INTERACTIONS BETWEEN CHONDROITIN SULFATE C AND POLY-L-LYSINE: PRELIMINARY REPORT

by

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SUMMARY: Ionic interactions occur between chondroitin sulfate C and poly-L-lysine in dilute aqueous solution which force the polypeptide to adopt the α-helical conformation rather than the 'charged coil' form expected at neutral pH in the absence of the polysaccharide.

Conformation-directing interactions between oppositely charged polyelectrolytes have been shown to occur in several biological systems, such as the nucleohistones (1,2). As a model for the interactions between connective tissue proteins and polysaccharides, we are investigating the properties of mixtures of charged polypeptides and mucopolysaccharides in aqueous salt solution. In our initial work we selected poly-L-lysine and chondroitin sulfate C as a model system. Chondroitin sulfate C is an alternating copolymer of N-acetyl-D-galactosamine-6-sulfate and D-glucuronic acid; both the sulfate and carboxyl groups are ionized at physiological pH, and an ionic interaction is to be expected with the positively charged ε-amine groups of poly-L-lysine. This paper is a report on our preliminary studies on this system, using circular dichroism spectroscopy (CD).

The circular dichroism spectrum of an 0.15% aqueous solution of poly-L-lysine and chondroitin sulfate C is shown in Fig. 1-Curve A. The solution contained equal proportions of lysine and disaccharide residues in 0.0025M sodium cacodylate at pH 7.2. This spectrum is quite different from that predicted from the data for the individual components under equivalent conditions. Poly-L-lysine at neutral pH shows a CD spectrum which is considered to be

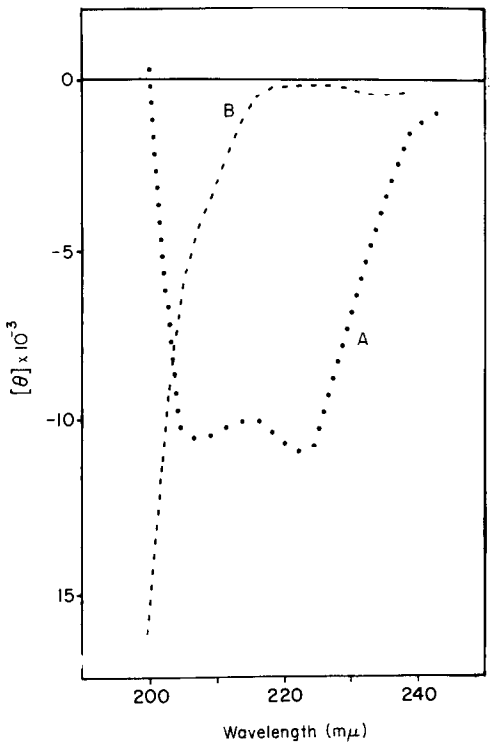

Figure 1- A Circular dichroism spectrum of a 1:1 mixture (molar concentration of polypeptide residues: molar concentration of mucopolysaccharide disaccharide residues) of poly-L-lysine and chondroitin sulfate C in 0.0025 M sodium cacodylate at pH 7.2.

B Circular dichroism spectrum calculated for a 1:1 mixture of poly-L-lysine and chondroitin sulfate C in 0.0025 M sodium cacodylate at pH 7.2., assuming no interaction between the species.

characteristic of the 'charged-coil' conformation (3,4). The CD spectrum of chondroitin sulfate C has a single trough at 208 mμ, and is very similar to those for the other connective tissue mucopolysaccharides (5). If there were no interactions between these materials in solution, the spectrum of a mixture should be that produced by combination of the spectra for the two components. Such a predicted spectrum is shown in fig. 1-curve B, for a mixture containing equal mole residue proportions of poly-L-lysine and chondroitin sulfate C. This is very different from the spectrum observed

for the mixture (Fig. 1-curve A), and strongly suggests that an interaction has taken place, which probably involves a conformational change in one or both of the components.

The single trough in the spectrum of chondroitin sulfate C is thought to be due to an electronic transition of the amide group of the galactose residue. Changes in the backbone conformation of the mucopolysaccharide should give rise to transitions in the region below 180 mμ, while the spectrum above this frequency should be essentially unchanged. Changes in temperature, pH, or salt concentration, produce only minor changes in the ellipticity of chondroitin sulfate C in aqueous solution. Thus we have assumed that the observed spectrum for the mucopolysaccharide is independent of the backbone conformation, and the subtraction of the chondroitin sulfate C curve from the spectrum of the mixture should yield the curve for the poly-L-lysine fraction alone. Fig. 2 shows the data presented in Fig. 1-curve A after subtraction of the curve predicted for the mucopolysaccharide fraction.

The curve in Fig. 2 is very similar to the observed spectrum for poly-L-lysine in the α-helical form. Thus, our results indicate that an interaction occurs between poly-L-lysine and chondroitin sulfate C, which forces the former to adopt the α-helical conformation, rather then the charged coil form expected at this pH. The circular dichroism spectra of mixtures of poly-L-lysine and hyaluronic acid, and of poly-L-lysine and desulfated chondroitin sulfate were very similar to curve B in fig. 1. There is no evidence for any conformation-directing interaction between poly-L-lysine and these two mucopolysaccharides, which both contain ionized carboxyl groups. Thus we conclude that the sulfate group is necessary for the interaction between chondroitin sulfate C and poly-L-lysine.

Present work suggests that the interaction is at a maximum when the sulfate and ε-amine groups are present in equal concentrations. Further details of these studies will be published in due course along with data concerning the effect on the interaction of changes in temperature and pH.

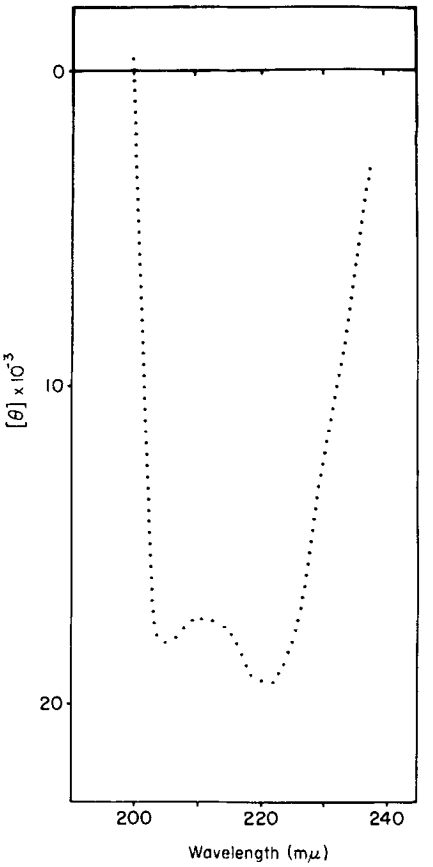

Figure 2. Circular dichroism spectrum presented in figure 1-curve A after removal of the chondroitin sulfate C contribution. Thus, the spectrum is for the poly-L-lysine in a 1:1 mixture in 0.0025 M sodium cacodylate at neutral pH.

Material precipitated from interacting mixtures is also being investigated by x-ray, infrared spectroscopy and electron microscope techniques.

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