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The Gibbs Energies, Enthalpies, and Entropies of the Dilution of Aqueous Sodium Chondroitin-4-sulfate and -6-sulfate

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The osmotic coefficients of a solvent, ϕ, in aqueous solutions of sodium chondroitin-4-sulfate (NaChS-A) with a relatively low molecular weight and -6-sulfate (NaChS-C) with various molecular weight were measured by means of vapor-pressure osmometry. The molar Gibbs energes of dilution, $\Delta_{\rm dil}G$, were determined from the thermodynamic analysis of the ϕ values. The molar entropies of dilution, $\Delta_{\rm dil}S$, were calculated through the substitution of the molar dilution enthalpies, $\Delta_{\rm dil}H$, which had been measured previously, into $\Delta_{\rm dil}G$. These thermodynamic quantities of dilution were discussed by taking Manning's limiting law into consideration. The concentration and temperature dependencies of the observed $\Delta_{\rm dil}H$ agreed well quantitatively with those of the electrostatic $\Delta_{\rm dil}H$ calculated by Manning's limiting law, when the distance between the neighboring charges of the polyion, b, was assumed to be 4.8 Å, whereas the observed $\Delta_{\rm dil}G$ and $\Delta_{\rm dil}S$ values did not agree quantitatively with the theoretical ones based on electrostatic interactions. From a comparison of the experimental $\Delta_{\rm dil}S$ values with the theoretical ones, the non-electrostatic dilution entropy was evaluated and discussed.

Chondroitin-4-sulfate and -6-sulfate, whose repeating units are composed of *N*-acetyl-D-galactosamine-4- and -6-sulfates, together with D-glucuronic acids, respectively, are known to be important components of mammalian connective tissues and are expected to interact specifically with the ions in biological environments according to their polyelectrolyte nature. To elucidate their behavior as biopolyelectrolytes, we have previously investigated their physicochemical properties in simple aqueous solutions with various counter-cations, such as osmotic coefficients,[1] partial molar volumes,[2] and the enthalpies of dilution.[3]

This paper concerns the Gibbs energies, enthalpies, and entropies of the dilution of sodium chondroitin-4-sulfate (NaChS-A) with a relatively low molecular weight and -6-sulfate (NaChS-C) with various molecular weights. The Gibbs energies were determined from the thermodynamic analysis of the osmotic coefficients measured by vapor-pressure osmometry. Since the enthalpies had been measured in a preceding work,[3] the entropies of dilution were calculated through the substitution of the enthalpies into the Gibbs energies. These thermodynamic quantities of dilution were discussed by taking Manning's limiting law[4] into consideration.

Experimental

Materials. Sodium chondroitin-4-sulfate (NaChS-A) was obtained commercially from the Seikagaku Kogyo Co., Ltd. (Tokyo, Japan); its average molecular weight $\overline{M}$, was 2.0×10^4. Four kinds of sodium chondroitin-6-sulfate (NaChS-C) with different molecular weights were used, two of them being commercial ones from the Seikagaku Kogyo Co., Ltd. ($\overline{M}=6.5\times10^4$ and 7.3×10^4), and the others having been offered by the Kaken Yakukako Co., Ltd. (Tokyo. Japan) ($\overline{M}=5.7\times10^4$ and 1.1×10^4). Their molecular weights were determined by viscosity measurements.[5,6] Distilled and deionized water was used for the preparation of aqueous ChS salt solutions.

Osmotic Coefficient. The osmotic coefficients were measured by means of vapor-pressure osmometry using an Hitachi-Perkin-Elmer Model 115 Molecular Weight Apparatus.[1] Aqueous sodium chloride solutions were used as reference solutions, and their osmotic coefficients, ϕ', were obtained from the literature.[7] When the solvent vapor pressure of a reference electrolyte solution is equal to that of a polyelectrolyte solution, the osmotic coefficient of the polyelectrolyte, ϕ, is given by

$$\phi = \frac{\nu' m' \phi'}{\nu_{\rm p} m_{\rm p}}, \tag{1}$$

where ν' and $\nu_{\rm p}$ are the total numbers of cations and anions produced by the dissociation of NaCl and the polyelectrolyte, and where m' and $m_{\rm p}$ are the molalities of NaCl and the polyelectrolyte respectively. The factors of the denominator, $\nu_{\rm p}$ and $m_{\rm p}$, can be written as follows;

$$\nu_{\rm p} = Z/Z_{\rm g} + 1 \tag{2}$$

and;

$$m_{\rm p} = 2m/Z, \tag{3}$$

where Z, $Z_{\rm g}$, and m are the stoichiometric charge number per polymer, the charge number of a counter ion, and the molality of the polymer on a repeating unit basis (mol kg^{-1}) respectively. Then,

$$\phi = \frac{\nu' m' \phi'}{(Z/Z_{\rm g}+1)(2m/Z)}. \tag{4}$$

Results and Discussion

The Osmotic Coefficients of NaChS Salts. Figures 1, 2, and 3 show the linear relationships between the ϕ values and polymer concentrations for various ChS samples at 298.15, 310.15, and 323.15 K respectively. They also show that the ϕ values depend on the polymer molecular weights; that is, higher-molecular-weight samples have smaller ϕ values. However, ϕ seems to approach a definite value with the increase in the molecular weight.

The Calculation of the Gibbs Energies, Enthalpies, and Entropies of Dilution. The molar Gibbs energy of dilution per repeating unit containing two ionized groups, $\Delta_{\rm dil}G$, from the initial concentration, $m_{\rm i}$, to the final concentration $m_{\rm f}$, ($m_{\rm i} \gg m_{\rm f}$), can be written as follows;

$$\Delta_{\rm dil}G = 2(1/Z+1/Z_{\rm g})RT\ln(a_{\pm,{\rm f}}/a_{\pm,{\rm i}}) \tag{5-a}$$

$$= 2(1/Z+1/Z_{\rm g})RT\ln\left(\frac{2m_{\rm f}\gamma_{\pm,{\rm f}}/Z}{2m_{\rm i}\gamma_{\pm,{\rm i}}/Z}\right), \tag{5-b}$$

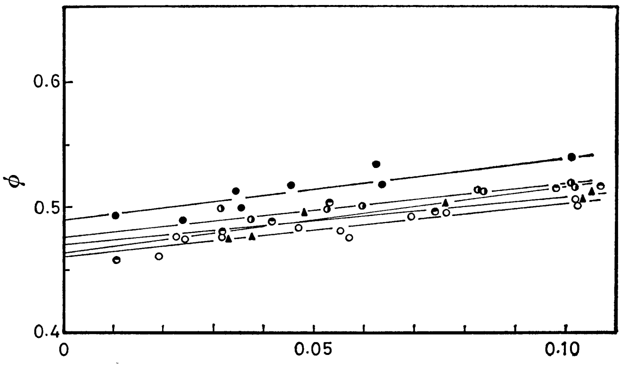

Fig. 1. Osmotic coefficients (ϕ) *vs.* concentration of NaChS-A and -C at 298.15 K.
◐: NaChS-A ($\overline{M}=2.0\times10^4$), ●: NaChS-C ($\overline{M}=1.1\times10^4$), ○: NaChS-C ($\overline{M}=5.7\times10^4$), ▲: NaChS-C ($\overline{M}=6.5\times10^4$), ◓: NaChS-C ($\overline{M}=7.3\times10^4$).

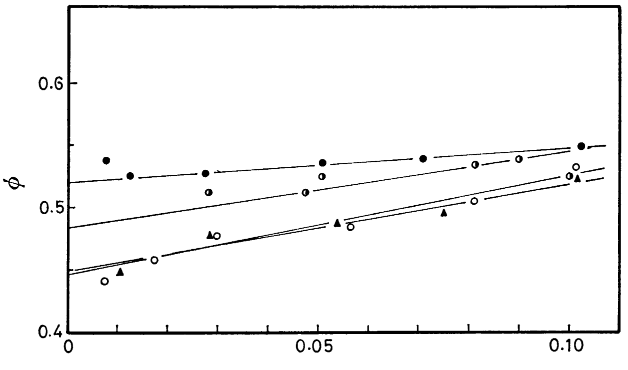

Fig. 2. Osmotic coefficients (ϕ) *vs.* concentration of NaChS-A and -C at 310.15 K.
Symbols are the same as in Fig. 1.

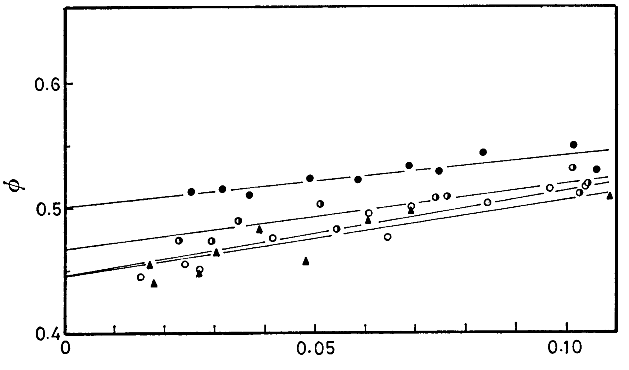

Fig. 3. Osmotic coefficients (ϕ) *vs.* concentration of NaChS-A and -C at 323.15 K.
Symbols are the same as in Fig. 1.

where $a_{\pm}$ and $\gamma_{\pm}$ are the mean activity and the mean activity coefficient of a polyelectrolyte respectively. To obtain $\Delta_{\text{dil}}G$ from the relation between m and ϕ, the mean activity coefficients in Eq. 5-b were calculated by means of the Gibbs-Duhem equation:

$$\ln(\gamma_{\pm,\text{f}}/\gamma_{\pm,\text{i}}) = (\phi_\text{f}-\phi_\text{i}) + 2\int_{m_\text{f}}^{m_\text{i}}\frac{1-\phi}{\sqrt{m}}\mathrm{d}\sqrt{m}\,. \tag{6}$$

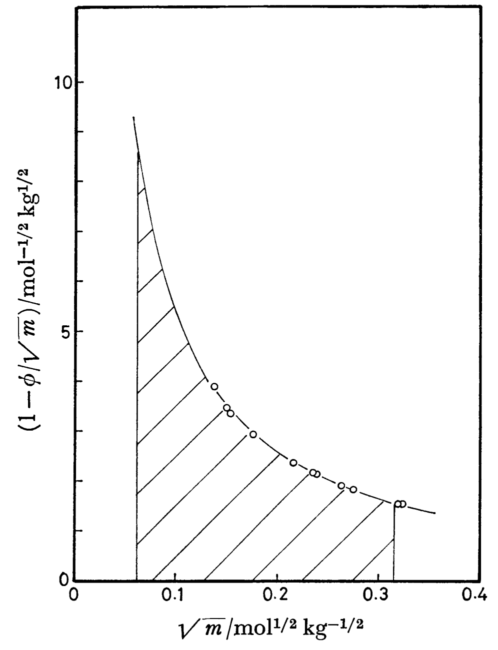

Fig. 4. Graphic integration of Eq. 3 at 310.15 K. Sample: NaChS-C ($\overline{M}=1.1\times10^4$).

In calculating $\int_{m_\text{f}}^{m_\text{i}}\frac{1-\phi}{\sqrt{m}}\mathrm{d}\sqrt{m}$ by graphic integration, the relations between ϕ values and m were assumed to be linear for the extrapolation of ϕ to the lower-m region. An example of the graph for integration is shown in Fig. 4.

When the experimental results of the molar dilution enthalpies per repeating unit, $\Delta_{\text{dil}}H$, in the previous paper[3] are used with the $\Delta_{\text{dil}}G$ thus obtained, the molar entropies of dilution, $\Delta_{\text{dil}}S$, can be obtained from Eq. 7:

$$\Delta_{\text{dil}}S = (-\Delta_{\text{dil}}G+\Delta_{\text{dil}}H)/T. \tag{7}$$

Table 1 shows $\Delta_{\text{dil}}G$, $\Delta_{\text{dil}}H$, and $\Delta_{\text{dil}}S$ values from $m_\text{i}=0.10$ and $0.08\ \text{mol kg}^{-1}$ to $m_\text{f}=3.85\times10^{-3}\ \text{mol kg}^{-1}$. These quantities can be divided into nonelectrostatic and electrostatic terms, as expressed by Eq. 8.[3,8] The former is the contribution from the dilution of a polyelectrolyte solution in a hypothetical reference state in which all the ions are discharged and is indicated by the superscript "o," while the latter is the contribution from the electrostatic interaction of polyions with counterions and is indicated by the superscript "el."

$$\Delta_{\text{dil}}G = \Delta_{\text{dil}}G^{\text{el}} + \Delta_{\text{dil}}G^{\text{o}} \tag{8-a}$$

$$\Delta_{\text{dil}}H = \Delta_{\text{dil}}H^{\text{el}} + \Delta_{\text{dil}}H^{\text{o}}, \tag{8-b}$$

and

$$\Delta_{\text{dil}}S = \Delta_{\text{dil}}S^{\text{el}} + \Delta_{\text{dil}}S^{\text{o}}. \tag{8-c}$$

According to Manning's limiting law, $\Delta_{\text{dil}}G^{\text{el}}$, $\Delta_{\text{dil}}H^{\text{el}}$, and $\Delta_{\text{dil}}S^{\text{el}}$ per mol of the repeating disaccharide unit can be evaluated as follows in the case of $\xi\geq1.0$:[3]

$$\Delta_{\text{dil}}G^{\text{el}} = -(RT/\xi)\ln(m_\text{i}/m_\text{f}) \tag{9-a}$$

$$\Delta_{\text{dil}}H^{\text{el}} = (RT/\xi)\left(1+\frac{T\mathrm{d}\varepsilon}{\varepsilon\mathrm{d}T}\right)\ln(m_\text{i}/m_\text{f}), \tag{9-b}$$

and

TABLE 1. THE VALUES OF $\Delta_{\rm dil}G$, $\Delta_{\rm dil}H$, AND $\Delta_{\rm dil}S$ FOR NaChS-A AND -C

	Sample	$\overline{M}$	298.15 K	310.15 K	323.15 K
$\Delta_{\rm dil}G$/kJ mol^{-1}	NaChS-A	2.0×10^4	−8.25	−8.80	−8.86
			−7.59	−8.09	−8.14
	NaChS-C	1.1×10^4	−8.65	−9.25	−9.37
			−7.97	−8.56	−8.65
		5.7×10^4	−7.69	−8.30	−8.59
			−7.09	−7.60	−7.87
		6.5×10^4	−8.06	−8.63	−8.67
			−7.48	−7.88	−7.96
		7.3×10^4	−7.97	—	—
			−7.32	—	—
$\Delta_{\rm dil}H$/kJ mol^{-1}	NaChS-A	2.0×10^4	−1.69	−2.59	−3.00
			−1.79	−2.76	−2.62
	NaChS-C	1.1×10^4	−1.58	−2.59	−2.57
			−1.83	—	−2.23
		5.7×10^4	−1.84	−2.86	−2.87
			−1.85	—	−2.57
$\Delta_{\rm dil}S$/J mol^{-1} K^{-1}	NaChS-A	2.0×10^4	22.0	20.0	18.1
			19.5	17.2	17.1
	NaChS-C	1.1×10^4	23.7	21.5	21.0
			20.6	—	19.9
		5.7×10^4	19.6	17.5	17.7
			17.6	—	16.4

The upper values for each sample: $m_i=0.10$ mol kg^{-1}. The lower values for each sample: $m_i=0.08$ mol kg^{-1}. $m_f=3.85\times10^{-3}$ mol kg^{-1}.

$$\Delta_{\rm dil}S^{\rm el} = (2R/\xi)\left(1+\frac{T{\rm d}\varepsilon}{2\varepsilon{\rm d}T}\right)\ln\,(m_i/m_f), \qquad \text{(9-c)}$$

where R is the gas constant, T is the absolute temperature, ε is the permittivity of the solvent (water), and ξ is the charge density parameter, which is defined by:

$$\xi = e^2/4\pi\varepsilon kTb, \qquad \text{(10)}$$

where e is the elementary charge, k is the Boltzmann constant, and b is the distance between the neighboring charges of a polyion.

In a preceding paper,[3] treating the dilution enthalpy of NaChS, we assumed implicitly that the non-electrostatic enthalpy of dilution, $\Delta_{\rm dil}H^\circ$, is negligible; then, so as to fit the theoretical values obtained from Eq. 9-b with the experimental $\Delta_{\rm dil}H$ values over as wide range of temperatures and concentrations as possible, we selected b=4.8 Å, which was in agreement with the value estimated from the X-ray diffraction measurement of NaChS film. The fit between the experimental $\Delta_{\rm dil}H$ and theoretical $\Delta_{\rm dil}H^{\rm el}$ values at 298.15 K is illustrated in Fig. 5. In this paper, we continued to use 4.8 Å as b for the reason mentioned above and calculated the values of $\Delta_{\rm dil}G^{\rm el}$ and $\Delta_{\rm dil}S^{\rm el}$ from the initial concentration, *i.e.*, 0.10 and 0.08 mol kg^{-1}, to the final concentration of 3.85×10^{-3} mol kg^{-1} at various temperatures, using Eqs. 9-a and -c. The results are tabulated in Table 2, along with the $\Delta_{\rm dil}H^{\rm el}$ values calculated in the preceding paper.

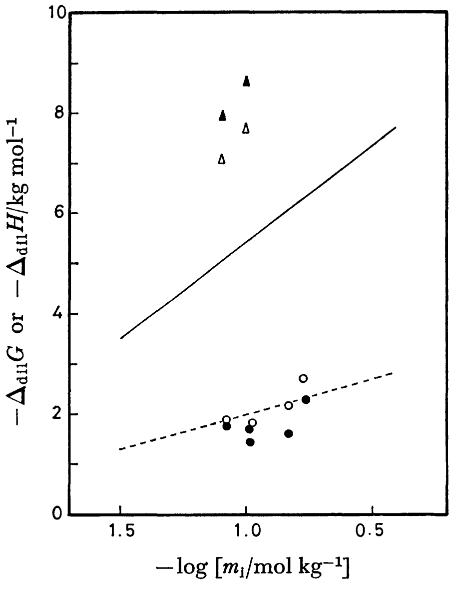

Fig. 5. $\Delta_{\rm dil}G$ and $\Delta_{\rm dil}H$ estimated from the Manning's limiting law for NaChS-C having two different molecular weights at 298.15 K.

△: Observed $\Delta_{\rm dil}G$ ($\overline{M}=5.7\times10^4$), ▲: observed $\Delta_{\rm dil}G$ ($\overline{M}=1.1\times10^4$), ○: observed $\Delta_{\rm dil}H$ ($\overline{M}=5.7\times10^4$), ●: observed $\Delta_{\rm dil}H$ ($\overline{M}=1.1\times10^4$), ——: $\Delta_{\rm dil}G^{\rm el}$ calculated from Eq. 9-a, ----: $\Delta_{\rm dil}H^{\rm el}$ calculated from Eq. 9-b.

The Evaluation of Non-electrostatic Terms. By substituting the above-mentioned electrostatic quantities, which were evaluated according to Manning's law, and the experimental over-all quantities of dilution into Eqs. 8-a, -b, and -c, we obtained the non-electrostatic contributions of dilution, $\Delta_{\mathrm{dil}}G^{\circ}$ and $\Delta_{\mathrm{dil}}S^{\circ}$, shown in the upper and middle sections of Table 3. Also, the values of $\Delta_{\mathrm{dil}}S^{\circ}$ can be calculated from $-\Delta_{\mathrm{dil}}G^{\circ}/T$ when $\Delta_{\mathrm{dil}}H^{\circ}$ is considered to be zero. The values thus calculated, as tabulated in the lower section of Table 3, reflect only the difference in experimental ϕ or $\Delta_{\mathrm{dil}}G$ values between samples of different molecular weights, while the values in the middle column involve additionally the differences in the experimental $\Delta_{\mathrm{dil}}H$ between different samples.

Next, we will examine the effect of polymer-chain

TABLE 2. THE VALUES OF $\Delta_{\mathrm{dil}}G^{\mathrm{el}}$, $\Delta_{\mathrm{dil}}H^{\mathrm{el}}$, AND $\Delta_{\mathrm{dil}}S^{\mathrm{el}}$, AS CALCULATED FROM MANNING'S LIMITING LAW

	298.15 K	310.15 K	323.15 K
$\Delta_{\mathrm{dil}}G^{\mathrm{el}}$/kJ mol^{-1}	−5.43	−5.60	−5.68
	−5.06	−5.18	−5.29
$\Delta_{\mathrm{dil}}H^{\mathrm{el}}$/kJ mol^{-1}	−2.02	−2.44	−2.90
	−1.90	−2.27	−2.70
$\Delta_{\mathrm{dil}}S^{\mathrm{el}}$/J mol^{-1} K^{-1}	11.4	10.1	8.6
	10.6	9.4	8.0

The distance between the neighboring charges of the polyion, b, is 4.8 Å. The upper values: $m_i=0.10$ mol kg^{-1}. The lower values: $m_i=0.08$ mol kg^{-1}. $m_f=3.85\times10^{-3}$ mol kg^{-1}.

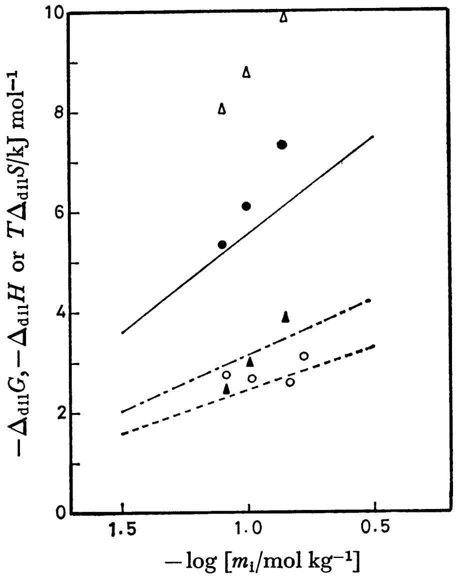

Fig. 6. Thermodynamic quantities of dilution of NaChS-A at 310.15 K.

△: Observed $\Delta_{\mathrm{dil}}G$, ○: observed $\Delta_{\mathrm{dil}}H$, ●: $T\Delta_{\mathrm{dil}}S$ calculated from Eq. 7, ▲: $T\Delta_{\mathrm{dil}}S^{\circ}$ calculated from Eqs. 7,8-c, and 9-c, ——: $\Delta_{\mathrm{dil}}G^{\mathrm{el}}$ calculated from Eq. 9-a, ----: $\Delta_{\mathrm{dil}}H^{\mathrm{el}}$ calculated from Eq. 9-b, —·—: $T\Delta_{\mathrm{dil}}S^{\mathrm{el}}$ calculated from Eq. 9-c.

TABLE 3. THE VALUES OF $\Delta_{\mathrm{dil}}G^{\circ}$ AND $\Delta_{\mathrm{dil}}S^{\circ}$ FOR NaChS-A and -C

	Sample	$\overline{M}$	298.15 K	310.15 K	323.15 K
$\Delta_{\mathrm{dil}}G^{\circ}$/kJ mol^{-1}	NaChS-A	2.0×10^4	−2.82	−3.20	−3.18
			−2.53	−2.91	−2.84
	NaChS-C	1.1×10^4	−3.22	−3.69	−3.69
			−2.91	−3.38	−3.36
		5.7×10^4	−2.26	−2.74	−2.91
			−2.03	−2.42	−2.58
		6.5×10^4	−2.63	−3.07	−2.98
			−2.42	−2.71	−2.67
		7.3×10^4	−2.54	—	—
			−2.27	—	—
$\Delta_{\mathrm{dil}}S^{\circ}$/J mol^{-1} K^{-1} a)	NaChS-A	2.0×10^4	10.6	9.9	9.5
			8.9	7.8	9.1
	NaChS-C	1.1×10^4	12.3	11.4	12.4
			10.0	—	11.9
		5.7×10^4	8.2	7.4	9.1
			7.0	—	8.4
$\Delta_{\mathrm{dil}}S^{\circ}$/J mol^{-1} K^{-1} b)	NaChS-A	2.0×10^4	9.5	10.4	9.8
			8.5	9.4	8.8
	NaChS-C	1.1×10^4	10.8	11.9	11.4
			9.8	10.9	10.4
		5.7×10^4	7.6	8.8	9.0
			6.8	7.8	8.0
		6.5×10^4	8.8	9.9	9.2
			8.1	8.7	8.3
		7.3×10^4	8.5	—	—
			7.6	—	—

The upper values for each sample: $m_i=0.10$ mol kg^{-1}. The lower values for each sample: $m_i=0.08$ mol kg^{-1}. $m_f=3.85\times10^{-3}$ mol kg^{-1}. a) Calculated from Eqs. 7 and 8-c. b) Calculated by assuming $\Delta_{\mathrm{dil}}H=\Delta_{\mathrm{dil}}H^{\mathrm{el}}$ or $\Delta_{\mathrm{dil}}S^{\circ}=-\Delta_{\mathrm{dil}}G^{\circ}/T$.

length on these thermodynamic quantities. As may be seen in Table 1, the polymers with lower molecular weights have larger values of $-\Delta_{\mathrm{dil}}G$ and $\Delta_{\mathrm{dil}}S$ than the higher-molecular-weight polymers. As compared with the differences in $-\Delta_{\mathrm{dil}}G$ and $\Delta_{\mathrm{dil}}S$ between samples of different molecular weights seems to be negligible. Therefore, the magnitudes of electrostatic $\Delta_{\mathrm{dil}}H^{\mathrm{el}}$, $\Delta_{\mathrm{dil}}G^{\mathrm{el}}$, and $\Delta_{\mathrm{dil}}S^{\mathrm{el}}$ can be considered to be approximately invariant with the change in the chain length of the polymer. This situation explains the agreements between the corresponding values of $\Delta_{\mathrm{dil}}S^{\circ}$ in the middle and lower sections of Table 3. After all, the effects of the chain length on various thermodynamic quantities can be concentrated in $\Delta_{\mathrm{dil}}S^{\circ}$. The discussion of $\Delta_{\mathrm{dil}}G^{\circ}$ can, then, be reduced to that of $\Delta_{\mathrm{dil}}S^{\circ}$. The values of $\Delta_{\mathrm{dil}}S^{\circ}$ increase significantly with the decrease in the chain length in Table 3. We cannot explain it clearly in this stage, but can only suggest that the effect of the chain length on $\Delta_{\mathrm{dil}}S^{\circ}$ is attributable to the problem of the conformation of ChS. With respect to the value of $\Delta_{\mathrm{dil}}S^{\circ}$ of a sufficiently long chain, the discussion is very important because of the comparatively large value of $\Delta_{\mathrm{dil}}S^{\circ}$ in $\Delta_{\mathrm{dil}}G^{\circ}$. However, in order to elucidate the nature of $\Delta_{\mathrm{dil}}S^{\circ}$ more clearly, the structural study of ChS in solution, including that of the solvent, is considered to be necessary.

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