THE MOLECULAR SIZE, SHAPE AND WEIGHT OF MUCOPROTEIN FROM CARTILAGE

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INTRODUCTION

The chondroitin sulfate-protein complex (CS-P) from cartilage accounts for at least a third of the chondroitin sulfate of cartilage, the remaining two thirds most likely being linked to collagen. The chondroitin sulfate and protein contents of CS-P have been estimated to be 60-75% and 25-40%, respectively, for various preparations3. Chondroitin sulfate is a linear acidic mucopolysaccharide4, whose repeating unit is a disaccharide consisting of α-glucuronic acid and N-acetyl-D-glucosamine. The glucuronic linkage is a β-linkage; the glucosamine linkage involves C4 and C6. The molecular weight of chondroitin sulfate is 20,000. The protein moiety of CS-P shows an amino acid composition different from that of collagen. The nature of the linkage between the protein and polysaccharide is unknown.

In the present work, the aim was to obtain information about the molecular size, shape and weight of CS-P.

EXPERIMENTAL

Preparation of CS-P

All operations were carried out at 4°C. A kg of fresh beef nasal septum cartilage, carefully cleaned, was homogenized in a Waring blender, which was operated intermittently to avoid heating, suspended in 1 l of 30%, HCl and mechanically shaken for two days. The suspension was squeezed through cheesecloth and centrifuged at 20,000 rpm for 15 min in a Spinco centrifuge. The supernatant showed two components in the analytical ultracentrifuge: the concentration of the faster one was negligible compared to that of the slower component. The supernatant was then dialyzed against running tap water for three days. A sample (5 g) and two volumes of absolute alcohol were successively added. The precipitate that formed was allowed to stand overnight and was then recovered by centrifugation at 20,000 rpm, for 15 min. Afterwards it was dried with alcohol and ether and suspended in 200 ml of phosphate buffer (M/15, pH 7.0). The turbid suspension was centrifuged at 20,000 rpm for 1 h, a very small quantity of precipitate being formed. The clear, viscous supernatant, exhibited in the analytical ultracentrifuge only a sedimenting boundary; when it was added to the solution was stored at 4°C for use in the physical studies, which were, therefore, performed with phosphate buffer (M/15, pH = 7.0) as the solvent.

Analytical and chromatographic results

Our CS-P solution had a concentration (dry weight at 110°C) of 0.72 g/l. The nitrogen content of CS-P determined by the micro-Kjeldahl method, was 5.85 g/l. Qualitative data on the amino acid composition of the protein of CS-P were obtained by paper chromatography.

References p. 52.
Sample of CS-P were dissolved in 0.1 N NaCl at 10 C for 16 h, and one- and two-dimensional amas, were prepared and scanned in gelatin and hydroxy-
philic glass and with wave in the two-dimensional analysis were used to trace. Amino acids were identified by develop-
ing the chro-matogram with ninhydrin, by U.V. Absorption by specific reaction (histidine and proline).

It was possible to identify the following amino acids by their k values: aspartic acid, serine, glycine, glutamic acid, threonine, alanine, leucine (141, 141); cysteine, lysine, valine, phenylalanine (141, 141); histidine, tyrosine, proline (141). The evaluation of the intensity of spots, as indicated by d/d, (+ +), (+ + +), is to be considered as only tentative and is based on the intensity of the spot corresponding to alanine. Protein is possibly due to trace contamination with collagen. Further spots pertaining to the carboxylate moiety of CS-P were not investigated.

**Physical methods and results**

Viscosities were determined with a Carver apparatus at a velocity gradient g = 0.166 sec^-1, and a temperature T = 21 C ± 0.05. The viscosity results are shown in Fig. 1.

![Viscosity plot](image)

**Fig. 1.** Viscosity plots against concentration.

The intrinsic viscosity was found to be [g] = 0.07 gsp units.

A model E Spinco ultracentrifuge operating at 60,000 r.p.m. was used in the sedi-
mentation analysis; sedimentation coefficients corrected to standard conditions were computed in the usual way. The sedimentation coefficient at infinite dilution, s0.7, was obtained by linear extrapolation of the plot of 1/s against s to s = 0 (Fig. 2). Only reconstituent concentrations below 0.4% were used. The sedimentation boundary showed considerable spreading and the peak was very asymmetric. s0.7 was found to be 6.86 x 10^-18 gsp units.

The flow birefringence of CS-P was measured at 21°C with a velocity gradient ranging from 1 to 396 sec^-1. For a 0.72% solution of CS-P at a velocity gradient g = 350 sec^-1, the streaming birefringence was 5.3 x 10^-4. Therefore, the intensity streaming birefringence, defined as I = (dn/dn) [target - background], was < 10^-4.

References p. 52.

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The double extrapolated in a diagram p. (8) to.

Selected References: Add 1

In order to correct an e value, should be multiplied correctly to compare relative size and shape reported in F. In mass composition in Reference p. 52.
ERRATUM
In order to correct an error in the labelling of Figs. 3 and 4, the $k_i R$ values should be multiplied by 1.55. The actual points in the figures are correctly placed relative to the new scale. The values of molecular weight, size and shape reported in the paper are correctly calculated.

**Vol. 26 (1957)**

MUCOPROTEIN FROM CARTILAGE

The refractive index increment was measured at $\lambda = 5460 \AA$ with a Rayleigh interferometer; the specific refractive index was $n = 1.368$ per g/ml.

Angular light scattering was measured at wavelength $\lambda = 5460 \AA$ with the apparatus of WIPKENS AND SCHERER. Solutions of CS-P with concentrations ranging from 2.86 to $1.4 \times 10^{-5}$ g/ml were used in these measurements. Scattering data were extrapolated to zero angle and zero concentration by the method of ZIMM. The angular scattering envelope obtained is shown in Fig. 3. The anisotropy was negligible, which is important for the following discussion. From the light-scattering measurements it was possible to obtain the following data: the weight average molecular weight, $M_w = 1.8 \times 10^6$, the disymmetry, $I_{40}/I_{50} = 2.15$, the $D$-average radius of gyration, $R_g = 1.86 \AA$.

$$\begin{array}{c|c|c|c}
0 & 2 & 4 & 6 \\
0 & 2 & 4 & 6 \\
\end{array}$$

r.p.m. was used in the sedimentation studies with the assumption that the velocity gradient $g = 0.960$

cells are shown in Fig. 1.

**Fig. 3. Zimm plot obtained with CS-P.**

The double extrapolation of the Zimm plot obtained for our CS-P was reworked in a diagram $P^2/(\alpha) vs. \beta$R where $P^2/(\alpha)$ is the reciprocal $P$ scattering factor and $\beta$ is the angle between the incident and scattered beams, in order to compare the plot with the curves pertaining to monodisperse systems of globular, rods and sphere, respectively (Fig. 4). It is known (Renes) that the curves of polydisperse systems are lower than those of the monodisperse ones.

We shall now discuss the three possibilities as to the shape of CS-P macromolecules, namely polydispersity, monodispersity, and sphere. As evident from Fig. 4, the sphere model seems to be quite improbable; furthermore, it would require a high specific volume $V = 4150$ m$^3$/g, which appears to be enormously high; the given value of $V$ is $1.4 \times 10^{-5}$ m$^{-3}$.

References: 52

- WIPKENS AND SCHERER
- ZIMM
- RENES
and the analytical volume (assuming a partial specific volume for CS$_2$ of $v = 0.60$ ml/g). $V_a = 1.77000$. All these data decidedly rule out the possibility of a system of spheres.

![Graph](image)

Fig. 2. Curves 1, 2, 3 pertinent to monodisperse systems of spheres, rods and disks, respectively. Curve 1 is the Zimm plot extrapolation to $c = 0$. Rods, anisotropic at the origin, 3, and disks, isotropic, 6.

On the other hand, a polydisperse system of rods, such as was put forward by Mathews and Lozowsky, must receive serious consideration. It is, indeed, by no means incompatible with the light scattering envelopes. The length of the rods, as deduced from $R_h$ would be $L \approx 4000$ A. For such a system it is possible to obtain approximate values for the number average molecular weight, $M_n$, and number average length of rods, $L_n$, by the method of Holzer, which is an application to rod systems of the method originally developed by Svedberg and Konowal. For polydisperse systems of rods, these values are $M_n = 1.3 \times 10^6$ and $L_n = 2.330$ A, consequently $M_n/M_w = 1.34$. We can now compute the value of the rotary diffusion constant for such rods. The volume intrinsic viscosity is easily obtained for a suspension of rods by dividing the weight specific viscosity by the partial specific volume (assumed to be $v = 0.60$ ml/g); we find $[\eta]_w = 3.09 \times 10^6$ a. By applying Svedberg's formula as modified by Svedberg for rod-like particles, we obtain an axial ratio $a \approx 30$; the diameter of the rods is also $d \approx 60$ A, and the rotary diffusion constant $D = 3 \times 0.5 \times 3 = 0.2$ sec$^{-1}$. Such a system should show a very good orientation already at velocity gradients as low as $v \approx 0.2$ sec$^{-1}$, because in the case the ratio $v/D$ is equal to unity, and the system could hardly fail to exhibit a strong flow birefringence in the gradient range examined. On the contrary, we found a negligible streaming birefringence, a result in agreement with the negative data of Wassen and Bayley for an analogous product.

All the above considerations show that the rod model is also highly improbable and that the hypothesis of a polydisperse system of random coils appears to be the most satisfactory. On the other hand, it has been possible to obtain the $M_w$ of the rods by the sedimentation rate at high concentration $c = 10^{-4}$ g/ml, which yielded a value of $M_w = 1.1 \times 10^6$ (see also p. 49).

The results of the present work on the behavior of a polydisperse system of rods and disks and a monodisperse system of rods and disks, respectively, for the stabilization of chymotrypsin by dodecyl sulfoacetate. The results are in agreement with the data of Wassen and Bayley.

The author wishes to express his gratitude to Dr. J. Levy for the critical examination of the manuscript and for the prepublication suggestions.
most satisfactory. On the assumption that we were dealing with such a system, we
were able to obtain other information about the CS-P macromolecule. Firstly, it may
be said that there are indications that there is not branching or at most at only an
very slight extent. Indeed, Bessiot* showed that branching increases the general upward
curvature of curve 2, while our curve 4 exhibits a strong downward curvature (Fig. 4).
This latter is substantially due to the wide polydispersity of the system. The pos-
ibility of a system of non-gaussian coils, which could also explain such a curvature,
seems to be ruled out by the high value of the ratio $A/A$, where $A$ is the length of
the filament (which may be estimated on the basis of an end-to-end arrangement
of chondroitin sulfate molecules and polypeptide chains in the macromolecule of CS-P),
and $r$ is the mean root-square end-to-end distance of the coil (computable from $R_0$).
Finally, using the method of Salmon and Bessiot 18, we found an approximate
value for $M_c$ (strictly speaking, its upper limit); we obtained $M_c = 480,000$; therefore,
$M_c/M_o \approx 4$, a result which confirms the wide polydispersity observed in the sediment-
analyses analysis. With the aim of checking the mutual consistency of our experimental
data as well as our conclusions, we applied the following equation proposed by Flory: 17:
$\Phi^1 \cdot \rho_{-3} = \Phi \cdot \rho_{-2}$, where $\Phi^1 \cdot \rho_{-3}$ should be a universal constant independent
of solvent, temperature and nature of the polymer. Introducing into the above equa-
tion our values $\Phi$, $M_c$, $\rho$, and the assumed value of $\Phi$, we found $\Phi^1 \cdot \rho_{-3} = 2.1 \cdot 10^6$;
the good agreement between this value and the theoretical one seems to confirm the
consistency of our experimental results and the validity of the proposed model.

CONCLUSION

The results of the present study on CS-P support the view of an end-to-end arrange-
ment of chondroitin sulfate linear chains and polypeptide chains in the macromolecule
of CS-P; the same conclusion but on a different basis, was reached by Weber and
Ravelli 16 for a product analogous to our CS-P. Such a structure would account satis-
factorily for the stabilizing and cementing function of CS-P on collagen.

It is suggested that such a function of CS-P involves more collagen structural
units than chondroitin sulfate does 16, 19; therefore, the first is extractable by milder
methods than the latter.

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comments on the preparation of CS-P.

SUMMARY

The chondroitin sulfate — protein complex of cartilage was studied by viscosity, streaming
birefringence, sedimentation and light scattering. It showed a $M_o = 1.3 \times 10^6$; a z-average radius
of gyration $R_o = 1.17 A$, the molecular shape is probably linear with a tendril coil configuration
in the native solution.

References p. 57.
SUR LA DÉNATURATION THERMIQUE ET LA DÉNATURATION DE SURFACE DE LA CARBOXYPEPTIDASE A DU PANCRÉAS*. Influence du TWEEN 80

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1 Voir nomenclature dans 1.

Bibliographie p. 60.