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Hydration of Potassium Hyaluronate

Yasuko SUZUKI and Hisashi UEDAIRA

Research Institute for Polymers and Textiles, 4 Sawatari, Kanagawa-ku, Yokohama

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Hyaluronic acid is one of the mucopolysaccarides, whose structure consists of a repeating disaccaride unit, D-glucronic acid and 2-acetamide-2-deoxy-D-glucose. It occurs in various animal tissues and fluids such as (bovine) vitreous humour, (human) umbilical cord and synovial fluid. One important function is to bind water in interstitial spaces of tissue and have a definite resistance to compression.[1,2] Jacobson and Laurent[3] and Laurent[4] carried out studies of streaming dielectric property and X-ray diffraction on aqueous hyaluronic acid solution, but no positive information on the interaction between hyaluronic acid and water molecules was obtained.

We present here the ultrasonic velocity data and partial specific volume for the aqueous potassium hyaluronate solutions. The adiabatic compressibility of the solution is calculated and the hydration number of hyaluronate ion is estimated.

Experimental

Potassium hyaluronate used was obtained from bovine vitreous humour (Tokyo Kasei G. R.). It was stirred in water under a stream of nitrogen at 60°C for 2 hr, filtered by Milipore filter (pore size $1.2 \pm 0.3\ \mu$), lyophilized and then dissolved in deionized water.

Ultrasonic velocity of solution was measured by an ultrasonic interferometer, at 5 MHz in frequency. Density was determined by picnometer (10 cc) at (25±

1) J. H. Fessler, *Biochem. J.*, **76**, 124 (1960).
2) J. S. Brimacombe and J. M. Webber, "Mucopolysaccharides Chemical Structure, Distribution, Isolation," Elsevier Publ. Co., New York (1964), Chapter 3.
3) B. Jacobson and T. C. Laurent, *J. Colloid Sci.*, **9**, 36 (1954).
4) T. C. Laurent, *Ark. Kemi*, **11**, 503 (1957).

0.01) and (30±0.01)°C. Adiabatic compressibility was calculated from the data of ultrasonic velocity and density of the solutions. Concentrations of the solutions were determined by gravimetric method and by measurement of the refractive increment using a Zeiss interferometer. The molecular weight of potassium hyaluronate was determined by a Shimazu light scattering instrument at 23°C, where the same sample solution as above was subjected to ultracentrifuging at 2.4×10^4 g and 10°C for one hour and filtered with a Corning ultrafine glass filter for optical clearance.

Results and Discussion

The weight average molecular weight of potassium hyaluronate, M_w, is obtained as 2.71×10^5 from the Zimm plot of light scattering data. The partial specific volume of potassium hyaluronate at infinite dilution, $\bar{v}_{20}$, was obtained from solution density. The values of $\bar{v}_{20}$ at 25 and 30°C are given in the table.

TABLE 1. THE AMOUNT OF HYDRATION OF POTASSIUM HYALURONATE

t (°C)	$M\times10^{-5}$	$\bar{v}_{20}$ (cc)	$\bar{\kappa}_{20}\times10^{12}$ (cm²/dyn)	n_{h1} (mol/base mol)	n_{h2} (mol/base mol)
25	2.71	0.51	−35.0	9.22 (6.7)*	18.4 (15.9)*
30		0.55	−66.5	19.4 (16.2)*	30.8 (27.6)*

* hydration number of hyaluronate ion.

The relation between adiabatic compressibility κ, ultrasonic velocity c and density of solution ρ is given by

$$\kappa = 1/\rho c^2 \quad (1)$$

The amount of hydration is obtained from the equations

$$\omega = (n_h v_1) = \frac{\bar{\kappa}_{20} - \kappa_2}{\kappa_h - \kappa_1}\bar{v}_{20} \quad (2)$$

$$\bar{\kappa}_{20} = -\frac{1}{\bar{v}_{20}}\left(\frac{\partial \bar{v}_2}{\partial \rho}\right)_0 = -\frac{1}{\bar{v}_{20}}\left(-\bar{v}_{20} - \frac{1}{\kappa_1}\frac{d\kappa}{dx}\right) \quad (3)$$

where ω and n_h are the volume and number of water molecule bounded to one gram of solute, respectively.[5] v_1 and v_2 are the specific volumes of solvent and solute, respectively, and $\bar{v}_{20}$ the partial specific volume of solute at infinite dilution. κ_1, κ_2 and κ_h are the adiabatic compressibilities of solvent, solute and hydrated water respectively and $\bar{\kappa}_{20}$ the partial specific compressibility of solute at infinite dilution. The relations between the compressibilities and the concentrations of solutions at 25 and 30°C are given in Fig. 1.

5) H. Nomura and Y. Miyahara, *J. Appl. Polym. Sci.*, **8**, 1643 (1964).

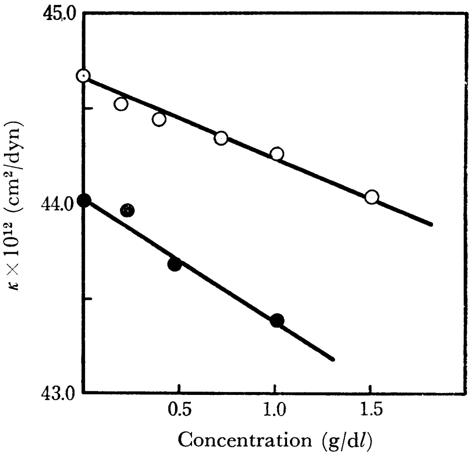

Fig. 1. The relations between adiabatic compressibilities of potassium hyaluraonate and its concentrations at 25 (open circle) and 30°C (closed circle).

In the calculation of the hydration number from Eq. (3), the following two limiting values of hydration number are obtained.

(1) When it is assumed that the solute and the water molecules very near the solute have zero compressibility (*viz.* $\kappa_h=0$ and $\kappa_2=0$), the lower limiting value of hydration number (n_{h_1}) is obtained. The values of n_{h_1} at 25 and 30°C are given in the forth column of the table.

(2) When it is assumed that the solute and bound water molecules participate in compression, the upper limiting value of hydration number (n_{h_2}) is obtained. The value of compressibility of potassium hyaluronate is not available, and so we assume its value to be the same as that of dextrin, 12×10^{-12} cm²/dyn.[6] Following Shiio, Ogawa and Yoshihashi,[6] we assume that the compressibility of hydrated water is the same as that of ice I, 1.2×10^{-11} cm²/dyn.[7] The values of n_{h_2} at 25 and 30°C are given in the fifth column of the table.

The hydration numbers of potassium ion determined from ultrasonic velocity are 2.5 and 3.2 mol/mol ion at 25 and 30°C, respectively.[8,9] It follows that the values of n_{h_1} of hyaluronate ion at 25 and 30°C are 6.7 and 16.2 mol/base mol (or 0.32 and 0.77 cc/g), respectively, and the values of n_{h_2} at 25 and 30°C are 15.9 and 27.6 mol/base mol (or 0.70 and 1.21 cc/g), respectively.

The values of n_{h_2} for glucose, sucrose, maltose and raffinose at 25°C are 0.35, 0.20, 0.22 and 0.22 cc/

6) H. Shiio, T. Ogawa and H. Yoshihashi, *J. Amer. Chem. Soc.*, **77**, 4980 (1955).
7) "International Critical Tables," Vol. 3, McGraw-Hill Book Co., New York (1928), p. 50.
8) T. Yasunaga and T. Sasaki, *Nippon Kagaku Zasshi*, **72**, 87, 89, 366 (1951).
9) *Z. Phys. Chem. Neue Folge*, **47**, 24 (1965).

g,[10] respectively. Thus, the hyaluronate ion is hydrated more strongly than sugars.

The amount of hydration of hyaluronate ion increases with increasing temperature, as in the case of polyvinyl alcohol.[11] One of the authors (H.U.) together with Uedaira[12] showed that sugars oriented the water molecules around them, and that the degree of hydration of sugars was increased by the addition of structure breaking solute. According to Samoilov's theory on salting-out,[13] the break of water structure increases the degree of hydration of the structure making solute. With increasing temperature, the water structure is destroyed by heat motion of molecule. Thus, the water molecules around hyaluronate ion are oriented more easily, and the amount of hydration increases. It is suggested that the hyaluronate ion strongly affects the hydration of ions in organism (for example, Ca^{2+}, Na^{+}, K^{+} ions).

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10) H. Shiio, *J. Amer. Chem. Soc.*, **80**, 70 (1958).

11) H. Nomura and Y. Miyahara, *Nippon Kagaku Zasshi*, **88**, 504 (1967).

12) H. Uedaira and H. Uedaira, Meeting of Colloid and Surface Chemistry (1968); *J. Phys. Chem.*, **74**, 1931 (1970).

13) O. Ya. Samoilov, "Sostoyanie i Rol' Vodny v Biologicheskikh Ob'ektakh," Nauka, Moskva (1967).