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SMALL-ANGLE NEUTRON SCATTERING STUDIES FROM SOLUTIONS
OF BOVINE NASAL CARTILAGE PROTEOGLYCAN*

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Small-angle neutron scattering, SANS, of the proteoglycan subunit of bovine nasal cartilage in 0.15N LiCl at 25°C yielded the radius of gyration, R_g , radius of gyration of the cross-section, R_q , persistence length, $\bar{a}$, and the molecular weight, M . The following values were obtained: $M = 3.9 \times 10^6$, $R_g = 745 \text{ \AA}$, $R_q = 34.6 \text{ \AA}$ and $\bar{a} = 35.2 \text{ \AA}$. These values compare favorably with those that were obtained from small angle x-ray scattering, SAXS, of a similar extract. The scattering curve of the proteoglycan subunit in D_2O showed a characteristic broad peak in the specified angular range similar to that observed from SAXS, thus confirming the polyelectrolyte nature of the proteoglycan.

1. Introduction

Proteoglycans are constituents of all types of connective tissues, such as blood vessels, skin, cartilage, and corneal stroma. In cartilage, a polypeptide of about 2000 amino acids forms the backbone of the proteoglycan molecule with many polysaccharide chains covalently attached as pendant groups [1]. These chains, comprising the glycosaminoglycans chondroitin sulfate and keratan sulfate,

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are linear sequences of alternating carbohydrates bearing carboxyl groups and sulfate esters, and the whole proteoglycan is, therefore, highly polyanionic.

Such molecules are essential components of connective tissues, but their structures and interactions are far from being understood completely. The structural patterns of proteoglycans have been shown to change in developing systems, for example, there are an increased ability to aggregate, increased length of keratan sulfate chains, decreased length of chondroitin sulfate chains, and changes in size of the proteoglycan, during the development of chick limb bud cell cultures [2].

Since physical methods such as SAXS and SANS can give information about macromolecular structures in solution under mild conditions, they may eventually be valuable in establishing the significance of these properties to their changes in the organization and function of various tissues.

2. Experimental Procedures

Bovine nasal cartilage was extracted with 4M guanidine hydrochloride in the presence of protease inhibitors, and proteoglycan was isolated by sequential sedimentation equilibrium in CsCl density gradients, first under associative and then under dissociative conditions, to give an A1D1 preparation as defined by Heinegard [3]. The material is referred to as proteoglycan subunit, PGS. PGS was further purified by passage through a column of sepharose CL-2B equilibrated with 0.5N lithium acetate, pH= 6.2 [3]. The PGS zone was divided into three parts in order to minimize the polydispersity of the sample. It was dialyzed against water and lyophilized, and the third fraction of

lowest molecular weight was used to prepare a solution of 11.925 mg/ml in 0.15N LiCl in 99% D₂O. The solution was dialyzed against solvent several times and the dialyzate was used as buffer.

Neutron scattering measurements were performed on the 30-m small-angle neutron scattering instrument at the National Center for Small-Angle Scattering Research, Oak Ridge National Laboratory. The instrument selects neutrons of $\lambda = 4.75 \text{ \AA}$ with a $\Delta\lambda/\lambda$ of 6%. The detector to sample distance was 17.02m which allowed us to detect a large radius of gyration from suitable slits combination. The short geometry involved a sample to detector distance of 1.75m and a 4 cm beam stop. This geometry permitted acquisition of high angle scattering data for the purpose of observing a characteristic peak previously seen in SAXS experiments [4]. The scattered neutrons are detected by a two-dimensional position sensitive ³He detector with 64 x 64 1-cm² elements. Sample and buffer were run in a pair of matched quartz cells to avoid error due to cell thickness and transmission. The detailed procedure is as described in a previous paper [5].

3. Theory

The M was obtained according to the equation [6,7]

$$\frac{I(0)}{I_{inc}(0)} = f\left(\frac{T_s}{1-T}\right) 4\pi \lambda M N_a t c \left[\left(\frac{1}{N_a} (\Sigma b - \rho_s V)\right)\right]^2 \quad (1)$$

where $I(0)$ is the coherent intensity scattered at zero angle which is obtained from the extrapolation of the Guinier plot, $I_{inc}(0)$ is the incoherent scattering of H₂O in the forward direction, f is the correction factor for the anisotropy in the incoherent scattering of H₂O for wavelength 4.75 $\text{\AA}$, T_s and T are transmission coefficients of the sample and the empty quartz cell, respectively, M is molecular weight, N_a is Avogadro's number, t is the cell thickness, c is the concentration in g/ml, b is the

scattering length of the molecule (about 93% polysaccharide and 7% proteins for the proteoglycan), ρ_s is the scattering density and V is the partial specific volume of the solute. The following values obtained were used in assessing M from Eq. (1) for the proteoglycan: $f = 0.7295$, $T_s = 0.857$, $T = 0.56$, $t = 0.2$ cm, $I(0) = 0.599$, $I_{inc}(0) = 0.1012$, $\Sigma b/N_a = 6.58 \times 10^{-14}$ cm/dalton, $\rho_s = 6.36 \times 10^{10}$ cm⁻², $V = 0.55$ cm³/gm.

The R_g and R_q are obtained from the equations [8],

$$\ln(I) = \ln I(0) - R_g^2 Q^2/3 \quad \text{and} \quad (2)$$

$$\ln(IQ) = \ln IQ(0) - R_q^2 Q^2/2 \quad (3)$$

where I is the scattered intensity and $Q = (4\pi/\lambda) \sin \theta$, the scattering vector. Figure 1 shows the Guinier plot of the bovine nasal cartilage proteoglycan in 0.15N LiCl-D₂O.

The $\bar{a}^*$ was calculated from the equation below [8].

$$\bar{a}^* = 2.3/Q^* \quad (4)$$

where Q^* is the transition between the I/Q^2 and I/Q portions of the scattering curve. Figure 2 shows the plot of IQ^2 vs. Q and the value of Q^* . Table 1 shows the values of M , R_g , R_q , and $\bar{a}^*$ obtained from the above equations for the proteoglycan subunit in 0.15N LiCl-D₂O.

4. Results and Discussion

The values of $M = 3.9 \times 10^6$, $R_g = 745$ Å, $R_q = 34.6$ Å and $\bar{a}^* = 35.2$ Å, obtained from SANS for the PGS in 0.15N LiCl at 25°C, compare favorably to those obtained from SAXS of another bovine nasal cartilage PGS preparation in the same solvent at 25°C [4], cf. Table 1. Values of $V = 2.5 \times 10^6$ [9] and $M = 3.97 \times 10^6$ [10] were reported from sedimentation equilibrium,

SE, and Pasternack *et al.* [11] reported the values of $M = 2.3 \times 10^6$ and $R_g = 570 \text{ \AA}$ from light scattering, LS, measurements of similar PGS in 4.0M guanidine hydrochloride, GHC1, at 25°C . Since the SAXS and SANS studies, including those from SE and LS, were made on separate PGS preparations, the values cannot be directly compared. However, Hascall and Sajdera [12] reported that bovine nasal cartilage PGS is highly polydispersed with M ranging from $2.5\text{--}5.0 \times 10^6$. It is interesting to note that Hayashi and co-workers [13] reported that the value of M of a polyisobutylene sample determined from SANS is higher than the value obtained from GPC/LS. Perkins *et al.* [14] reported $M = 81,000 \pm 20,000$ for pig laryngeal cartilage proteoglycan derived from SANS, which was in satisfactory agreement with biochemical estimates of 83,000. It is to be noted that these investigators used the region $Q = 0.009$ to $0.26 \text{ \AA}^{-1}$ whereas we worked in the range of $Q = 0.00163$ to $0.32 \text{ \AA}^{-1}$.

In a recent paper by Koyama [15] it was reported that the curve from SANS and SAXS of polyelectrolyte solutions with no added salts have a broad peak in the region $Q = 10^{-2} - 10^{-1} \text{ \AA}^{-1}$. Interestingly enough we observed the peak both in SANS, Fig. 3, and SAXS [4] curves of PGS in D_2O and H_2O , respectively, in the same angular range, thus confirming that proteoglycan is a polyelectrolyte in these solvents. Further, the investigation of the height and the position of the peak should enable the estimation of the effective coulomb potential. However, to do this would require the acquisition of scattering curves as a function of molarity of added salt.

References

- [1] J.F. Kennedy, Proteoglycans-Biological and Chemical Aspects in Human Life, (Elsevier Scientific Pub. Co., 1979). pp. 17.
- [2] S. DeLuca, D. Heinegord, V. C. Hascall, J. H. Kimura, and A. I. Caplan, J. Biol. Chem. 252 (1977) 6600.

- [3] D. Heinegord, Biochim. Biophys. Acta. 285 (1972) 181.
- [4] A. Patel, S. S. Stivala, B. Khorramian, S. P. Damle, and J. D. Gregory, to be published.
- [5] E. C. Uberbacher, V. Ramakrishnan, D. E. Olins, and G. J. Bunick, Biochemistry. 22 (1983) 4916.
- [6] B. Jacrot and G. Zaccai, Polymers. 20 (1981) 2413.
- [7] M. Satre and G. Zaccai, FEBS Letters. 102, 2 (1979) 244.
- [8] S. S. Stivala, M. Herbet, O. Kratky, and I. Pilz, Arch. Biochem. Biophys. 127 (1968) 795.
- [9] V. C. Hascall and S. W. Sajdera, J. Biol. Chem. 244 (1969) 2384.
- [10] H. Reihanian, A. M. Jamieson, L. H. Tang, and L. Rosenberg, Biopolymer. 18 (1979) 1727.
- [11] S. G. Pasternack, A. Veis, and M. Bren, J. Biol. Chem. 249 (1974) 2206.
- [12] V. C. Hascall, S. W. Sajdera, J. Biol. Chem. 245 (1970) 4920.
- [13] H. Hayashi, P. J. Flory, and G. D. Wignall, Macromolecules. 16 (1983) 1328.
- [14] S. J. Perkins, A. Miller, T. E. Hardingham, and H. Muir, J. Mol. Biol. 150 (1981) 69.
- [15] R. Koyama, Macromolecules. 17 (1984) 1594.

Running Title: Neutron Scattering from Proteoglycan

- Key Words : Neutron Scattering, Proteoglycan,
Macromolecular parameters, Polyelectrolyte

TABLE 1
Physico-Chemical Parameters of
Bovine Nasal Cartilage PGS at 25°C

Method	Medium	$M \times 10^{-6}$	$R_g, \text{\AA}$	$R_q, \text{\AA}$	$a, \text{\AA}$	Ref.
SANS	0.15N LiCl	3.9	745	34.6	35.2	4
SAXS	0.15N LiCl	2.25	493	25.0	29.9	
Sedimentation equilibrium	4.0M GHC1 ^a	2.5				9
Light Scattering	4.0M GHC1	2.3	570			11

^a = Guanidine hydrochloride

Figure Captions:

- Fig. 1. Guinier plot of the PGS in 0.15N LiCl-D₂O.
- Fig. 2. Plot of IQ^2 versus Q for persistence length of PGS in 0.15N LiCl-D₂O.
- Fig. 3. Scattering curve of PGS in D₂O.

Fig-1

Fig. 2

Fig. 3