the many different factors that can influence the transformation temperature–concentration relation. For a three-component system $\Delta\alpha_{2,\mathrm{p}}$, $\Delta\bar{H}$ and a_2 need to be independently known to be able to analyze quantitatively the results. When more species are present not only do additional preferential interactions with the polymer need to be taken into account but the interaction of the monomeric species with each other can also play an influential role. It is clear from eq 3 that, depending on the sign and magnitude of these interaction parameters, a wide spectrum in temperature–composition relations can be accommodated which could include monotonic increases, monotonic decreases, and the existence of extremals. Therefore, it is not unexpected that a supernatant solution composed of ATP and salts displays a complex and unusual behavior. An explanation of the observations that for a supernatant solution containing Mg^{2+} and ATP there is a minima in the transition temperature as a function of salt concentration while the Ca^{2+}–ATP systems behave quite differently must be sought in the nature of the interaction coefficients. It is premature to attempt a quantitative analysis of these data without this information. However, it is important to note in this connection that both Ca^{2+} and Mg^{2+} form complexes with ATP and the equilibrium constants are approximately the same for both species.[28, 29] On the other hand Hammes and Levison[30] have reported that the forward rate constant for the complexing reaction is at least a hundredfold greater for Ca^{2+} than for Mg^{2+}. There are, therefore, features in the aqueous chemistry of these ions which are different and which could reflect themselves in differences in the activity coefficient and interaction coefficients that are involved.

(28) R. A. Alberty, *J. Biol. Chem.*, **243**, 1337 (1968).

(29) M. M. Taqui Khan and A. E. Martell, *J. Phys. Chem.*, **66**, 10 (1962).

(30) G. G. Hammes and S. A. Levison, *Biochemistry*, **3**, 1504 (1964).

Studies on Aqueous Solutions of Sodium Poly-L-glutamates. Determinations of Mean Activity Coefficient, Osmotic Coefficient, Transference Number, and Partial Molal Volume

Norio Ise and Tsuneo Okubo

Department of Polymer Chemistry, Kyoto University, Kyoto, Japan.

Received February 3, 1969

ABSTRACT: The mean activity coefficient of sodium poly-L-glutamate, NaPGA, in the two-component system water–NaPGA was investigated by the isopiestic vapor pressure and/or emf measurements. The transference and related measurements were also carried out for the determination of the net valency and the transference number of PGA ions. The degrees of neutralization of PGA with NaOH were 0.3, 0.6, and 1.0, and the solution temperature studied was between 25 and 50°. The fraction of free gegenions and the transference number of PGA ions were concentration insensitive, and dependent on the degree of neutralization and solution temperature, being 0.6∼0.95 and 0.2∼0.4, respectively. The osmotic coefficient was between 0.5 and 1.3, varying with the degree of neutralization, polymer concentration, and temperature, and increased sharply by the helix–coil transition induced by rising temperature. The mean activity coefficient decreased with concentration at low concentrations; the decreasing tendency was more remarkable for the helical structure than for the coiled state. The two coefficients indicated the strong water structure-forming tendency of the PGA ions. The partial molal volume was further measured for various salts of PGA. The individual molal volume of PGA ions was calculated to be 85.7 ml/monomol; the electrostrictional hydration effect and the hydrophobic structural effect were −7.7 and −11.7 ml/monomol, respectively. From this, it is concluded that the monomer unit of PGA is hydrated electrostrictionally by three water molecules.

Poly-L-glutamic acid, a simple model compound for proteins, has been investigated by using various experimental techniques. Most of them are concerned with the change in physicochemical properties associated with the helix–coil transition.[1–5] However, the mean activity coefficient, which is one of the most fundamental thermodynamic quantities, has not been measured. The mean activity coefficients of several synthetic polyelectrolytes have been investigated by emf measurements of a concentration cell with transference, and isopiestic vapor pressure measurements by us and Dolar, *et al.*[6–14] In the present paper, we report the activity, transference, and density data for sodium salts of poly-L-glutamic acid, NaPGA, at various degrees of neutralization and temperatures.

Experimental Section

Materials. A Koch-Light Laboratories sample of a sodium salt of poly-L-glutamic acid (NaPGA) (mol wt

---

(1) P. Doty, A. Wada, J. T. Yang, and E. R. Blout, *J. Polym. Sci.*, **23**, 851 (1957).

(2) K. Imahori and J. Tanaka, *J. Mol. Biol.*, **1**, 359 (1959).

(3) A. Wada, *Mol. Phys.*, **3**, 409 (1960).

(4) E. R. Blout, I. Schnier, and N. S. Simmons, *J. Amer. Chem. Soc.*, **84**, 3193 (1962).

(5) G. Holtzworth and P. Doty, *ibid.*, **87**, 218 (1965).

(6) N. Ise and T. Okubo, *J. Phys. Chem.*, **69**, 4102 (1965).

(7) N. Ise and T. Okubo, *ibid.*, **70**, 1930 (1966).

(8) N. Ise and T. Okubo, *ibid.*, **70**, 2400 (1966).

(9) N. Ise and T. Okubo, *ibid.*, **71**, 1287 (1967).

(10) N. Ise and T. Okubo, *ibid.*, **71**, 1886 (1967).

(11) T. Okubo, N. Ise, and F. Matsui, *J. Amer. Chem. Soc.*, **89**, 3697 (1967).

(12) (a) N. Ise and T. Okubo, *J. Phys. Chem.*, **72**, 1361 (1968); (b) D. Dolar and H. Leskovsek, *Makromol. Chem.*, **118**, 60 (1968).

(13) N. Ise and K. Asai, *J. Phys. Chem.*, **72**, 1366 (1968).

(14) N. Ise and T. Okubo, *ibid.*, **72**, 1370 (1968).

40,000 ~ 100,000) was used throughout. A dilute solution of NaPGA (<10^{-4} monomol l.$^{-1}$) was passed through columns of cation- and anion-exchange resins to the acid form. Completeness of the conversion was assured by a flame test. Then sodium salt was prepared by neutralization with aqueous NaOH (reagent grade). The resulting solution was concentrated to about 0.05 monomol l.$^{-1}$ below 50° using a rotary vacuum evaporator. The polymer concentration was determined by conductometric titration. Conductivity water was used in preparing all of the solutions.

Transference Experiments. The principle was based on the method developed earlier by Huizenga, Grieger, and Wall,[15] and the detail of the experiments has been described in a previous paper.[16] An H-type glass tube divided into two compartments by a sintered-glass frit sealed at the center was used as the electrolytic cell. Two kinds of cells having different dimensions were used for measurements in high and low concentration ranges. The polymer concentrations before and after the electrolysis were determined by conductometric titration. The electric conductance of the solutions was measured by using a Jones and Ballinger type of cell[17] and a Wheatstone bridge at 1000 cps. The experiments were carried out in a thermostat at 25 and 45 ± 0.02°. The accuracy of transference number data is estimated to be ±3%. Both the transference number of sodium ions and the equivalent conductance of NaCl at 45° were taken from the data by Gordon, *et al.*[18,19]

Isopiestic Measurements. The isopiestic measurements were carried out at 25, 35, and 50 ± 0.005° by using an apparatus described before.[11] The apparatus consisted of a desiccator containing a copper block in good thermal contact with 14 silver cups. The time required for the solutions to reach the equilibrium was found by preliminary runs to be about 3 days at high dilutions. The error of our isopiestic measurements was at the highest 3% of the concentration value. The reference electrolyte was sodium chloride. The osmotic coefficients of NaCl solutions were taken from the experiments by Janz and Gordon,[20] and Smith.[21]

Emf Measurements. The details of the measurements of the mean activity coefficient were reported in the previous paper.[6] A concentration cell with transference was set up with sodium glass electrodes as

$$\begin{array}{l|l:l|l} \text{Na glass} & \text{NaPGA} & \text{NaPGA} & \text{Na glass} \\ \text{electrode} & \text{solution 2} & \text{solution 1} & \text{electrode} \end{array} \qquad \text{(I)}$$

The emf (E) of the cell is given by

$$E = \frac{(1+\alpha)RT}{\alpha F}\int_{a_2}^{a_1} t_{2p}\, \mathrm{d} \ln a \qquad (1)$$

where a_1 and a_2 are the mean activities of polyelectrolyte in solutions 1 and 2, respectively, R the gas constant, T the temperature, F the Faraday charge, t_{2p} the transference number of macroions, and α the number of free gegenions dissociated from one macroion. By combining the transference number and the emf data, we can determine the mean activity coefficient, γ_α. The details of the computation were described previously.[6] The sodium glass electrodes used in cell I were the products of the Horiba Manufacturing Co., Kyoto, Japan. The measuring circuit consisted of a precision potentiometer (Type K-2 of Shimazu Manufacturing Co., Kyoto) and a vibrating-reed electrometer (TR-85 manufactured by the Takeda Riken Industry Co., Tokyo).

Density Measurements. The apparent molal volumes of various salts of PGA were determined by pycnometric density measurements at 25 ± 0.005° with an apparatus employed earlier.[22] The density measurements were believed to be precise within ±0.00005, and the experimental uncertainty of the apparent molal volume was at the highest 1%.

Results and Discussion

Temperature Dependence of the Helical Content in PGA Conformation. The helix–coil transition of the PGA conformation has been investigated by using several methods.[23] The temperature dependence was found to be not so sharp. The helix content decreased by about 30% by increasing temperature from 10 to 60°.[23] Recently the Cotton effect having a minimum at 233 mμ was measured in this laboratory for the determination of the temperature dependence of the helix content of various salts of PGA at various degrees of neutralization in pure water.[24] The specific rotations at 233 mμ, $[m']_{233}$, were −15,500 and −800 for the helix and coil conformations, respectively. These values are in good agreement with those previously reported.[23,25,26] Our results show that the helix contents of, for example, LiPGA (degree of neutralization = 0.30) at 25, 35, 45, and 50° were 55, 46, 37, and 30%, respectively; the helix content increased by about 25% with increasing temperature from 25 to 50°.[27,28] Thus, the helix–coil transition by temperature was found to be not so sharp. However, the conformational transition induced by adding a third component, namely acid or alkali, however, is inappropriate to the investigation of the thermodynamic solution properties. We intended, therefore, to study the helix–coil transition by changing temperature.

Transference Data. The results of the transference and related experiments are compiled in Table I. The symbols are defined as follows: C, polymer concentration (monomol l.$^{-1}$); κ, specific conductance; Λ_{2g}, ionic conductance due to sodium ion; q_2, increase in equivalents of macroion in anode compartment; N_e, total equivalents of electricity; f_{2g}, fraction of sodium ions not associated with macroion; f_{2p}, polymer charge fraction; t_{2p}, transference number of macroion.

As seen from the ninth column of Table I, the fraction of free gegenions, f_{2g}, was concentration insensitive, increased with temperature, and decreased with increasing degree of neutralization. The concentration insensitivity of f_{2g} was previously found for several syn-

---

(15) J. R. Huizenga, P. F. Grieger, and F. T. Wall, *J. Amer. Chem. Soc.*, **72**, 2636 (1950).

(16) T. Okubo, Y. Nishizaki, and N. Ise, *J. Phys. Chem.*, **69**, 3690 (1965).

(17) G. Jones and M. Ballinger, *J. Amer. Chem. Soc.*, **53**, 411 (1931).

(18) R. W. Allgood and A. R. Gordon, *J. Chem. Phys.*, **10**, 124 (1942).

(19) H. E. Gunning and A. R. Gordon, *ibid.*, **10**, 126 (1942).

(20) G. J. Janz and A. R. Gordon, *J. Amer. Chem. Soc.*, **65**, 218 (1943).

(21) R. P. Smith, *ibid.*, **61**, 500, 1123 (1939).

(22) N. Ise and T. Okubo, *J. Amer. Chem. Soc.*, **90**, 4527 (1968).

(23) See, for example, P. Doty, A. Wada, J. T. Yang, and E. R. Blout, *J. Polym. Sci.*, **23**, 851 (1957); N. S. Simons, C. Cohen, A. G. Szent-Györgyi, D. B. Wetlaufer, and E. R. Blout, *J. Amer. Chem. Soc.*, **83**, 4766 (1961); E. R. Blout, L. Schmier, and N. S. Simons, *ibid.*, **84**, 3193 (1962).

(24) N. Ise and T. Okubo, unpublished results.

(25) J. T. Yang and T. Samejima, *J. Biol. Chem.*, **238**, 3262 (1962).

(26) J. T. Yang and W. J. McCabe, *Biopolymers*, **3**, 209 (1965).

(27) These results agree with those found by P. Doty, *et al.*,[23] and by Y. Go, *et al.*[28]

(28) Y. Go, Y. Kondo, E. Yamabe, and T. Okamura, *Kobunshi Kagaku*, **25**, 477 (1968).

TABLE I
TRANSFERENCE AND RELATED MEASUREMENTS ON NaPGA SOLUTIONS

Deg of neutralizn	Temp, °C	10^2C, monomol l.$^{-1}$	pH	10^4 mho cm^{-1}	10^5q_2, equiv	10^5N_e, equiv	Λ_{2g}	f_{2g}	f_{2p}	t_{2p}
1.0	25	3.07	8.38	10.50	2.85	7.46	45.4	0.59	0.59	0.22
		2.63	8.24	8.98	8.04	22.4	45.7	0.62	0.62	0.21
		2.40	8.10	8.23	1.93	5.60	45.8	0.60	0.60	0.21
		1.54	7.96	5.34	2.45	5.60	46.6	0.56	0.56	0.25
		0.672	7.58	2.65	1.11	2.80	47.6	0.64	0.64	0.25
		0.146	7.00	0.761	0.410	0.933	48.8	0.62	0.62	0.27
1.0	45	3.07		15.19	2.03	8.08	66.3	0.63	0.63	0.16
		3.07		15.19	6.55	22.4	66.4	0.61	0.61	0.18
		0.672		3.65	2.20	7.46	70.0	0.63	0.63	0.19
		0.146		0.878	1.12	3.73	71.8	0.67	0.67	0.20
0.6	25	2.61	6.79	6.93	4.72	5.60	46.4	0.65	0.39	0.33
		2.04	6.78	5.49	4.18	4.73	46.8	0.64	0.38	0.34
		1.31	6.78	3.57	3.29	4.20	47.3	0.66	0.40	0.31
		0.572	6.61	1.61	1.78	2.04	48.2	0.65	0.39	0.34
		0.125	6.44	0.345	0.490	0.58	49.2	0.64	0.38	0.32
0.6	45	2.61		10.37	4.99	9.33	67.8	0.75	0.45	0.24
		2.24		8.28	7.19	11.19	68.5	0.67	0.40	0.26
		2.04		7.52	2.68	4.79	68.8	0.69	0.41	0.23
		0.125		0.618	0.81	1.24	72.2	0.79	0.48	0.31
0.3	25	3.53	5.92	6.40	5.81	3.73	46.6	0.81	0.24	0.38
		2.76	5.87	5.08	6.85	4.48	47.0	0.82	0.25	0.38
		1.77	5.97	3.37	4.36	2.80	47.6	0.82	0.25	0.39
		0.772	5.72	1.55	2.46	1.49	48.3	0.82	0.25	0.41
		0.168	5.82	0.302	3.41	2.24	49.3	0.85	0.26	0.36
0.3	45	3.53		10.06	10.28	8.39	68.3	0.92	0.28	0.34
		2.76		7.40	5.26	4.73	68.9	0.91	0.27	0.30
		1.77		4.89	14.86	11.19	69.8	0.87	0.26	0.35
		0.772		2.29	9.54	7.46	71.0	0.91	0.27	0.35
		0.168		0.533	2.19	1.87	72.4	0.96	0.29	0.34

thetic polyelectrolytes[16, 29, 30] in the two-component system water + polyelectrolyte. The dependence of f_{2g} with degree of neutralization was similar to that previously reported for NaPAA.[15] The increase of f_{2g} with increasing temperature may be due to enhanced Brownian motion of ions, and also due to lowering of the electrostatic interaction between macroions and their gegenions by the conformational transition from helix to coil. Figure 1 shows the f_{2g} values of NaPGA (degree of neutralization, *DN*, = 1.0), NaPAA (*DN* = 1.0),[16] and PEI·HCl (*DN* = 0.8).[29] The f_{2g} values of NaPGA, NaPAA, and PEI·HCl are 0.61, 0.44, and 0.23, respectively, in the concentration range studied (<0.05 monomol l.$^{-1}$). The large value of f_{2g} of NaPGA may be caused mainly by its large volume of the repeating unit having one ionizable group. The smallest f_{2g} value of PEI·HCl would be due to the weakest electrostriction of water molecules by this macrocation than by other macroions; only one water molecule per monomer unit of the PEI cation was found to be hydrated from the measurement of the partial molal volume.[22] The PEI cation is therefore apt to bind its gegenions (chloride ions in the present case) rather than solvent water molecules.

As seen from the tenth column of Table I, the polymer charge fraction, f_{2p}, shows similar dependence on temperature, degree of neutralization, and polymer concentration as f_{2g} has shown.

The transference number of PGA anions, t_{2p}, is shown in the last column of Table I. The t_{2p} value decreased with increasing degree of neutralization and temperature. Furthermore, it slightly decreased with increasing polymer concentration.

Osmotic Coefficient. The measured concentrations of isopiestic solutions of NaPGA and NaCl (reference electrolyte) are given in Table II. The osmotic coefficient determined by the isopiestic vapor pressure measurements can be defined in two different ways, namely on the basis of the stoichiometric number of ions (φ_z) and of the effective number of free ions (φ_α) as

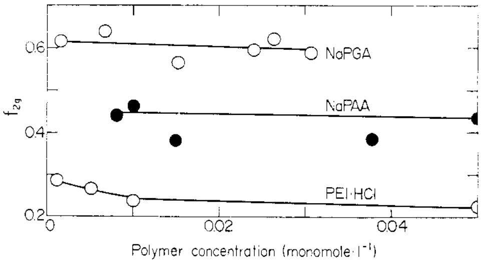

Figure 1. The fraction of free gegenions of NaPGA, NaPAA, and PEI·HCl at 25°. Data on NaPAA and PEI·HCl were taken from ref 16 and 29, respectively.

---

(29) N. Ise and T. Okubo, unpublished data on hydrochlorides of polyethylenimine (PEI·HCl).

(30) R. L. Darskus, D. O. Jordan, and T. Kurucsev, *Trans. Faraday Soc.*, **62**, 2876 (1966).

Table II
Concentrations of Isopiestic Solutions of NaCl and NaPGA

Temp, °C	m_0, (m)	m (monomol/l.) deg of neutralizn 0.3	0.6	1.0
25	0.0138	0.110	0.108	
	0.0155	0.117	0.118	0.0919
	0.0166	0.123	0.124	0.0958
	0.0176	0.128	0.131	0.0999
	0.0203	0.142	0.147	0.110
	0.0288	0.182	0.197	0.143
	0.0447	0.250	0.275	0.192
35	0.0147	0.106	0.108	0.0853
	0.0165	0.115	0.119	0.0922
	0.0221	0.142	0.145	0.108
	0.0343	0.197	0.194	0.140
	0.0451	0.269	0.261	0.183
	0.0585	0.375	0.352	0.243
50	0.0170	0.110	0.115	0.0901
	0.0209	0.127	0.137	0.105
	0.0257	0.149	0.167	0.124
	0.0321	0.178	0.201	0.147
	0.0525	0.271	0.302	0.207

$$\varphi_z = 2m_0\varphi_0/(z/z_{2g} + 1)(m/z) \qquad (2)$$

and

$$\varphi_\alpha = 2m_0\varphi_0/(\alpha/z_{2g} + 1)(m/z) \qquad (3)$$

where m_0 is the molality of the reference electrolyte solution, m the isopiestic concentration of the polysalt (equiv/1000 g of water), z and α are the stoichiometric and net valencies of macroions, respectively, z_{2g} is the valency of gegenions of the polysalt, and φ_0 is the practical osmotic coefficient of the reference electrolyte at m_0. If one assumes that $\alpha = f_{2g}z$, the φ_α value can be determined by the isopiestic measurement using f_{2p} value.

The φ_z values of NaPGA are shown in Figure 2. It is clear that φ_z increased with increasing temperature and concentration. The increase of φ_z with temperature is steeper at lower degrees of neutralization. This temperature dependence of φ_z of NaPGA is qualitatively similar to that of simple electrolytes, for example, sodium chloride in concentrated regions (m > 0.5 mol l.$^{-1}$).[31,32] It is obvious that the observed concentration dependence and the large values of φ_z, particularly at low degrees of neutralization and comparatively concentrated regions, indicate a dominant contribution of the nonelectrostatic interactions compared with the electrostatic ones. The PGA ions have various groups having strong influence on the structure of water, for example, methylene, carboxyl, and imino groups. These groups give rise to hydrophobic interactions and hydrogen bonds between the groups and solvent. The φ_z values of NaPGA at a degree of neutralization = 1.0 are between 0.3 and 0.5 at 25° in the investigated range of concentration (0.1~0.3 monomol/1000 g of water) as seen in Figure 2. The φ_z of sodium polystyrenesulfonate (NaPSt),[12a,33] sodium polyphosphate (NaPP),[14] NaPAA,[9] and sodium polyethylenesulfonate (NaPES)[13] were approximately 0.6, 0.4, 0.2, and 0.2, respectively. According to the water structure theory, the observed φ_z values of NaPGA suggest that the salting-out effects between PGA and Na ions are weaker than those between PSt and Na ions being similar to those between PP and Na ions, and stronger than those between PAA or PES and Na ions. This relative magnitude of the osmotic coefficients of the various polyelectrolytes can reasonably be accounted for by a strong hydrophobic structure-forming tendency of the PGA anion caused by the methylene groups on one hand and solvent-solute interactions by hydrogen bond (imino groups) and by π electrons of carboxyl groups on the other.

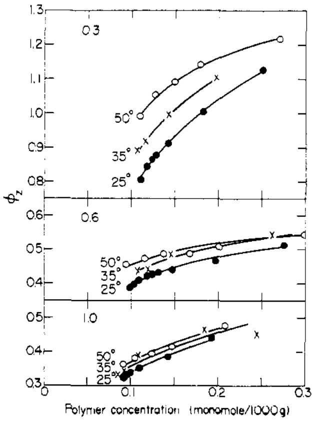

Figure 2. Stoichiometric osmotic coefficient of NaPGA. The degrees of neutralization are 0.3, 0.6, and 1.0 from the top to the bottom.

As seen from Figure 3, the φ_α values showed an interesting feature at a degree of neutralization = 0.3; φ_α at 50° (coil rich) is larger than that at 25° (helix rich), whereas φ_α at degrees of neutralization of 0.6 and 1.0 does not change with temperature. The relative contributions of the conformational and temperature effects to the osmotic coefficient cannot be easily separated. The coincidence of φ_α at 25 and 50° for NaPGA of degrees of neutralization of 0.6 and 1.0, however, may suggest a negligible role of the temperature effect. Thus, we may say that the increase of φ_α with increasing temperature at a degree of neutralization = 0.3 is due to enhanced solvent-solute interactions as follows. The nonpolar part of a PGA side chain tends to fold

---

(31) H. S. Harned and L. F. Nims, *J. Amer. Chem. Soc.*, **54**, 423 (1932).

(32) H. S. Harned and B. B. Owen, "The Physical Chemistry of Electrolytic Solutions," Reinhold Publishing Corp., New York, N. Y., 1958, Chapter 12.

(33) Independently, the φ_z of sodium polystyrenesulfonate was measured using differential osmometry by Chu and Marinsky [*J. Phys. Chem.*, **71**, 4352 (1967)]. Some discrepancies between these authors' data and ours are notable. For example, their φ_z was about 0.24 whereas ours about 0.6 at 0.2 equiv/l. Recently performed isopiestic measurements of the NaPSt–water and NaPSt–NaCl–water system confirmed our data given above (K. Kaji and N. Ise, to be published). The impurity factor in our sample was ruled out by elementary analysis and the possibility of the degradation of the polymer in solution was rejected on the basis of viscosity measurements. Furthermore, we checked the technique adopted by Chu and Marinsky. The results showed that leakage of gegenions through the Visking membrane took place to an appreciable amount in a short time, even more quickly when polyethylene glycol solutions were used as a reference solution. In the light of such a fundamental deficiency, we concluded that the osmometry was not as reliable as the isopiestic method (in which leakage of gegenions cannot occur), in particular *when foreign simple salt is not present.* Detailed accounts of this matter will be given in a separate note, together with those of other discrepancies (on the gegenion specificity) which are most likely to be related with the leakage problem.

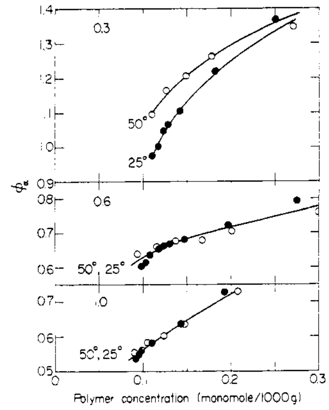

Figure 3. Thermodynamic osmotic coefficient of NaPGA. The degrees of neutralization are 0.3, 0.6, and 1.0 from the top to the bottom.

against the main chain so as to minimize the extent of contact with water, and form hydrophobic bonds with neighboring nonpolar groups.[34–36] Thus, the hydrophobic interactions between solute and water are minimized in the helical state, whereas in the coiled state the nonpolar methylene groups turn to interact with water. This may be one of the reasons why the solvent activity is decreased with increasing temperature. Furthermore, the oxygen and nitrogen atoms in the PGA molecule bonded by hydrogen bond in the helical conformation[37] turn to interact with water by the conformational change from helix to coil. Also, the solvent–solute interactions induced by π electrons of the C–N bond[37] may also affect the activity of water as was observed.

Mean Activity Coefficient. The essential data of the mean activity coefficients of NaPGA obtained from emf of a concentration cell with transference are given in Table III. The first and second columns give the degree of neutralization and solution temperature, respectively. The third column indicates the concentration of solution 2 in cell I (monomol/1000 g of water), solution 1 being the most concentrated one in each set of solutions. The transference numbers, t_{2p}, are given again in the fifth column. In the last column, the observed mean activity coefficients, γ_α, are shown in the logarithmic form. These γ_α values were the relative ones determined on the basis of the $\gamma_{2g,\alpha}$ value at m = 0.01 by the same convention as adopted in the preceding papers.[6–8] The mean activity coefficient of NaPGA always decreased with increasing concentration in dilute regions, and even more sharply at lower temperatures (Table III and Figures 4–6). This rapid decrease of γ_α with concentration at 25° indicates that the helical structure exerts stronger electrostatic influence than the coiled state. In Figures 4–6, the concentration is given in the cube root. The logarithms of the mean activity coefficients do not decrease linearly with the cube root of the polymer concentration, in contrast with previously studied polyelectrolytes, *i.e.*, NaPAA,[9] NaPES,[13] NaPP,[14] sodium salts of poly(vinyl alcohol) partially acetalized with glyoxylic acid[7] and hydrochlorides of PEI and its low molecular weight analogs.[8] This cube-root rule was qualitatively interpreted by the local regularity of ionic distribution, which was believed to be resulted from the purely electrostatic interactions between ions. Though this rule was found to hold also for the equivalent conductance of NaPAA and for the diffusion constant of HPSt,[38] it is true that this rule holds only in highly diluted concentration regions or is not valid for some polyelectrolytes having strong solvent–solute interactions, *e.g.*, various salts of PSt.[12a] The failure of the cube-root rule for NaPGA may be due to strong nonelectrostatic solvent–solute interactions as was demonstrated in the previous section.

TABLE III
MEAN ACTIVITY COEFFICIENTS OF NaPGA BY THE EMF METHOD AT 25 AND 45°

Deg of neutralizn	Temp, °C	10^2m, monomol/1000 g of water	Emf, mV	t_{2p}	Log γ_α[a]
1.0	25	0.146	15.80	0.27	0.014
		0.279	12.35	0.26	0.045
		0.672	8.27	0.25	−0.162
		1.02	5.61	0.24	−0.163
		2.40	1.26	0.21	−0.200
		3.07	0	0.22	−0.208
1.0	45	0.146	14.30	0.20	−0.087
		0.279	11.30	0.19	−0.127
		0.672	7.02	0.19	−0.152
		1.54	3.20	0.18	−0.183
		2.40	1.02	0.18	−0.183
		3.07	0	0.17	−0.197
0.6	25	0.125	17.72	0.32	0.118
		0.238	13.79	0.33	0.132
		0.572	9.75	0.34	−0.140
		0.871	7.34	0.34	−0.203
		2.04	1.61	0.34	−0.287
		2.61	0	0.33	−0.312
0.6	45	0.125	19.21	0.31	−0.025
		0.238	15.23	0.25	−0.080
		0.572	9.53	0.25	−0.101
		1.31	4.38	0.25	−0.135
		2.04	1.50	0.23	−0.127
		2.61	0	0.24	−0.133
0.3	25	0.168	20.11	0.36	0.208
		0.321	15.76	0.38	0.126
		0.772	9.91	0.41	−0.004
		1.77	4.63	0.39	−0.137
		3.53	0	0.38	−0.232
0.3	45	0.168	24.00	0.34	0.090
		0.321	19.20	0.34	0.031
		0.772	11.98	0.35	−0.020
		1.77	5.10	0.35	−0.064
		2.76	1.90	0.30	−0.101
		3.53	0	0.34	−0.114

[a] The γ_α values were determined using a convention, $\gamma_\alpha = \gamma_{2g,\alpha}$ at m = 0.01.

The mean activity coefficient can also be determined indirectly from the osmotic coefficient by using the Gibbs–Duhem equation

---

(34) G. Némethy and H. A. Scheraga, *J. Phys. Chem.*, **66**, 1773 (1962).

(35) G. Némethy, I. Z. Steinberg, and H. A. Scheraga, *Biopolymers*, **1**, 43 (1963).

(36) C. Tanford, *J. Amer. Chem. Soc.*, **84**, 4240 (1962).

(37) L. Pauling, R. B. Corey, and H. R. Branson, *Proc. Nat. Acad. Sci.*, **37**, 729 (1951).

(38) N. Ise and Y. Okubo, unpublished results.

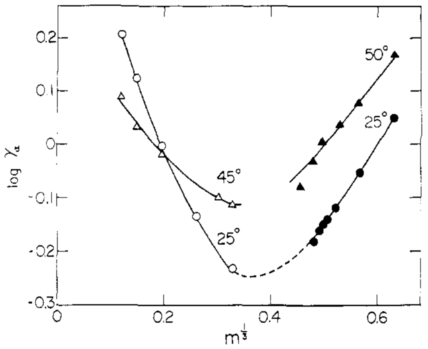

Figure 4. Mean activity coefficient of NaPGA at a degree of neutralization of 0.3: emf method ○ (at 25°), △ (45°); isopiestic method ● (25°), ▲ (50°).

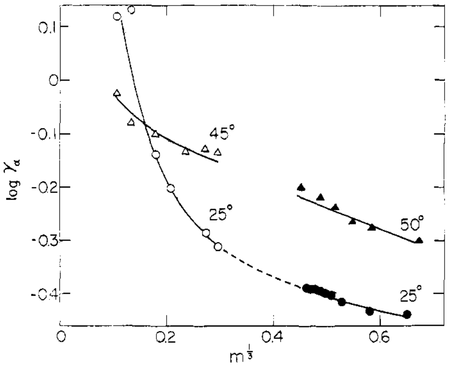

Figure 5. Mean activity coefficient of NaPGA at a degree of neutralization of 0.6: emf method ○ (at 25°), △ (45°); isopiestic method ● (25°), ▲ (50°).

$$\ln(\gamma_{\alpha,1}/\gamma_{\alpha,2}) = \varphi_{\alpha,1} - \varphi_{\alpha,2} + 2\int_m^{m_2}[(1-\varphi_\alpha)/\sqrt{m}]\,\mathrm{d}\sqrt{m} \quad (4)$$

where the suffixes 1 and 2 of the coefficients refer to concentrations m_1 and m_2, respectively. The relative value of the mean activity coefficient, $\gamma_{\alpha,1}/\gamma_{\alpha,2}$, thus determined was conveniently standardized by an extrapolation to the γ_α values obtained from the emf method. The results are shown in Figures 4–6 by solid marks. The mean activity coefficient of NaPGA of a degree of neutralization of 0.3 increased with increasing concentration in the comparatively concentrated regions as clearly shown in Figure 4. This is due to the preponderance of hydrophobic interactions over the coulombic ones at low degrees of neutralization.

Partial Molal Volume. As reported previously,[22] the solvent–solute interactions, particularly, the electrostriction of solvent, and the iceberg water structure formation can be discussed separately and quantitatively by using the partial molal volume. Thus the density was also measured for detailed studies on the solvent–solute interactions. The results are compiled in Table IV. The polymer concentration was not changed, since the apparent molal volumes of polyelectrolytes were practically concentration independent.[22] The last column gives the apparent molal volumes, which can be assumed to be equal to the partial molal volumes, $\bar{V}_2{}^0$. The $\bar{V}_2{}^0$ is comprised of three contributions, *i.e.*, the intrinsic ionic volume including dead space between ion and solvent molecules (V_{int}), the contribution from the electrostrictional hydration of water (E), and the hydrophobic structural contribution (P), as[22]

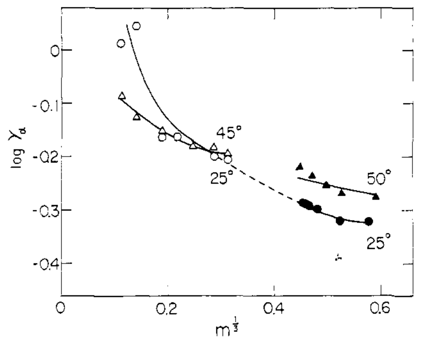

Figure 6. Mean activity coefficient of NaPGA at a degree of neutralization of 1.0: emf method ○ (at 25°), △ (45°); isopiestic method ● (25°), ▲ (50°).

Table IV
Densities and Apparent Molal Volumes of Various Salts of PGA at 25°

Salt	Deg of neutralizn	Concn, monomol l.$^{-1}$	Density	$\phi_v/z(=\bar{V}_2{}^0)$
NaPGA	1.0	0.0757	1.00219	83.7
	0.8	0.0352	0.99928	83.9
	0.6	0.0863	1.00216	83.1
	0.45	0.0472	0.99972	83.2
	0.3	0.0506	0.99971	83.0
LiPGA	0.3	0.0843	1.00089	85.2
KPGA	0.3	0.0536	0.99967	92.3
$(CH_3)_4$NPGA	0.3	0.1064	1.00057	117.8
$(C_2H_5)_4$NPGA	0.3	0.0457	0.99856	135.7

$$\bar{V}_2{}^0 = [V_{\text{int},2g} + P_{2g} + E_{2g}] + [V_{\text{int},2p} + P_{2p} + E_{2p}] \quad (5)$$

where 2g and 2p denote gegenion and macroion, respectively. The P_{2g} term is negligible for metal salts. Using eq 5 and 6 of ref 22, the $[V_{\text{int},2g} + P_{2g} + E_{2g}]$ term is estimated, and the $[V_{\text{int},2p} + P_{2p} + E_{2p}]$ term is found to be 89.4 ml monomol^{-1} for NaPGA of a degree of neutralization = 1.0. The $V_{\text{int},2p}$ term can be calculated as 108.8 ml monomol^{-1} by using Conway's equation (eq 7 of ref 22), where the length of monomer and its radius were taken as 4.0 and 3.5 Å, respectively. For salts of weak polyelectrolytes, the $[V_{\text{int},2p} + P_{2p}]$ value can be estimated by the measurements of $\bar{V}_2{}^0$ at a degree of neutralization = 0, at which E_{2p} vanishes. The poly-L-glutamic acid (HPGA), however, is not stable in water. We expediently assume therefore the $[V_{\text{int},2p} + P_{2p}]$ of HPGA to be equal to that of the corresponding monomer, glutamic acid (HGA), 97.1 ml mol^{-1}.[39] Thus the P_{2p} and E_{2p} of PGA can be estimated to be −11.7 and −7.7 ml monomol^{-1}, respectively. As noted in the previous paper,[22] the P term of nonpolar hydrocarbons was about −20 ml mol^{-1}, independent

---

(39) E. J. Cohn and J. T. Edsall, "Proteins, Amino Acids and Peptides," Reinhold Publishing Corp, New York, N. Y., 1943, Chapter 16.

of the length of the chain,[40] and this value was also found for PAA, PEI, and its analogs. On the other hand, the P_{2p} of PSt and PP were -6.0 and 0, respectively, being larger than -20. These large P_{2p} values suggested that the PSt and PP ions had the strong interactions with water molecules by π electrons of the benzene ring and the phosphate group, respectively. Similarly, the comparatively large P_{2p} value of PGA suggests the strong contribution of the interactions from hydrogen bonds and π electrons mentioned above. At full degree of neutralization, the PGA ion was found to be hydrated by three water molecules per monomer unit from the E_{2p} by the same method as that of the previous paper.[22] The extent of the electrostriction of PGA ions is seen to be about the same as that of PAA ions likewise having carboxyl groups.

Acknowledgments. This work was supported in part by the grant-in-aid of the Ministry of Education.

---

[40] W. L. Masterton, *J. Chem. Phys.*, 22, 1830 (1954).

Mean and Single-Ion Activity Coefficients and Transference Data of the Sodium Salt of a Deoxyribonucleic Acid in Aqueous Solution[1]

Tsuneo Okubo and Norio Ise

Department of Polymer Chemistry, Kyoto University, Kyoto, Japan.
Received March 10, 1969

ABSTRACT: The mean activity coefficient of the sodium salt of a deoxyribonucleic acid, NaDNA, in two- and three-component systems, *i.e.*, NaDNA + H_2O, and NaDNA + NaCl + H_2O, was investigated by the emf method. The transference and related measurements were also carried out for the determination of the net valency and the transference number of DNA anions. The polymer charge fraction, f_{2p}, was concentration insensitive, decreased with increasing concentration of added salt, and was larger in the denatured state than in the native one. The transference number of DNA anions in the NaDNA + H_2O system was about 0.5, being insensitive toward the polymer concentration. The mean activity coefficient of NaDNA increased sharply with polymer concentration ($0.0007 \sim 0.003$ equiv $l.^{-1}$), indicating the presence of the strong short-range interactions between DNA molecule and solvent water. A similar tendency was also noticed for the single-ion activity coefficient of DNA anions. The single-ion activity coefficient of gegenions slightly decreased with polymer concentration.

The solution properties of deoxyribonucleic acid (DNA) have been intensively investigated by using various experimental techniques, *i.e.*, light scattering,[2] viscosity,[3] sedimentation,[4] Donnan equilibrium,[5] electrophoresis,[6] etc. However, no measurement has been reported on the mean activity coefficient, which is one of the most fundamental thermodynamic quantities. We have measured this quantity for several synthetic polyelectrolytes by emf measurements of a concentration cell with transference,[7-9] and by isopiestic vapor pressure measurements.[10-15] Recently Dolar and Leskovsek[16] investigated the mean activity coefficients of polystyrenesulfonic acid by the emf method. In a previous paper,[17] sodium salts of poly(L-glutamic acid), NaPGA, were studied by the measurements of the mean and single-ion activity coefficients, osmotic coefficient, transference properties, and partial molal volume. The contribution of the short-range solvent–solute interactions on NaPGA properties was noted. In the present paper, we report the activity and transference data for sodium salts of native and denatured DNA in the binary and ternary systems, *i.e.*, NaDNA + H_2O and NaDNA + NaCl + H_2O, and compare the results with those previously obtained for synthetic polyelectrolytes. It should be noted that the present work is the first of a series of the thermodynamic investigations of DNA and its biological function from this laboratory. This work will be followed by the study of the catalytic action of DNA in ionic reactions (the primary salt effects), in which the information on the mean activity coefficient given in the present work is indispensable.

Experimental Section

Materials. The sodium salt of salmon sperm DNA (NaDNA) employed in these investigations was purchased from Calbiochem Corp., Los Angeles, Calif., U.S.A. (Lot. 50211). This material was purified by washing with 85% ethanol (the pH was adjusted to 7∼8 by adding 0.001 *N* NaOH), then with pure ethanol, and pure acetone cooled to 0∼5°. Then the NaDNA was dried for about 20 hr under

---

[1] (a) Presented at the 18th Annual Meeting of the Society of High Polymers, Japan, May 1969, Kyoto. (b) Supported in part by the Grant in Aid of the Ministry of Education.

[2] See, for example, P. Doty and B. H. Bunce, *J. Amer. Chem. Soc.*, 74, 5029 (1952); G. L. Brown, M. B. M'Ewen, and M. I. Pratt, *Nature*, 176, 161 (1955); J. Hermans, Jr., *J. Phys. Chem.*, 63, 175 (1959).

[3] See, for example, B. E. Conway and J. A. V. Butler, *J. Polym. Sci.*, 42, 441 (1960).

[4] See, for example, A. R. Peacocke and H. K. Schachman, *Biochim. Biophys. Acta.*, 15, 198 (1954); K. V. Shooter and J. A. V. Butler, *Trans. Faraday Soc.*, 52, 734 (1956); P. F. Davison, *Proc. Nat. Acad. Sci. U. S.*, 45, 1560 (1959).

[5] See, for example, U. P. Strauss, C. Helfgott, and H. Pink, *J. Phys. Chem.*, 71, 2550 (1967).

[6] See, for example, P. D. Ross and R. L. Scruggs, *Biopolymers*, 2, 79 (1964).

[7] N. Ise and T. Okubo, *J. Phys. Chem.*, 69, 4102 (1965).

[8] N. Ise and T. Okubo, *ibid.*, 70, 1930 (1966).

[9] N. Ise and T. Okubo, *ibid.*, 70, 2400 (1966).

[10] N. Ise and T. Okubo, *ibid.*, 71, 1287 (1967).

[11] N. Ise and T. Okubo, *ibid.*, 71, 1886 (1967).

[12] T. Okubo, N. Ise, and F. Matsui, *J. Amer. Chem. Soc.*, 89, 3697 (1967).

[13] N. Ise and T. Okubo, *J. Phys. Chem.*, 72, 1361 (1968).

[14] N. Ise and K. Asai, *ibid.*, 72, 1366 (1968).

[15] N. Ise and T. Okubo, *ibid.*, 72, 1370 (1968).

[16] D. Dolar and H. Leskovsek, *Makromol. Chem.*, 118, 60 (1968).

[17] N. Ise and T. Okubo, *Macromolecules*, 2, 401 (1969).