urea and 1.43 dL/g for 8 M urea, an apparent increase of only 3.6%, which is within experimental error. Similar analysis of the set at 3.01 M ionic strength gives a value of 0.585 dL/g at zero urea and 0.599 at 8 M urea, an increase of only 2.4%, well within experimental error.

The values of Huggins' k' are not shown, but are also quite unspectacular. No particular trend was found with either salt or urea concentration; at 0.11 M ionic strength the average value is 0.43 with a standard deviation of 0.05, and at 3.01 M, the average is 0.44 with a standard deviation of 0.10.

This insensitivity of the intrinsic viscosity to urea is quite contrary to our expectations. If urea stabilizes the randomly coiled form of polypeptides, natural or synthetic, by a strong favorable interaction with the peptide groups, or side chains, for that matter, then it would seem to follow that a $(Glu)_n$ random coil in urea is in a substantially "better solvent" than one of comparable charge in the absence of urea. The resulting "long-range" interactions ought to result in an expansion factor appreciably greater than 1, with the usual accompanying enhancement of intrinsic viscosity. We are not unaware of the pitfalls in extending these ideas, developed for uncharged polymers in rather apolar solvents, to polyelectrolytes in complex aqueous solvent media, but predictions made on this basis are usually qualitatively correct.

It might be reasoned that the $(Glu)_n$ random coil at 0.11 M ionic strength is already so expanded through charge–charge interactions, i.e., the polyelectrolyte effect, that the putative strong interactions with urea can produce very little further expansion. We believe, however, that the absence of influence of urea at ionic strength 3.01 M, where charge–charge interactions are considerably suppressed, makes this explanation untenable.

For a time, we considered the possibility that the indifference of $(Glu)_n$ intrinsic viscosity to urea implies that the well-documented susceptibility of ordered protein conformations to urea is not shared by $(Glu)_n$, i.e., that earlier data on the influence of urea on the helix–coil transition of $(Glu)_n$ might be in error. To test this hypothesis, we performed the following experiment. First, the titration curves of our sample of $(Glu)_n$ were determined in 0.1 M NaCl at 0.00 and at 8.00 M urea, in order to determine the pH, in each case, at which the polymer is precisely half-ionized. Circular dichroism spectra were then taken for each at that point. This experiment allowed comparison of the α-helix content of $(Glu)_n$ molecules of precisely the same charge and at the same ionic strength in the absence and presence (8.00 M) of urea. Using the minimum near 220 nm as a measure of helix content, we found the amount of helix in 8 M urea to be only about one-fourth as great as in its absence, the measured ellipticities being -2950 deg cm^2 $dmol^{-1}$ in 8 M urea and $-12\,100$ sans urea. Clearly, then, urea *does* favor the randomly coiled structure in $(Glu)_n$ as well as in proteins.

The possibility exists that urea, in addition to any influence it may have through putative solvation of the peptide groups, alters charge–charge interactions through its influence upon the dielectric constant of the medium. If this dielectric constant effect is such as to cause a contraction of the coil, it may cancel out the expected expansion due to solvation, leaving the essentially zero net effect actually found. This possible explanation can be examined by using a recently developed theory of the dimensions of polyelectrolyte molecules.[7] From the theory and the knowledge that 8.0 M urea enhances the aqueous dielectric constant by ~25%, the ratio of intrinsic viscosities with and without urea may be calculated. We find $[\eta]_{8\,M}/[\eta]_{0\,M} = 1.06$ when the ionic strength is 0.11 M. Thus, the effect of urea on the dielectric constant and hence on the polyelectrolyte dimension leads to a predicted change in intrinsic viscosity that is quite comparable in its positive sign and small magnitude to that observed, leaving little room for belief that it acts to offset a large positive solvent effect.

It is also possible that strong, specific binding of urea to the polymer chain alters the "short-range" interactions so as to reduce the dimensions sufficiently to offset the putative "good-solvent" effect of the "long-range" interactions. However, it is rather difficult to believe that a polymer chain bristling with bound urea molecules could be more locally flexible than the unsolvated chain.

At present, we have no cogent explanation for the observations here recorded but are tempted to venture, tentatively and somewhat vaguely, that urea exerts its denaturing action, at least on $(Glu)_n$, not by strong stabilizing interactions with the random coil but by strong destabilizing action on the helix. This might come about, say, by urea molecules directly competing with the helix's carboxyl–carboxyl hydrogen bonds (if there are such) or, indirectly, by weakening such hydrogen bonds through enhancement of the dielectric constant. Perhaps other workers can develop other explanations of these rather puzzling observations. Clearly, more experiments are also called for, with studies of the molecular weight dependence of $[\eta]$ in urea and extension to water-soluble polymers of other charge types (e.g., poly(L-lysine) and poly[(hydroxyethyl)-L-glutamine] being the most obvious). It is perhaps noteworthy that a similar puzzle arises in the case of randomly coiled $(Glu)_n$ in solutions containing guanidinium chloride, the intrinsic viscosity being somewhat *lower* in, say, 1 M guanidinium chloride than in 1 M NaCl.[8]

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Trapped Entanglements vs. Dissociable Junctions in Networks Cross-Linked in Strained States

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Many rheological properties of uncross-linked polymers of high molecular weight can be interpreted in terms of a temporary network structure.[1] In most systems, there is strong evidence that the virtual network is the result of topological restraints, usually conceived as entanglements, rather than dissociable junctions between loci of specific attraction on the molecules.[1] One of the most direct manifestations of the reality of an entanglement network is the behavior of a polymer cross-linked near the glass transition temperature in a state of strain, e.g., in simple extension with a stretch ratio λ_0. After release, it retracts to a state of ease (stretch ratio λ_s) in which the elastic forces associated with the cross-links and with entanglements trapped by the cross-links are equal and opposite.[2,3] If the original temporary network consisted of dissociable junctions, the forces associated with them should disappear at equilibrium.

It was thought at first that the very existence of a state of ease demonstrated the permanence of the trapped entanglements and that the equilibrium strain would remain at λ_0 if the original temporary junctions could dissociate.[3] However, that is not quite the case. The system would correspond to that treated by Flory[4] in which two stages of cross-links are introduced, one in the unstrained and the second in a strained state, and all of the first-stage cross-links are subsequently removed. Then a memory of the first-stage cross-links remains in the structure and there is an equilibrium state of ease with a strain λ_s smaller than λ_0. Nevertheless, it can be shown from data of our previous studies[5,6] that the observed retraction to λ_s is different from that expected for dissociable junctions, so quantitatively there is still clear evidence for permanent trapping of entanglements by cross-links, as illustrated in the present note.

We utilize some of the same data for 1,2-polybutadiene that have already been interpreted in terms of the trapped entanglement model and recalculate them in terms of a model of temporary, dissociable junctions. The concentration of temporary network strands in moles per cubic centimeter in the original uncross-linked polymer, ν_e, is calculated in either case from the time-dependent Young's modulus $E(t)$ measured in stress relaxation at a standard

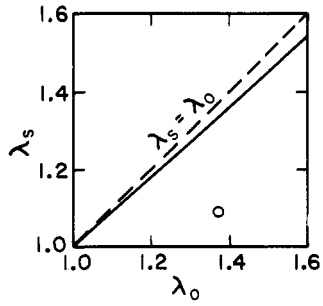

Figure 1. Plot of λ_s against λ_0 as predicted from the dissociable junction model for experiment C255 of Table I (solid curve). The open circle is an experimental point.

time t = 3 min, corresponding to the relaxation period allowed at 0 °C before the stretched polymer is chilled below the glass transition temperature for cross-linking by γ irradiation. The small-strain relaxation modulus $E(t)$ is obtained by extrapolation of stress data to small strains by use of the Mooney–Rivlin equation. Assuming a front factor of unity,

$$\nu_e = E(3\text{ min})/3RT \tag{1}$$

In the entanglement model, ν_e is the concentration of strands terminated by entanglements; in the dissociable junction model, it is the concentration of strands terminated by temporary junctions.

In the dissociable junction model, ν_e may be identified with the first-stage cross-links of the Flory theory.[4] In this theory, Gaussian statistics are assumed and there are no free ends (infinite initial molecular weight). If, after ν_x moles of strands are introduced by cross-linking while strained in simple extension with a stretch ratio λ_0, all the original junctions are dissociated, the system then behaves as though composed of ν_{1e} strands with reference state $\lambda = 1$ and ν_{2e} strands with reference state λ_0, where

$$\nu_{1e} = \Phi\nu_x \tag{2}$$

$$\nu_{2e} = (1 - \Phi)\nu_x \tag{3}$$

and Φ is a rather complicated function of ϕ_2, which is defined as

$$\phi_2 \equiv \nu_x/(\nu_x + \nu_e) \tag{4}$$

We have taken the function appropriate for a random distribution of cross-linked units. The equilibrium state of ease is then calculated from Gaussian statistics[2,4] to be given by

$$\lambda_s = [(1 + \lambda_0 R_0)/(1 + R_0\lambda_0^{-2})]^{1/3} \tag{5}$$

where $R_0 \equiv \nu_{2e}/\nu_{1e}$. From these equations, the relation between λ_0 and λ_s expected for dissociable junctions can be calculated and compared with experiments on 1,2-polybutadiene.

For this purpose, ν_{1e} is calculated from the tensile stress of the final cross-linked network when it is returned to a stretch ratio λ_0 and the strands with reference state λ_0 make no contribution to the stress. The same equation[3] is used as for calculating ν_N, the concentration of strands terminated by trapped entanglements, in the entanglement model; in the dissociable junction model, it provides the concentration of effective strands whose reference state is $\lambda = 1$. (There is a slight inconsistency in that this calculation of ν_{1e} implies certain deviations from neo-Hookean behavior[3] and the subsequent application of the Flory theory is based on neo-Hookean relations, but the difference in ν_{1e} is small (order of 15%) compared with the large difference between the predictions of the dissociable

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