Interactions of an Intact Proteoglycan and its Fragments with Basic Homopolypeptides in Dilute Aqueous Solution

By ROBERT A. GELMAN* and JOHN BLACKWELL†

*Department of Macromolecular Science, Case Western Reserve University,
Cleveland, Ohio 44106, U.S.A.*

and MARTIN B. MATHEWS

Department of Pediatrics and Biochemistry, University of Chicago, Chicago, Ill. 60637, U.S.A.

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The interactions between a proteoglycan and cationic polypeptides have been investigated by the use of circular-dichroism spectroscopy. The interaction produces an induced conformational change for poly(L-arginine) and poly(L-lysine), similar to the effects previously reported for mucopolysaccharide-polypeptide mixtures. For bovine nasal septum proteoglycan, the interactions are similar to those for chondroitin 4-sulphate, which comprises approximately 63% of the total polysaccharide. The results also suggest that the interactions produce a conformational change in the protein core. Similar studies for the Smith-degradation product show that the protein core can adopt a substantial α -helical content and is capable of interactions with poly-(L-arginine). The interactions for chondroitin sulphate 'doublets' are significantly different from those for the separated chains, indicating that the arrangement of the polysaccharide side chains in pairs (and larger groups) along the protein backbone contributes to the interaction properties of the intact proteoglycan.

Glycosaminoglycans (mucopolysaccharides) occur in the extracellular matrix of connective tissue, where they are bound to protein chains in covalent complexes known as proteoglycans. The comb-like model for the proteoglycan, first proposed by Mathews & Lozaityte (1958), consists of a protein core of about 2000 amino acid residues to which linear polysaccharide chains are covalently linked; the protein core contains approximately 30% charged amino acids, with two acidic residues for every basic amino acid, and approximately 10% proline. About 100 polysaccharide chains are attached as side chains; many of these form pairs linked together by less than ten amino acids and separated by some 35 amino acids from adjacent pairs (Mathews, 1971). The polysaccharide components of proteoglycans can be any of the connective-tissue polysaccharides; for bovine nasal septum cartilage they are chondroitin 4-sulphate, chondroitin 6-sulphate and keratan sulphate. The lengths of the polysaccharide chains and their distribution along the protein core are not uniform (Brandt & Muir, 1971).

Proteoglycans exist as aggregates in connective tissue (Sajdera & Hascall, 1969b); the question of the involvement of hyaluronic acid and other

molecular species in this aggregation is the subject of considerable interest (Hardingham & Muir, 1973; Gregory, 1973; Wells & Serafini-Francassini, 1973). Circular-dichroism (c.d.) studies (Eyring & Yang, 1968) show that the protein core has a random conformation, and viscosity measurements (Mathews & Lozaityte, 1958; Hascall & Sajdera, 1970) indicate that the macromolecule occupies a large domain in solution. The polyelectrolyte nature is evidenced by the observed contraction in the presence of salt (Eyring & Yang, 1968).

Proteoglycans, and also the component polysaccharides, undergo ionic interactions with tropocollagen (DiSalvo & Schubert, 1966; Toole & Lowther, 1967, 1968a; Mathews & Decker, 1968; Stevens *et al.*, 1969; Mathews, 1970; Öbrink, 1973; R. A. Gelman & J. Blackwell, unpublished results); these interactions are effected by the cationic side chains of collagen molecules and the negatively charged sulphate groups of the proteoglycan, and lead to the formation of 'native-type' fibrils (Toole & Lowther, 1968b). The predominant factors in the collagen-proteoglycan systems are electrostatic interactions (Sajdera & Hascall, 1969b; Mathews, 1970).

The present paper describes studies of the interactions of proteoglycan with cationic homopolypeptides, in dilute aqueous solution, as models for proteoglycan-protein systems. Glycosaminoglycans undergo an electrostatic interaction with cationic

* Present address: Polymer Department, The Weizmann Institute of Science, Rehovot, Israel.

† To whom all enquiries should be addressed.

polypeptides (Gelman *et al.*, 1972, 1973*a,b*; Gelman & Blackwell, 1973*a,b,c*, 1974). C.d. spectroscopy shows that positively charged polypeptides undergo a conformational change when mixed with glycosaminoglycans in neutral aqueous solution. The strength of the interaction, as judged by the temperature at which the interactions are disrupted, varies with the chemical structure of both the polysaccharide and the polypeptide. For the polypeptide, the strength increases with the length of the side chain along the series: poly-(L-ornithine) < poly-(L-lysine) < poly-(L-arginine). Charge delocalization of the guanidinium group is probably an additional factor contributing to the strength of interaction of poly-(L-arginine). The interactions also depend on the number and position of the anionic groups, and the nature of the glycosidic linkages; the glycosaminoglycans in order of increasing strength of interaction are: hyaluronic acid < chondroitin 4-sulphate < heparitin sulphate < chondroitin 6-sulphate < keratan sulphate-1 $\leq$ dermatan sulphate < heparin.

We have now extended these studies to observe the effects of bovine nasal septum proteoglycan on the conformation of cationic polypeptides. By analogy with the polypeptide-glycosaminoglycan systems, we have investigated the constraints imposed by the proteoglycan structure. In particular, we have studied the effects of the constraints imposed by linkage of the polysaccharide chains to the protein core, i.e. localization of many polysaccharide chains, and an increased molecular weight. The question of direct interaction between the protein core and the polypeptides was also investigated.

Experimental

Materials

A proteoglycan sample from bovine nasal septum cartilage was isolated by extraction with guanidinium chloride (Sajdera & Hascall, 1969*a*; Hascall & Sajdera, 1970). This specimen contained 9% protein, 6% keratan sulphate and 85% chondroitin sulphate; the ratio of 4- to 6-sulphate was 3:1. A degradation product of proteoglycan ('protein-core' sample), prepared by Smith degradation (periodate oxidation), consisted of the protein core and keratan sulphate chains; all the chondroitin sulphate chains have been removed. A specimen of chondroitin sulphate 'doublets', i.e. pairs of chondroitin sulphate chains linked by a small section of the protein core, was prepared from bovine nasal septum cartilage proteoglycan by hydrolysis with trypsin and chymotrypsin (Mathews, 1971).

Poly-(L-lysine) hydrobromide (lot L-96), poly-(L-arginine) hydrochloride (lot AG-6) and poly-(L-ornithine) hydrobromide (lot O-29) were obtained from New England Nuclear Corp., Boston, Mass., U.S.A., and had reported molecular weights of

100000, 65000 and 105000 respectively. Methanol (spectral grade) was obtained from Matheson, Coleman and Bell, East Rutherford, N.J., U.S.A.

Preparation of mixtures

Separate dilute aqueous solutions were prepared of the polypeptides and various proteoglycan samples; in each case, the concentration was 0.5mM with respect to amino acid and/or disaccharide residues, on the basis of the weight of the dissolved material. Mixtures containing different proportions of the constituents were prepared by slow dropwise addition of the polypeptide solution to the proteoglycan solution, with vigorous mixing. The relative proportions of the two components are generally quoted as the residue ratio, i.e. the molar concentration of the amino acid residues of the polypeptide/molar concentration of disaccharide residues of the proteoglycan. (As such, these ratios do not take the protein core into consideration.) A 1:1 mixture of poly-(L-arginine) and proteoglycan, for example, has total concentration of 0.022% (w/v) and contains one arginine residue for each disaccharide residue. The final pH values of the mixtures were in the range 6.7–7.5. The effects of methanol were investigated by the addition of the solvent to previously prepared solutions.

Circular dichroism

C.d. spectra of the mixtures at various ratios were recorded at room temperature by using a JASCO J-20 recording spectropolarimeter equipped with a quartz cell of path-length 5mm. The c.d. data are reported in terms of degrees $\cdot \text{cm}^2 \cdot \text{decimol}^{-1}$, on the basis of the molecular weights of the amino acid and/or the disaccharide residues. The mixtures were very slightly turbid, to an extent that varied with component ratio; such turbidity may make qualitative estimation of the molar ellipticity difficult, but the general qualitative features of the c.d. bands should be unaffected (Urry & Ji, 1968; Urry *et al.*, 1970).

Temperature studies

The c.d. spectra were recorded after equilibration at successively higher temperatures. The temperatures were raised in increments by circulating water through a jacketed quartz cell (path-length 5mm) at selected temperatures accurate to $\pm 0.2^\circ\text{C}$. Equilibrium was judged to be attained when the spectrum showed no further changes over a 15min period.

Results

Proteoglycan

The c.d. spectrum of bovine nasal septum cartilage proteoglycan is shown in Fig. 1 (curve A). The spectrum exhibits a single trough at about 203nm,

Fig. 1. C.d. data for bovine nasal septum proteoglycan

Curve A (—), c.d. spectrum of proteoglycan, in dilute aqueous solution at neutral pH values. The bar represents the extent of the noise in the experimental data. Curve B (·····), calculated contribution of the glycosaminoglycan component of the proteoglycan. Curve C (●), difference spectrum calculated for the protein core by subtracting curve B from curve A. The ellipticity, $[\theta]_x$, is calculated on the basis of the mean residue molecular weight of the component; the scale on the left applies to curves A and B and the scale on the right to curve C.

and is similar to that reported by Eyring & Yang (1968). On the basis of the analyses of the proteoglycan, the spectral contribution of the polysaccharide component can be calculated by combination of the spectra of the individual glycosaminoglycans according to their proportions in the sample (R. A. Gelman & J. Blackwell, unpublished results). The c.d. spectra of glycosaminoglycans in the 200–250 nm region remain approximately constant as the pH, temperature, salt concentration, etc. are varied; these are all changes that can be expected to change the backbone conformation. Thus the spectra of the polysaccharides can be considered to be insensitive to the chain conformation, and therefore the spectrum of the polysaccharide component can be calculated with reasonable certainty as shown in Fig. 1 (curve B). Subtraction of this spectrum from that of the proteoglycan gives the curve for the protein core (Fig. 1, curve C), which is characteristic of the 'random' conformation of polypeptides (Chen *et al.*, 1972). The fact that there is almost no change in the c.d. spectrum with temperature also suggests an unordered conformation.

Fig. 2(a) shows the c.d. spectra for various mixtures of poly-(L-arginine) and proteoglycan in neutral aqueous solution. These spectra for the mixtures are strikingly different from the calculated spectra for these mixtures, obtained by combination of the spectra of the proteoglycan and poly-(L-arginine) in the appropriate ratio. Curve E in Fig.

2(a) is such a calculated spectrum for a 1:1 mixture and is quite different from the observed spectrum for this ratio, curve C. These results suggest that the interaction has produced a conformational change in one or both of the macromolecules. As in previous work (cf. Gelman *et al.*, 1973a), we have assumed that the spectral contribution of the polysaccharide component in the interacting mixture remains constant. Consequently, any changes in the c.d. spectra produced on interaction of the polypeptide and proteoglycan must be due to conformational changes in the polypeptide and/or the protein core of the proteoglycan. If we assume for the moment that the specimen of the entire proteoglycan is constant, i.e. the contribution of the protein core is also unaffected by the interactions, then subtraction of the proteoglycan spectrum from the curves in Fig. 2(a) yields the data in Fig. 2(b). These spectra match those for polypeptide solutions containing a high α -helical content and imply that an interaction has occurred between the proteoglycan and polypeptide, which has induced a large proportion of the latter to adopt the α -helical form, as in the polypeptide-glycosaminoglycan systems.

The apparent helix content remains constant as the proportion of polypeptide is increased up to a ratio of $0.60 \pm 0.05:1$ (i.e. 0.6 arginine residues/disaccharide residue). Thereafter small increases occur in the ellipticity at 222 nm, $[\theta]_{222}$, which correspond to a slight decrease in helical content, up to the ratio of $1.00 \pm 0.05:1$. Above this ratio, much larger changes occur in the spectrum, corresponding to a progressive and rapid decrease in the helical content. This behaviour is in contrast with our previous observations for most polypeptide-glycosaminoglycan interactions (Gelman & Blackwell, 1973b,c, 1974; Gelman *et al.*, 1973a,b), where a sharp maximum in the interaction at a particular ratio can be delineated. The only exception is the poly-(L-arginine)-keratan sulphate-1 system (Gelman & Blackwell, 1974), which shows a constant percentage helicity up to the ratio of 1:1, whereupon the helix content falls as additional polypeptide is added.

As indicated above, the percentage helicity for poly-(L-arginine) in the presence of the proteoglycan cannot be estimated, since the subtraction procedure used for Fig. 2(b) assumed that the proteoglycan spectrum would be unaffected by the interaction. Since there is a high content of negatively charged side chains in the protein core, interaction of this component with poly-(L-arginine) is to be anticipated; such an interaction should result in a conformational change detectable by c.d. methods. This will be discussed further below, but it is clear from the value of $[\theta]_{222} = -36000$ at maximum interactions, that the α -helix content must be very high.

Fig. 2. C.d. data for mixture of proteoglycan and poly-(L-arginine)

(a) C.d. spectra of dilute aqueous solution mixtures of proteoglycan and poly-(L-arginine) at different residue ratios: curve A (●), 0.55:1; curve B (■), 0.6:1; curve C (○), 0.1:1; curve D (—), 1.1:1; curve E (---), calculated spectrum for a 1:1 mixture, assuming no interaction. (b) Data in Fig. 2(a) after subtraction of the proteoglycan spectrum: curve A (●), 0.55:1; curve B (■), 0.6:1; curve C (○), 1:1; curve D (—), 1.1:1.

Fig. 3. A plot of ellipticity at 222 nm, $[\theta]_{222}$, as a function of temperature for a 1:1 mixture of poly-(L-arginine) and proteoglycan

For details see the text.

On increasing the temperature, the α -helix-directing effect breaks down and the polypeptide reverts to the 'charged coil' form. This is best seen in a plot

of the ellipticity at 222 nm, $[\theta]_{222}$, against temperature (Fig. 3). For mixtures of poly-(L-arginine) and proteoglycan, a sigmoidal 'melting' curve is obtained with a transition or 'melting' temperature (T_m) = $56.0 \pm 1.0^\circ\text{C}$; the value of T_m does not vary with the ratio. This value is very close to that of $54.5 \pm 1.0^\circ\text{C}$ reported for the poly-(L-arginine)-chondroitin 4-sulphate system, as compared with $76.0 \pm 1.0^\circ\text{C}$ and $>90^\circ\text{C}$ for equivalent mixtures of chondroitin 6-sulphate and keratan sulphate-1 respectively. The results imply that the chondroitin 4-sulphate component controls the interaction of the proteoglycan with poly-(L-arginine). The interaction of poly-(L-arginine) with a 3:1 mixture of chondroitin 4- and 6-sulphate, with a total arginine/disaccharide ratio of 1:1, was examined for comparison with the proteoglycan data. On increasing the temperature, the 'melting' curve showed a slow almost linear transition, in contrast with the sigmoidal curve for the proteoglycan system. Clear differences can be seen between the interactions of the proteoglycan and the chondroitin sulphate mixture. The proteoglycan interactions show a sharp breakdown, whereas for the chondroitin sulphates, the broad transition probably reflects the breakdown of separate interacting systems.

Fig. 4 shows the spectra of mixtures of poly-(L-

Fig. 4. C.d. data for mixtures of proteoglycan and poly-(L-lysine)

(a) C.d. spectra of dilute aqueous solution mixtures of proteoglycan and poly-(L-lysine) at different residue ratios: curve A (○), 0.52:1; curve B (●), 1.05:1; curve C (■), 1.15:1; curve D (—), 2.1:1. (b) Data in Fig. 4(a) after subtraction of the proteoglycan spectrum: curve A (○), 0.52:1; curve B (●), 1.05:1; curve C (■), 1.15:1; curve D (—), 2.1:1.

lysine) and proteoglycan in neutral aqueous solution. The results indicate that there is an interaction in this system, with maximum conformational effect at a ratio of $1.0 \pm 0.1:1$. However, the magnitude of the spectral changes are much less than for the poly-(L-arginine) system, and it appears that a lower helical content is induced for the poly-(L-lysine). This interaction again mirrors that for the poly-(L-lysine)-chondroitin 4-sulphate system, which contained only about 20% α -helix at maximum interaction, whereas the equivalent chondroitin 6-sulphate and keratan sulphate-1 systems induced greater than 80% helicity. The results for the proteoglycan-poly-(L-lysine) system are further indication of the controlling influence at the chondroitin 4-sulphate component of the interactions. The proteoglycan had no detectable effect on the conformation of poly-(L-ornithine), i.e. the spectra of the mixtures were the same as those predicted from the spectra of the components of neutral pH values. This is consistent with the results of the individual polysaccharides: chondroitin 6-sulphate, chondroitin 4-sulphate and keratan sulphate-1 have no effect on the conformation of poly-(L-ornithine) at these concentrations.

Protein core

In Fig. 5, curve A is the c.d. spectrum of the Smith-degradation product from bovine nasal septum cartilage proteoglycan. The spectrum is similar in shape to that observed for the non-degraded proteoglycan. Subtraction of the calculated contribution of the polysaccharide (40% keratan

Fig. 5. C.d. data for the Smith-degradation product of the proteoglycan, the 'protein-core' sample

Curve A (—), c.d. spectrum of the 'protein-core' sample in dilute aqueous solution at neutral pH values; Curve B (○), calculated c.d. spectrum for the keratan sulphate-1 component; curve C (●), difference c.d. spectrum calculated for the protein component. The ellipticity, $[\theta]_{\lambda}$, is calculated on the basis of the mean residue molecular weight of the component; the scale on the left applies to curves A and B and the scale on the right applies to curve C.

sulphate-1), which is shown as curve B (Fig. 5), produces curve C, the spectral component due to the protein core. This spectrum is characteristic of the 'random' conformation of a protein, indicating that

Fig. 6. C.d. data for the Smith-degradation product of the proteoglycan in aqueous methanol solutions

Curve A (—), c.d. spectrum of the 'protein-core' sample, in 67% (v/v) methanol; curve B (○), calculated c.d. spectrum for the keratan sulphate-1 component; curve C (●), difference c.d. spectrum calculated for the protein component. The data are presented in the same manner as in Fig. 5.

the protein core is unordered in solution. A similar result was obtained for the protein core of the intact proteoglycan (Fig. 1, curve C).

Addition of methanol causes a pronounced change in the c.d. spectrum. A progressive change was seen in the spectrum as the concentration of methanol was increased to approx. 67% (v/v), beyond which no further change occurred. Fig. 6 shows the spectral data obtained for the protein core in a solution containing methanol. Curve A is the c.d. spectrum for the sample in 67% (v/v) methanol; this spectrum is markedly different from that in Fig. 5 for the aqueous solution. Curve B (Fig. 6) is the calculated spectrum for the keratan sulphate fraction; subtraction of this yields curve C, which is the subtraction curve for the protein component. Comparison of this curve with those predicted for mixtures of different polypeptide conformations (Greenfield & Fasman, 1969; Chen *et al.*, 1972) indicates that the protein core for the degraded sample is 35–40% α -helical. Thus changes can be induced in the conformation of the protein core, which is capable of adopting a significant α -helical content. This effect must be allowed for in consideration of the α -helix-directing interaction between proteoglycans and the polypeptides. Small changes were observed in the c.d. spectrum of the intact proteoglycan after dilution with methanol. These changes were of smaller magnitude than those for the core sample, and probably were due to the low protein fraction. As a result, detailed inter-

Fig. 7. C.d. data for mixtures of poly-(L-arginine) with the Smith-degradation product of the proteoglycan

(a) C.d. spectra for dilute aqueous solution mixtures of poly-(L-arginine) and 'protein-core' sample at different arginine/disaccharide residue ratios: curve A (●), 0.25:1; curve B (○), 0.5:1; curve C (■), 1:1; curve D (—), 2.5:1. (b) Data in Fig. 7(a) after subtraction of the spectrum of the 'protein-core' specimen (i.e. curve A in Fig. 5): curve A (●), 0.25:1; curve B (○), 0.5:1; curve C (■), 1:1; curve D (—), 2.5:1.

Fig. 8. C.d. data for mixtures of poly(L-arginine) and chondroitin sulphate 'doublets'

(a) C.d. spectra for dilute aqueous solution mixtures of poly(L-arginine) and chondroitin sulphate 'doublets' at different residue ratios: curve A (—), 0.55:1; curve B (●), 1:1; curve C (■), 1.5:1; curve D (○), 1.67:1. (b) Data in Fig. 8(a) after subtraction of the contribution predicted for the 'doublet' specimen: curve A (—), 0.55:1; curve B (●), 1:1; curve C (■), 1.5:1; curve D (○), 1.67:1.

Fig. 9. A plot of ellipticity at 222 nm, $[\theta]_{222}$, as a function of temperature of a 1:1 mixture of poly(L-arginine) and chondroitin sulphate 'doublets'

For details see the text.

pretation in terms of induced conformational change for the protein core is not possible, but it can be surmised that changes similar to those for the Smith-degradation product have been induced.

The c.d. spectra for mixtures of poly(L-lysine) and the 'protein-core' specimen are the same as those predicted for non-interacting mixtures, indicating

that there is no conformation-directing interaction between these molecules. However, Fig. 7 shows that there is an α -helix-directing effect for mixtures of poly(L-arginine) and the 'protein-core' sample. The maximum interaction ratio, as judged by the largest conformation directing effect on the polypeptide, occurs at a residue ratio of approximately 0.25:1 (arginine residues/keratan sulphate disaccharide residues); this can also be expressed in terms of the protein-core as approx. 0.08:1 (arginine residues/amino acid residues in the core). These stoichiometries are difficult to interpret in terms of the chemical make-up of the polysaccharide or protein components of the 'protein-core' sample. No conformation-directing interactions were observed with poly(L-ornithine).

Chondroitin sulphate 'doublets'

In order to investigate the possible effect of the arrangement of the glycosaminoglycan chains in pairs along the protein core, we investigated the interactions of chondroitin sulphate 'doublets'. The 'doublet' sample consisted of pairs of chondroitin sulphate chains connected by a short peptide chain, a portion of the original protein core.

Fig. 8 shows the c.d. spectra for mixtures of poly(L-arginine) and the chondroitin sulphate 'doublet' sample at various ratios. The results indicate that the chondroitin 'doublets' induce the α -helical conformation for the polypeptide in the same manner as the intact proteoglycan. The difference

Fig. 10. C.d. data for mixtures of poly-(L-lysine) and chondroitin sulphate 'doublets'

(a) C.d. spectra for dilute aqueous solution mixtures of poly-(L-lysine) and chondroitin sulphate 'doublets', at different residue ratios: curve A (—), 0.2:1; curve B (○), 0.9:1; curve C (■), 1.0:1; curve D (●), 1.5:1. (b) Data in Fig. 10(a) after subtraction of the contribution due to the chondroitin sulphate 'doublets': curve A (—), 0.2:1; curve B (○), 0.9:1; curve C (■), 1.0:1; curve D (●), 1.5:1.

spectra (Fig. 8b) are virtually identical up to a ratio of $1.5 \pm 0.1:1$, above which the ellipticity decreases with increasing polypeptide concentration. Thus the percentage of induced α -helix in these mixtures is constant up to maximum interaction at a ratio of 1.5:1; this is in contrast with our previous observations for the poly-(L-arginine)-chondroitin 6-sulphate and -chondroitin 4-sulphate systems, where the helical content increased with the proportion of polypeptide up to the maximum interaction ratio, and then decreased (Gelman *et al.*, 1973b; Gelman & Blackwell, 1973b). Constant α -helix content in the presence of excess of polysaccharide was observed for the poly-(L-arginine)-keratan sulphate-1 system (Gelman & Blackwell, 1974); this was thought to be due perhaps to the lower charge density for the keratan sulphate-1 specimen, i.e. 1.17 anionic groups/disaccharide residue. A further difference from the individual polysaccharides was the maximum interaction ratio of 1.5:1, as compared with 2:1 for both chondroitin 4- and 6-sulphate.

The thermal stability of the poly-(L-arginine)-'doublet' system mirrors that for the proteoglycan interactions, with a sharp 'melting' effect at $T_m = 56.5 \pm 1.0^\circ\text{C}$, as can be seen in Fig. 9. Fig. 10 shows the c.d. spectra of mixtures of poly-(L-lysine) and chondroitin sulphate 'doublets' at various ratios.

These spectra are similar to those reported for the poly-(L-lysine)-proteoglycan and poly-(L-lysine)-chondroitin 4-sulphate systems. Further, no conformation-directing interaction is observed for mixtures of poly-(L-ornithine) and the 'doublet' specimen.

Discussion

The data presented here indicate that conformation-directing interactions occur between basic homopolypeptides [poly-(L-lysine) and poly-(L-arginine)] and bovine nasal septum proteoglycan. Fig. 2 shows that the induced α -helicity in the poly-(L-arginine)-proteoglycan mixture, at maximum interaction, is very high. In the subtraction curves (Fig. 2b), the value of $[\theta]_{222} = -36000$ at maximum interaction matches the value observed for 100% α -helical polypeptides (Greenfield & Fasman, 1969; Chen *et al.*, 1972). This value for $[\theta]_{222}$ is of considerably higher magnitude than any reported for the various polypeptide-glycosaminoglycan systems. For example, the polypeptide subtraction curves for the poly-(L-arginine)-chondroitin 6-sulphate, poly-(L-arginine)-chondroitin 4-sulphate and poly-(L-arginine)-keratan sulphate-1 systems had values of $[\theta]_{222}$, for their maximum interaction ratios, of -28000 , -24000 and -24000 respectively.

For each of the latter systems, the percentage helicity was judged to be greater than 80% in each case, on the basis of the shape of the curve rather than the absolute value of the ellipticity, since it was considered that the very slight turbidity had probably decreased the recorded values. The poly-(L-arginine)-proteoglycan specimens also showed slight turbidity with a result that the values of $[\theta]_x$ in Fig. 2(b) should have an even greater magnitude. The high values of $[\theta]_x$ imply that the assumption of a constant proteoglycan contribution to the spectrum is invalid and that the conformation of the protein core has changed as a result of the interaction, probably with a significant proportion adopting the α -helical form.

Addition of methanol to the solution of the 'protein-core' specimen indicates that the core can adopt an ordered conformation with up to 40% α -helical content. Since proline comprises approx. 10% of the residues, it seems reasonable that the remainder has an extended, approximately 3_1 helical conformation, for which the c.d. spectrum is similar to that for poly-(L-proline)II or the 'charged-coil' form. The α -helical content appears to be quite large, especially since the protein has such a high proline content. Since the protein core is capable of adopting a large proportion of α -helix, it could well contribute to the apparent large α -helical content of the poly-(L-arginine)-proteoglycan system. Recent work (V. C. Hascall, personal communication) indicates that approximately one-third of the protein core is virtually unsubstituted with polysaccharide chains. This region, which contains a lower proportion of proline residues, is involved in a very specific interaction with hyaluronic acid and other tissue components when proteoglycan subunits aggregate. The induced α -helical content, which is approximately 40% of the protein, may be entirely in this region, with the portion of the protein core to which the polysaccharide chains are linked being in an extended helix.

The spectra for the poly-(L-lysine)-proteoglycan mixtures (Fig. 3) show weak conformation-directing effects with the α -helical content estimated¹ at 20–25% (Greenfield & Fasman, 1969). This is a similar α -helical content to that for the poly(L-lysine)-chondroitin 4-sulphate system (Gelman & Blackwell, 1973a,b). The 'melting' temperature (T_m) for the poly-(L-arginine)-proteoglycan system is 56.0°C, which is almost the same as that for the poly-(L-arginine)-chondroitin 4-sulphate system (Gelman & Blackwell, 1973b). These results strongly suggest that chondroitin 4-sulphate, which comprises approx. 63% of the proteoglycan, is the predominant factor in the interaction. It is difficult to explain the maximum interaction ratio in terms of a simple stoichiometric relationship between anionic and cationic groups, as was found for the polypeptide-

glycosaminoglycan systems, and a more complex interaction involving shielding of many of the negatively charged groups is envisaged.

The interactions of the 'protein-core' sample with the polypeptides underscore the importance of the chondroitin sulphate component to the interactions of the proteoglycan. The poly-(L-arginine)-'protein-core' system shows strong interactions, with maximum effect at a relatively low ratio of approximately 0.25 amino acid (arginine) residues/disaccharide (keratan sulphate-1) residue. These results are in contrast with our results for the interactions of keratan sulphate-1, which induces a high helical content for both poly-(L-lysine) and poly-(L-arginine), with maximum interaction at a ratio of 1.2:1 (Gelman & Blackwell, 1974); this ratio corresponds to one amino acid residue/negatively charged (sulphate) group on the polysaccharide. The last mentioned results were for corneal keratan sulphate-1, but recent results (R. A. Gelman & J. Blackwell, unpublished work) show that similar interactions occur for cartilage keratan sulphate-2. However, it appears at present that the keratan sulphate component of the 'protein-core' sample does not interact with poly-(L-lysine), and, at best, with very small amounts of poly-(L-arginine). The molecular weight of the keratan sulphate chains attached to bovine nasal septum proteoglycan is reported to be as low as 3600 (Schubert & Hamerman, 1968), which may be too short to allow for specific conformation-directing interactions. It seems more likely that the interactions in the poly-(L-arginine)-'protein core' system are between the polypeptide and the protein core. Approximately 20% of the amino acids in this protein have negative charges at neutral pH values, and it has been shown that interactions between cationic and anionic polypeptides affect the conformation of both components (Hammes & Schullery, 1968).

The apparent lack of a conformation-directing interaction for the keratan sulphate fraction could be a key to the changes in mechanical properties of connective tissue with age. The increase in the proportion of keratan sulphate, and the concomitant decrease of chondroitin sulphate, in cartilage with age (Kaplan & Meyer, 1959; Mathews & Glagov, 1966), may result directly in the changes in the physical and mechanical properties of the tissue.

The c.d. spectra for the interaction of poly-(L-lysine) and the chondroitin sulphate 'doublets' are similar to that for the polypeptide-chondroitin 4-sulphate system. The c.d. spectra for the poly-(L-arginine)-'doublet' mixtures show a constant α -helical content up to a certain proportion of polypeptide. The apparent maximum ratio for this interaction does not follow the stoichiometric relationship found for the poly-(L-arginine)-chondroitin 4-sulphate system; the maximum interaction occurs at 1.5:1, instead of

2.0:1 as found for the model system. These results suggest that there is an effect owing to the 'doublet' arrangement; perhaps this arrangement leads to an effective shielding of some charges, which could result in a lower effective charge density, leading to the lower maximum interaction ratio. This lower effective charge density would be expected to cause the polysaccharide chains to be less extended, which could allow for the maximum α -helical-directing effect even in the presence of excess of polysaccharide.

The 'melting' temperature for the poly-(L-arginine)-chondroitin sulphate 'doublet' mixture of 56°C, which is within experimental error of the values for the poly-(L-arginine)-proteoglycan and the poly-(L-arginine)-chondroitin 4-sulphate systems, suggests both that chondroitin 4-sulphate may be the predominant glycosaminoglycan in controlling the interactions of the proteoglycan and that the 'doublets' behave as if they were still part of the proteoglycan molecule.

In conclusion, it is apparent that the protein core of a proteoglycan is capable of adopting an ordered conformation, and that interactions with cationic polypeptides probably have such a conformation-directing effect. It seems likely that the interaction of connective-tissue proteins with proteoglycans will have a similar effect, with a result that the protein core has an ordered conformation *in vivo*.

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