to demonstrate an action of ouabain on phosphoprotein metabolism. Since it has been found[7,8] that phosphorylation and dephosphorylation of these molecules appear to be dependent upon Na^+ or K^+, respectively, and since both are blocked by ouabain, it is obvious that under certain conditions no effect of ouabain would be observed. This might be due to the complex interactions of phosphoprotein, Na^+, K^+ and drug.

It is to be noted that the results of the present work have been obtained both by the assay of alkali-labile P as a measure of the phosphoprotein P and also by chromatographic separation of radioactive phosphorylserine from the protein residues of the tissue. Our previous conclusions that phosphoproteins are concerned with sodium transport[5-8] are thus further strengthened.

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The effect of urea and guanidine on the helix content of poly-N^5-(3-hydroxypropyl)-L-glutamine in aqueous-solvent systems

The remarkable changes in optical-rotatory properties of proteins during denaturation, brought about by agents such as acid, alkali, urea and guanidine, have been interpreted in terms of decrease in helical content of the protein[1]. It was, therefore, of interest to investigate whether the above denaturing agents are capable of influencing synthetic polypeptides in a similar way. Only few data on the effect of urea on optical-rotatory properties of polyamino acids have been published[1-3]. DOTY AND GRATZER[4] have reported recently that the helicity of a poly-L-alanine segment in a block copolymer in which the central block consisted of L-alanine residues and the two flanking blocks of DL-glutamic acid residues, dropped from 92% in water to 70% in 8 M urea. KULKARNI AND BLOUT[5] have shown that a copolymer of L-alanine

and γ-*N*-(2-morpholinylethyl)-α-L-glutamamide had a helix content of more than 50 % in 0.2 M NaCl, but no helical conformation in 8 M urea.

The synthesis[6] and some optical-rotatory-dispersion data[7] of poly-N^5-(3-hydroxypropyl)-L-glutamine have been reported. From values of the optical-rotatory constant[8], b_0, measured under various conditions it was concluded that this non-ionic polyamino acid has an α-helical conformation in dimethylformamide and methanol solutions and that the addition of water causes a decrease in helical content.

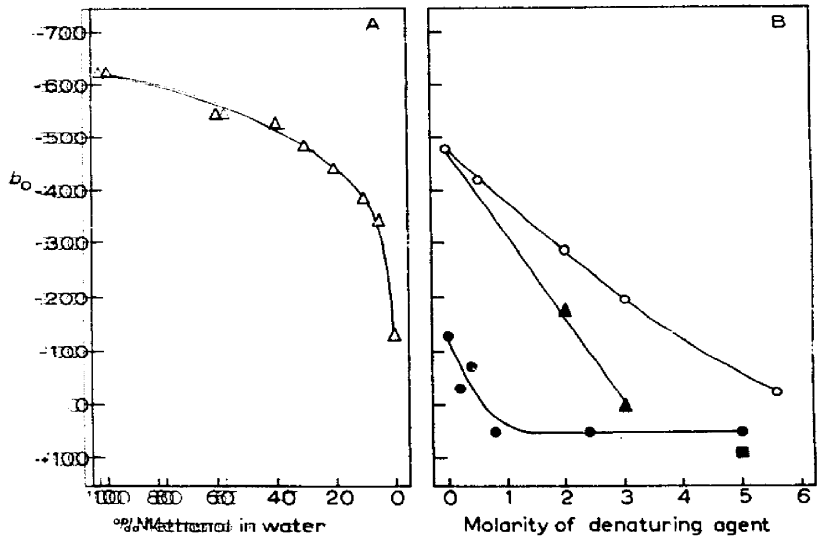

Fig. 1. A. The dependence of the optical-rotatory-dispersion constant[8], b_0, of poly-N^5-(3-hydroxypropyl)-L-glutamine (degree of polymerization is 250), on solvent composition, in the water–methanol system. B. The dependence of the optical-rotatory-dispersion constant, b_0, of poly-N^5-(3-hydroxypropyl)-L-glutamine (degree of polymerization is 250), on the concentration of denaturing agents. ○—○, urea in water–methanol (7:3, v/v); ●—●, urea in water; ▲—▲, guanidinium chloride in water–methanol (7:3, v/v); ■, guanidinium chloride in water.

Fig. 1 shows the values of b_0 of poly-N^5-(3-hydroxypropyl)-L-glutamine obtained in water–methanol mixtures (left) and those obtained in water and water–methanol (7:3, v/v) on the addition of increasing amounts of urea (right). The b_0 values were evaluated from linear MOFFITT plots[1,8] obtained from measurements of the optical-rotatory dispersion of meticulously clarified* solutions over the wavelength range 300–700 mμ. If changes in b_0 are correlated with changes in helical content[1] (without necessarily assuming strict proportionality between b_0 and degree of helicity), the curves obtained indicate that, as the water content of the methanol–water solvent system increases, the degree of helicity decreases. The rather abrupt change of b_0 in the narrow range of solvent composition—20 % methanol to pure water—indicates a rather drastic change in conformation in this region. The fact that addition of increasing amounts of urea to an aqueous solution of the polymer causes further

* The same solutions, before centrifugation for 3 h at 10 000 rev./min, give in the range of 300–700 mμ MOFFITT plots with a change of slope at about 400 mμ. The slope of the short-wavelength part of the plot was identical with that given by clear solutions over the whole wavelength range. The slope of the long-wavelength part gave higher absolute b_0 values. Previously published[6] b_0 values, measured only above 400 mμ and on solutions which were note meticulously clarified, corresponded to the long-wavelength part of the plot and are, therefore, somewhat too high.

change of b_0 in the direction correlated with the unfolding of the molecule, strongly suggests that poly-N^5-(3-hydroxypropyl)-L-glutamine still possesses partial helical conformation in water. The unfolding effect of urea can be observed more clearly in water–methanol (7:3), in which solvent the polypeptide is much more helical than in water (Fig. 1). In this case urea in a concentration higher than 5 M is necessary to destroy the helical conformation, as compared with less than 1 M in water. In the water–methanol (7:3) system guanidinium chloride is about twice as effective as urea. It is significant that these two compounds show a similar relative effectiveness in the denaturation of proteins. In anhydrous methanol the addition of urea to near saturation (2 M) causes no significant change of b_0.

The b_0 value of polyhydroxypropyl-L-glutamine in water remains unchanged in the presence of NaOH (1 N) and HCl (1 N). This finding indicates that the unfolding of helical proteins and polypeptides by acids and bases is due to repulsions of electrically charged groups in side chains and not due to any effect of hydroxyl or hydrogen ions on the hydrogen bonds of the α-helix. Also, as expected for the case of a non-ionizable polypeptide, polyhydroxypropyl-L-glutamine shows no increase of helical content when NaCl (0.5 M) is added to its aqueous solution.

Finally, we wish to present independent support for the assumption that poly-N^5-(3-hydroxypropyl)-L-glutamine has a helical conformation in methanol, whereas in water this structure is largely broken down. A polymer preparation of a degree of polymerization, DP = 250*, had an intrinsic viscosity $[\eta] = 0.29$ dl/g in water and $[\eta] = 0.58$ dl/g in methanol; a polymer of DP = 560* showed $[\eta] = 0.60$ dl/g in water and $[\eta] = 2.60$ dl/g in methanol. The fact that an increase in the degree of polymerization by a factor of 2.2 corresponds to an increase in $[\eta]$ in water by a factor of 2.0 and in methanol by a factor of 4.3 indicates that in the latter solvent the molecules are rodlike, the length of the molecule being proportional to the degree of polymerization, whereas in the former solvent the molecules assume a configuration, behaving hydrodynamically as a random coil which, however, may still contain short helical segments.

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* Determined from intrinsic sedimentation and diffusion coefficients measured both in water and methanol, and partial specific volume, $\bar{v} = 0.74$, measured in water.