ON THE NATURE OF INTERACTION OF DODECYL SULFATE WITH PROTEINS. EVIDENCE FROM UNCHARGED POLYPEPTIDES[1]

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SUMMARY: Circular dichroism and equilibrium dialysis measurements in aqueous solution reveal no strong interaction between dodecyl sulfate and the unionized polypeptides poly(N^5-ω-hydroxyethyl-L-glutamine), poly(N^5-ω-hydroxypropyl-L-glutamine) and poly(N^5-bis(ω-hydroxyethyl)-L-glutamine). Dodecyl sulfate does not affect the stability of the helical forms of poly(N^5-ω-hydroxypropyl-L-glutamine) and poly(N^5-bis(ω-hydroxyethyl)-L-glutamine) in water.

The hydrodynamic properties of the complexes formed by dodecyl sulfate and several reduced and denatured proteins have been interpreted as evidence for the existence of an ordered, perhaps rod-like, conformation (1). The changes produced in the optical activity of several proteins by dodecyl sulfate are of the type which would be expected for an increase in the net content of α helix, but the optical activity associated with a completely helical protein is not attained (1-4). An increase in the content of β structure also occurs in some proteins in the presence of dodecyl sulfate (4). Binding of dodecyl sulfate to proteins has been suggested to involve an electrostatic interaction between the detergent and cationic sites on the protein (5,6), an interaction of dodecyl sulfate with the peptide bond (7) and interaction between hydrophobic portions of the protein and the hydrocarbon chain of the detergent (8).

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Studies with ionic homopolypeptides support the existence of an important electrostatic contribution to the interaction between dodecyl sulfate and proteins. Dodecyl sultate induces a random coil to β structure and a random coil to α helix transition in poly(L-lysine) (9,10) and poly(L-ornithine) (10,11), respectively. In contrast, dodecyl sulfate exerts only a minor effect on charged poly(L-glutamic acid) (12). While providing convincing evidence of the importance of coulombic effects, it is not clear from these studies whether the emphasis should be placed on the attractive electrostatic interaction between dodecyl sulfate and poly(L-lysine) or on the repulsive electrostatic interaction between dodecyl sulfate and poly(L-glutamic acid). Our approach is to investigate whether any interaction occurs in aqueous solution between dodecyl sulfate and homopolypeptides of the structure $(-NHCHRCO-)_x$ when R does not bear groups which are charged in water near pH 7. The homopolypeptides studied are poly(N^5-ω-hydroxyethyl-L-glutamine) (R = $CH_2CH_2CONHCH_2CH_2OH$), poly(N^5-ω-hydroxypropyl-L-glutamine) (R = $CH_2CH_2CONHCH_2CH_2CH_2OH$) and poly($N^5$-bis(ω-hydroxyethyl)-L-glutamine) (R = $CH_2CH_2CON(CH_2CH_2OH)_2$). These polypeptides contain no charged groups except the single charges which may be present at the chain termini, thereby minimizing electrostatic interactions between the detergent and polypeptide. Both the α helix and β structure are sterically accessible to the polypeptides studied.

Materials and Methods. Poly(N^5-ω-hydroxypropyl-L-glutamine) was obtained from New England Nuclear. Sodium dodecyl sulfate was obtained from Matheson Scientific Co. and recrystallized from ethanol. Poly(N^5-ω-hydroxyethyl-L-glutamine) was synthesized from poly(γ-benzyl-L-glutamate) using a slight modification of the procedure described by Lotan et al. (13). Poly(N^5-bis(ω-hydroxyethyl)-L-glutamine) was prepared in a similar manner using diethanolamine instead of ethanol amine and reaction conditions of 70° for seven days. The buffer had a pH of 8.0 at 25° and was prepared from 0.05 M 2-amino-2-hydroxymethyl-1,3-propanediol and phosphoric acid. Circular dichroism was measured using a Durrum-Jasco Model J-20 recording spectro-

polarimeter calibrated as described by Cassim and Yang (14). The technique used for equilibrium dialysis has been described elsewhere (15).

Results with a disordered polypeptide. Poly(N^5-ω-hydroxyethyl-L-glutamine) adopts a statistical coil conformation at all temperatures in water (16-18). The circular dichroism in water at 30° exhibits a strong negative band at 198 nm and a weak positive band at 216 nm (19). The weak positive band decreases in intensity with increasing temperature (19), which is the expected result for a statistically coiling homopolypeptide with a $-CH_2R$ side chain (20). The effect of temperature on the ellipticity at 214 nm is shown by the symbol ● in Figure 1. Qualitatively similar circular dichroism spectra are

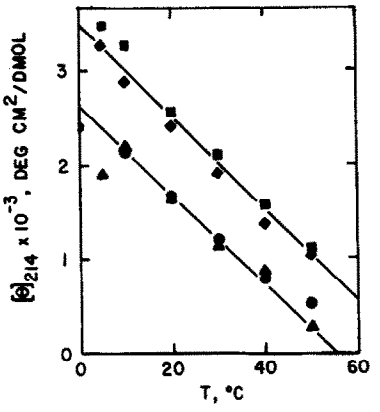

Figure 1. Mean residue ellipticity at 214 nm for poly(N^5-ω-hydroxyethyl-L-glutamine) in water (●), 0.1% dodecyl sulfate (▲), pH 8 buffer (■) and pH 8 buffer containing 0.1% dodecyl sulfate (◆).

observed as a function of temperature for poly(N^5-ω-hydroxyethyl-L-glutamine) in 0.1% dodecyl sulfate, in the pH 8 buffer, and in the pH 8 buffer containing 0.1% dodecyl sulfate. The mean residue ellipticities at 214 nm in these solvents are shown as a function of temperature in Figure 1. There is no evidence for any alteration in conformation in the presence of the detergent. The buffer exerts a larger effect on the mean residue ellipticity at a particular temperature than does the dodecyl sulfate. Equilibrium dialysis in the pH 8 buffer containing 0.1% dodecyl sulfate revealed no binding of the

detergent to poly(N^5-ω-hydroxyethyl-L-glutamine). Dodecyl sulfate exhibits no appreciable interaction with this uncharged disordered polypeptide under conditions where both proteins and positively charged homopolypeptides are markedly affected by the detergent.

Polypeptides undergoing a helix to random coil transition. Poly(N^5-ω-hydroxypropyl-L-glutamine) has been shown to be partially helical in water at low temperature and to become less helical upon heating (13,17,21,22). Poly(N^5-bis(ω-hydroxyethyl)-L-glutamine) behaves in a similar fashion, but is slightly less helical than poly(N^5-ω-hydroxypropyl-L-glutamine) at a specified temperature (23). Disruption of the helix by heating reduces the intensity of the negative circular dichroism band near 222 nm in both polypeptides. The temperature effect on the mean residue ellipticity of poly(N^5-ω-hydroxypropyl-L-glutamine) at 222 nm is shown in Figure 2 for the four solvents systems used with poly(N^5-ω-hydroxyethyl-L-glutamine). The dodecyl sulfate has only a very

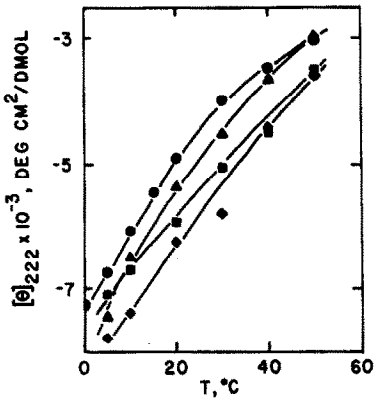

Figure 2. Mean residue ellipticity at 222 nm for poly(N^5-ω-hydroxypropyl-L-glutamine) in water (●), 0.1% dodecyl sulfate (▲), pH 8 buffer (■) and pH 8 buffer containing 0.1% dodecyl sulfate (◆).

small effect (less than that observed upon changing the solvent from water to the pH 8 buffer). The same conclusion is obtained from the analogous circular dichroism spectra measured with poly(N^5-bis(ω-hydroxyethyl)-L-glutamine). Since the completely helical polypeptide would have a mean residue ellipticity

of about -40,000 deg cm^2/dmol at 222 nm (21), while the result at this wavelength for the completely disordered polypeptide would be close to zero (19), it can be concluded that the increase in helical content upon the addition of the detergent in no case exceeds about 3%.

The Zimm-Bragg (24) initiation parameter, σ, is close to 0.0002 for poly(N^5-ω-hydroxypropyl-L-glutamine) (17,22). Consequently its helical content in the helix-coil transition region would be markedly affected by even small changes in the equilibrium constant, s, for the propagation of a helical segment (24). The data show that dodecyl sulfate has no significant effect on the stability of the helical form of poly(N^5-ω-hydroxypropyl-L-glutamine) and poly(N^5-bis(ω-hydroxyethyl)-L-glutamine).

Equilibrium dialysis measurements with poly(N^5-ω-hydroxypropyl-L-glutamine) and poly(N^5-bis(ω-hydroxyethyl)-L-glutamine) at 25° in the pH 8 buffer detected only weak binding of dodecyl sulfate, with the amount being about 0.1 g detergent per g of polypeptide.

The absence of an effect of dodecyl sulfate on the order-disorder transition of poly(N^5-ω-hydroxypropyl-L-glutamine) and poly(N^5-bis(ω-hydroxyethyl)-L-glutamine), coupled with the dramatic affect of this detergent on the order-disorder transitions of poly(L-lysine) (9,10) and poly(L-ornithine) (10,11), provides impressive evidence for the importance of electrostatic interactions between the detergent and cationic sites in synthetic polypeptides. The results reported here do not support the proposal that there is an interaction between dodecyl sulfate and the peptide bond (7).

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