OWL MONKEY VITREOUS: A NOVEL MODEL FOR HYALURONIC ACID STRUCTURAL STUDIES

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Received June 21,1976

Summary: Circular dichroism (CD) studies have been made on hyaluronic acid (HA) obtained from dialyzed owl monkey vitreous. The protein content of these samples is low enough not to interfere with the CD measurements of hyaluronic acid. The ellipticity values of vitreous HA are higher than those of HA from other tissues, indicating a higher degree of preferred order. Since the purification procedures involve only dialysis, the owl monkey vitreous can be a model tissue for structural studies of HA close to its native state.

Hyaluronic acid is a glycosaminoglycan present in the intercellular matrix of the connective tissue. It is found in relatively high concentration in the vitreous and synovial fluid. Its primary structure is made up of unbranched repeating disaccharide units of glucuronic acid and N-acetyl glucosamine with alternating β1-3 and β1-4 linkages. Chiroptical studies on purified HA solutions derived from human umbilical cord and rooster comb have revealed a highly ordered structure, probably a helix (1,2). The variety of conformations including helical structure of this polysaccharide in the solid state has been indicated by X-ray diffraction (3,4) and infrared studies (5). However, information on the structure of HA in solution close to its native state is of major interest. This knowledge could be gained by using hyaluronic acid from some tissue, the isolation of which does not involve extensive purification procedures. Such procedures can also cause changes in the hydrodynamic and natural conformational properties of HA. The liquid vitreous of the owl monkey provides such a tissue due to its high HA and low protein content. Purification of this HA is limited to the removal of low

molecular weight solutes by dialysis. This simple treatment would allow us to study the polymer close to its native state, whereas other sources such as gel vitreous, synovial fluid, umbilical cord or rooster comb demand more rigorous purification steps, especially for the removal of relatively high amounts of collagen and non-collagenous proteins.

The present communication reports the results of CD measurements in order to demonstrate the nature and applicability of owl monkey vitreous for structural studies of hyaluronic acid.

Materials and Methods: Liquid vitreous was obtained from anesthetized owl monkeys by aspiration of 1.0-1.5 ml via a needle inserted through the pars plana ciliaris. All optical and chemical measurements were performed after extensive dialysis of the vitreous samples against 0.15 M NaCl at 4°C. Purified umbilical cord hyaluronic acid was obtained from Biotrics, Inc., and dialyzed against 0.15 M NaCl in order to maintain same pH and salt concentration as the vitreous samples. The CD spectra were recorded in a Jasco ORD/UV-5 spectropolarimeter using 0.2 cm path length. The UV absorption was measured in a Beckman spectrophotometer Model DK-2. Hyaluronic acid concentration of the samples was calculated from the hexuronic acid content using a modified (6) carbazole reaction method (7). Viscosity measurements were carried out at 25°C in a Cannon-Ubbelohde semi-microdilution viscometer. The molecular weight (M) was calculated from the plot of intrinsic viscosity $[\eta]$ against known molecular weight samples on the basis of the Mark-Houwink relationship (8), $[\eta]=KM^a$. Protein was determined according to Folin's phenol method (9). Collagen content of the sample was estimated from hydroxyproline determination (10). Molar ellipticity values were calculated on the basis of the molecular weight of the disaccharide unit of hyaluronic acid.

Results and Discussion: Figure 1 shows the ultraviolet spectrum of a typical vitreous sample after dialysis against 0.15 M NaCl at 4°C for 24 hrs. No appreciable amount of protein absorption was detected at 280 nm. The absorption below 230 nm presumably is due to the amide chromophore of HA. The analyses and the limiting viscosity number determination (Table 1) show that the major macromolecular component of the vitreous samples is hyaluronic acid with a molecular weight in the range of 2 to 3 x 10^6. Table 1 summarizes the results of analyses and CD data of the different samples. The protein content of the samples ranges between 2 and 13% of the HA concentration. The total hydroxyproline content is less than 1 µg per 100 µg protein of the pooled vitreous sample. The contribution of collagen to the CD spectrum can thus be

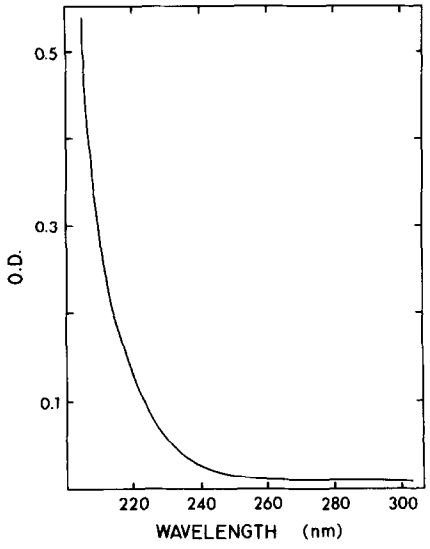

Fig. 1 The absorption spectrum of vitreous sample II. Hyaluronic acid, 520 µg/ml; protein, 70 µg/ml; path length, 0.1 cm.

Table I

Analyses and Physico-Chemical Properties of Dialyzed Owl Monkey Vitreous

Sample	Hyalronic Acid	Protein	Intrinsic Viscosity [η]	CD value (θ) x 10^{-3}
	µg/ml	µg/ml	cc/gm	$\frac{\text{deg. x cm}^2}{\text{decimole}}$
I	325	23	4450	18.1
II	520	70	4800	15.7
III	520	47	4300	12.1
IV	300	6	2200	19.7
V	400	8	4200	16.5

ruled out. Figure 2 shows the CD band of vitreous and purified umbilical cord HA. Both have a peak at 210 nm but the molar ellipticity value of vitreous HA

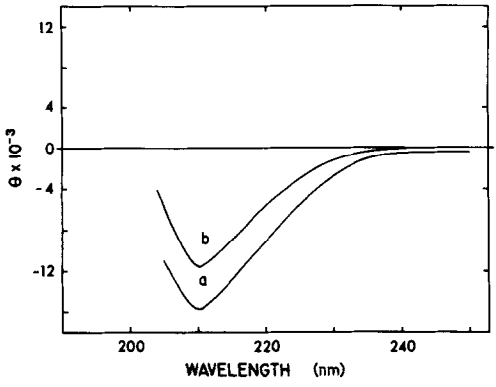

Fig. 2 (a) The circular dichroism spectrum of same vitreous sample as in Fig. 1. (b) The circular dichroism spectrum of purified hyaluronic acid from umbilical cord (intrinsic viscosity 4500 cc/gm, average mol. wt. 2.8 x 10^6); path length in both, 0.2 cm.

is 30% higher than that of purified umbilical cord HA. The average enhancement of ellipticity values of all analyzed vitreous samples as compared to purified HA is 36.6% (range 0 to 66%). The CD band of the sample with the highest protein content (sample II) does not show any shift of the 210 nm peak or a shoulder at 222 nm. If all of this protein is assumed to be in a 100% helical form with an accepted molar ellipticity value of 38,000 deg.cm^2/decimole (11) at 222 nm, one would expect to see a shoulder in the 222 nm region or a shift in the 210 nm band of this sample. Moreover, the varying amount of proteins is not correlated to the intrinsic viscosity and the ellipticity values of vitreous HA (Table 1). This concurs with the results of previous studies on hyaluronic acid preparations (12). We conclude therefore that the protein content in the samples studied does not interfere with the optical study of HA. Table I also shows that the intrinsic viscosity values of the owl monkey vitreous HA do not have any relation to the CD parameters, which is in agreement with our previous results on purified cord and rooster comb HA (13).

The enhanced molar rotation and ellipticity values of HA in comparison to its monomers (1,14) and oligomers (1) were strong evidence for a long-range

order in the polymer conformation. The higher ellipticity values of vitreous HA compared to purified HA (Fig. 2) can thus be attributed to the degree of order in this polymer. According to this concept the HA of the owl monkey vitreous has a significantly higher ordered structure than purified HA. At present, it is not known whether this difference is due to the difference in tissue origin or to a possible loss of ordered structure caused by the purification process.

The biological function of hyaluronic acid is not well defined and is probably tissue specific. Whether or not a structure-function relationship of this polymer exists is not yet known. Studies on normal and pathological vitreous hyaluronic acid close to its native state could provide a better understanding of this possible relationship. Our results demonstrate that the owl monkey vitreous provides such a model tissue.

Acknowledgements: Part of this investigation was supported by grants from the U.S. National Science Foundation BMS 75-15316, PHS EY 01760-01 and Deutsche Forschungsgemeinschaft (German National Science Foundation) Hu 194-1. We wish to gratefully acknowledge the technical assistance of Judith W. Appleby, and the editorial assistance of S. Flavia Blackwell.

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