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Enthalpies of Dilution of Aqueous Sodium Chondroitin 4-Sulfate and 6-Sulfate

Hideya TSUGE,* Masakatsu YONESE, and Hiroshi KISHIMOTO

Faculty of Pharmaceutical Sciences, Nagoya City University, Tanabe-dori, Mizuho-ku, Nagoya 467

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Enthalpies of dilution of aqueous sodium chondroitin 4- and 6-sulfate were measured by a microcalorimeter from the various initial concentrations m=0.16—0.07 monomole kg^{-1} to the final concentration m'=3.85×10^{-3} monomole kg^{-1} at various temperatures. The concentration and temperature dependencies of measured enthalpies well agreed quantitatively with the theoretical ones calculated by Manning's limiting law, when the distance between the neighboring charges of polyion, b, was assumed to be 4.8 Å, which was obtained from X-ray diffraction measurement.

Chondroitin 4- and 6-sulfate, whose repeating units are composed of *N*-acetyl-D-galactosamine 4- and 6-sulfate together with D-glucuronic acid, respectively, are known to be the important components of mammalian connective tissues and have been investigated from various points of view. In our laboratory, the counter ion binding of chondroitin sulfates was confirmed to exist at low concentrations from the measurements of two thermodynamic properties, molal osmotic coefficients[1] and partial molar volumes.[2] Also, the electrolyte permeability of the gelatin–chondroitin sulfate mixed membrane as a model of connective tissue has been investigated.[3] In this work, the enthalpies of dilution ($\Delta_{dil}H$) of aqueous chondroitin 4- and 6-sulfate were measured by a twin-type microcalorimeter, and the behaviors of chondroitin sulfate at moderate concentrations were discussed by applying Manning's limiting law[4,5] to our results.

Experimental

Materials. Chondroitin 4-sulfate (ChS-A), extracted from whale cartilage as sodium salt, was obtained from Seikagaku Kogyo Co., Ltd. (Tokyo, Japan). It has an average molecular weight of 2.0×10^4 as determined by viscosity measurement.[6,7]

Two kinds of chondroitin 6-sulfate (ChS-C), extracted from shark cartilage as sodium salts, were offered from Kaken Yakukako Co., Ltd. (Tokyo, Japan). Their molecular weights were determined to be 5.7×10^4 and 1.1×10^4, respectively. Distilled and deionized water was used for the preparation of aqueous ChS salt solutions.

Method.[8] The calorimeter used was a twin-type conduction microcalorimeter (CM 204 D1 of Rhesca. Co., Ltd.). A micro volt meter AM-1001 B of Ohkura Electric Co. was used as an output amplifier. Each solution cell (sample side or reference side) of the calorimeter consists of a reaction-vessel and an ampoule. The content of the ampoule can be mixed with that of the reaction-vessel by destroying the ampoule.

Sample Side: Aqueous solution of ChS salt (1—2 ml) in the ampoule. Water (30—50 ml) in the reaction-vessel.

Reference Side: The same amounts of water as the sample side in the ampoule and the reaction-vessel, respectively.

After a steady state had been reached (after about 20 h), ampoules of both sides were destroyed at the same time and the heats arised from mixing were measured. The heats correspond to the dilution enthalpies of ChS salts from the various initial concentrations m=0.16—0.07 monomole kg^{-1} to the final concentration m'=3.85×10^{-3} monomole kg^{-1}, where monomole means the molar amount of the repeating disaccharide unit of ChS. The heats divided by the amount of ChS in monomole give the dilution enthalpies per ChS monomole, $\Delta_{dil}H$.

Results

The exothermic quantities, *i.e.* $-\Delta_{dil}H$, of NaChS-A and NaChS-C at several temperatures were plotted against the initial concentrations m in Figs. 1 and 2, respectively. The value of $-\Delta_{dil}H$ increased monotonously with m.

Of NaChS-C samples having different molecular weights, the higher molecular weight sample showed the greater $-\Delta_{dil}H$ by about 400 J monomole^{-1} at different m and temperatures than the lower one.

Since the plots in Figs. 1 and 2 showed qualitatively the greater $-\Delta_{dil}H$ at the higher temperature, we plotted $-\Delta_{dil}H$ against temperature in Figs. 3 and 4 for the NaChS-A and NaChS-C, which were diluted from 0.1 monomole kg^{-1} to m'=3.85×10^{-3} monomole kg^{-1}, showing fairly linear relations.

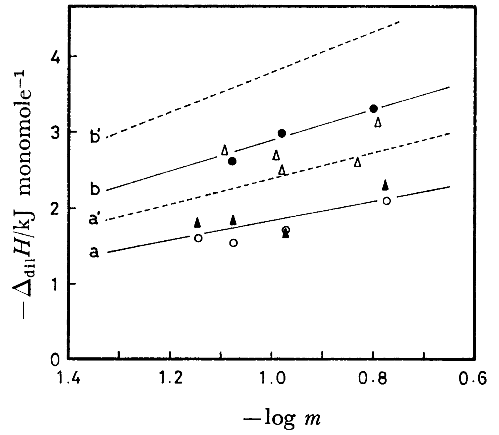

Fig. 1. Dilution enthalpies of NaChS-A *vs.* solute concentration

○: at 292.15 K, ▲: at 298.15 K, △: at 310.15 K, ●: at 323.15 K.

Theoretical values: solid lines in b=4.8 Å (a; at 292.15 K, b; at 323.15 K), broken lines in b=6.3 Å (a'; at 292.15 K, b'; at 323.15 K).

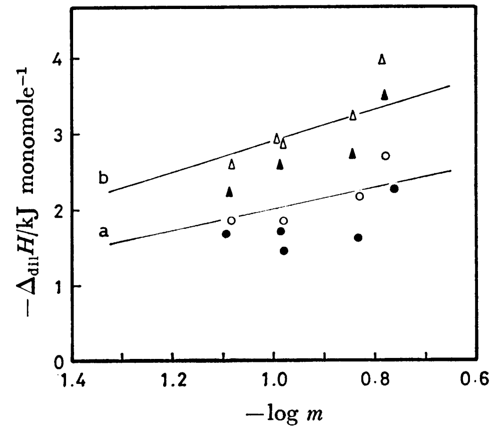

Fig. 2. Dilution enthalpies of NaChS-C *vs.* solute concentration
○: $M.W.=5.7\times10^4$ at 298.15 K, ●: $M.W.=1.1\times10^4$ at 298.15 K, △: $M.W.=5.7\times10^4$ at 323.15 K, ▲: $M.W.=1.1\times10^4$ at 323.15 K.
Theoretical values: a: $b=4.8$ Å at 298.15 K, b: $b=4.8$ Å at 323.15 K.

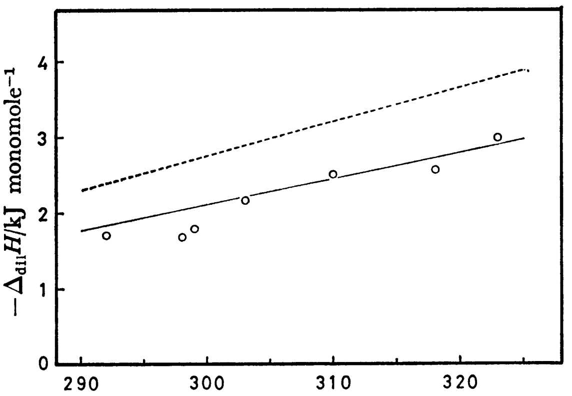

Fig. 3. Effect of temperature on the dilution enthalpies of NaChS-A from 0.1 monomole kg^{-1} to 3.85×10^{-3} monomole kg^{-1}. Solid line: $b=4.8$ Å, broken line: $b=6.3$ Å.

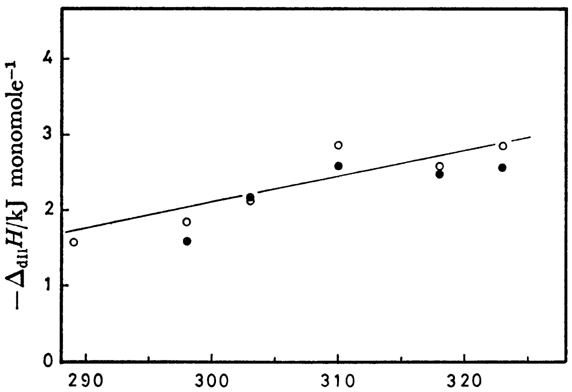

Fig. 4. Effect of temperature on the dilution enthalpies of NaChS-C from 0.1 monomole kg^{-1} to 3.85×10^{-3} monomole kg^{-1}. ○: $M.W.=5.7\times10^4$, ●: $M.W.=1.1\times10^4$.
Solid line: $b=4.8$ Å.

Discussion

To examine to what extent the enthalpies of dilution of aqueous NaChS solutions may be explained by the electrostatic interaction of polyion with counterions, $\Delta_{\text{dil}}H$ was divided as follows.[9,10]

$$\Delta_{\text{dil}}H = \Delta_{\text{dil}}H^{\circ} + \Delta_{\text{dil}}H^{\text{el}}, \qquad (1)$$

where $\Delta_{\text{dil}}H^{\circ}$ is the enthalpy of dilution of polyelectrolyte solution into a hypothetical reference state, in which all the ions are discharged and $\Delta_{\text{dil}}H^{\text{el}}$ is the contribution from the electrostatic interaction of polyion with counterion and can be evaluated by various electrostatic theories, *e.g.* Manning's limiting law.[4] Manning's theory replaces a polymer chain with infinitely long line charges and considers the two modes of interaction: Debye-Hückel "ion atmosphere" and condensation of counterions on the line charge. There, polyions are assumed to interact only negligibly with each other. Recently, this theory was applied to the thermodynamic properties of polyelectrolyte solutions, showing that this model is a good representation of the polyelectrolyte solution at low concentration.[11] T. Okubo *et al.* used successfully Manning's theory for the enthalpies of dilution of polyacrylate, carboxymethylcellulose, *etc.*

According to Manning, the electrostatic Helmholtz free energy F^{el} for this model[4] may be shown to be

$$F^{\text{el}}/VkT = -\xi n_{\text{e}} \ln \kappa, \qquad (2)$$

where V is total volume, k is Boltzmann constant, T is absolute temperature, ξ is charge density parameter, n_{e} is equivalent polyion concentration (charge/ml), and κ is Debye screening parameter. The value of κ is given by Eq. 3 at a salt-free solution.

$$\kappa^2 = \frac{4\pi e^2}{\varepsilon kT} n_{\text{g}} z_{\text{g}}, \qquad (3)$$

where e is a protonic charge, ε is the dielectric constant of solvent and n_{g} and z_{g} are the concentration (ion/ml) and valence, respectively, of counterion. The value of ξ is given by

$$\xi = e^2/\varepsilon kTb, \qquad (4)$$

where b is the distance between the neighboring charges of a polyion. Different theoretical treatments are given for the case of $\xi \geq 1$ and $\xi \leq 1$. For the critical value of $\xi = 1$, b is equal to 7.1 Å in water at 298.15 K. The ξ value of ChS salt equals to the case of $\xi \geq 1$ as can be presumed by its structure. Starting from Eq. 2, $\Delta_{\text{dil}}H^{\text{el}}$ could be finally expressed as follows.[12]

$$\Delta_{\text{dil}}H^{\text{el}} = RT/\xi\left(1+\frac{T\text{d}\varepsilon}{\varepsilon\text{d}T}\right) \ln{(m/m')}, \quad \xi \geq 1 \qquad (5)$$

There, the choice of ξ value, which is the only adjustable parameter in Manning's theory, of ChS salts is a subject of discussion.

Two theoretical values of b were discussed with experimental $\Delta_{\text{dil}}H$ as follows. One was a structural value which obtained from X-ray diffraction measurement.[13,14] It was reported that ChS salts have eight-fold, three-fold and two-fold single stranded helical conformations. Their disaccharide lengths are 9.3—9.8 Å. The value of $b=4.8$ Å, which is a half of 9.6 Å for disaccharide lengths of three-fold helical molecule of ChS-A and ChS-C salts, gives the structural value. Another was the value of $b=6.3$ Å. We previously measured the molal osmotic coefficients of ChS-A and ChS-C salts by vapor pressure osmometry. If the ξ value was considered as an adjustable parameter, experimental coefficients at infinite dilution ϕ_0 well agreed with the theoretical coefficients calculated from $\phi=(2z_g\xi)^{-1}$ using $b=6.3$ Å.[1]

Theoretical $-\Delta_{dil}H^{el}$, calculated from Eqs. 4 and 5, are shown by solid lines with $b=4.8$ Å in Figs. 1, 2, 3, and 4 and by broken line with $b=6.3$ Å, for the case of NaChS-A only, in Figs. 1 and 3, respectively. It was confirmed that the theoretical enthalpic values for $b=4.8$ Å well agreed quantitatively with experimental values. In the above-mentioned treatment, the non-electrostatic enthalpies of dilution had been implicitly assumed to be negligible. However, it can be safely stated that the assumption is at least relatively a self-consistent one over the experimental range of temperature and concentration.

As the conclusion, at least tentative, ChS salt in aqueous solution may behave in such a way that the polyion of ChS has a stretched conformation with disaccharide lengths of *ca.* 12.6 Å at infinite dilution and a conformation nearly like solid state at moderate concentration.

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