J. Am. Chem. Soc. **1980**, *102*, 7901–7906

Ordered Structure in Dilute Solutions of Ionic Biopolymers. 2. Small-Angle X-ray Scattering Study of Sodium Polyacrylate Solution[1a,b]

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Received March 31, 1980

Abstract: Small-angle X-ray scattering measurements using a position-sensitive proportional counter were carried out for aqueous dilute solutions (0.007–0.03g/mL) of fractionated sodium polyacrylates. Water and the polymer sample used were purified with great care. A well-defined peak was observed at scattering vectors between 0.03 and 0.09 Å^{-1}. The dimension of the repeating unit (or the Bragg distance) of the ordered structure increased with decreasing polymer concentration or degree of neutralization. It increased with increasing degree of polymerization of the macroions and salt concentration. When two fractions were mixed, a single peak (not two) was observed and the Bragg distance fell between those of the mother fractions. From these results the observed peak was concluded to be due to intermolecular ordering. The Bragg distance was found to be always smaller than the intermacroion distance calculated with the assumption of simple cubic distribution. This reflects the importance of the "attractive" interaction between macroions through the intermediary of their gegenions and suggests that a two-state structure is maintained in the dilute solution. In other words, the ordered state coexists with the disordered one. The ordered structure was discussed with reference to regular arrangements found for simple ions, proteins, and charged polymer latex particles.

Introduction

Crystals are undoubtedly the consequence of a regular packing of molecules or ions. When these molecules or ions are dissolved into a large amount of solvent, in other words when a very dilute solution is formed, such a regularity can no longer exist because of thermal agitation. The distribution of the solute particles in such a solution must be random. Between the two extreme cases of the crystal and infinite dilution lies the solute distribution at intermediate concentrations. When the solute concentration is raised from the infinite dilution, the randomness must diminish gradually. If the particles interact with each other, regular packing would come into existence at relatively low concentrations, even though it would not be completely regular. When the interaction is very strong, the threshold concentration may be fairly low.

With such a general situation in mind, it is interesting to recall the X-ray analysis by Bernal and Fankuchen[2] of the plant virus in wet and dry gel states and in concentrated solution. They found distinct intermolecular reflections and concluded that the virus particles were distributed in a regular manner in these states and also in solution. Subsequently, Riley and Oster[3] observed one or more well-defined small-angle X-ray diffraction bands for solutions (33–51% in wt/vol) of bovine serum albumin and human hemoglobin and claimed that the solutions of such electrically charged macromolecules possessed structure of almost crystalline regularity. The Bragg distance (60–85 Å for these protein molecules) was found to decrease with increasing concentration to a limiting value for the saturated solution (54.5 Å for the hemoglobin). Sodium salt of deoxyribonucleic acid, which may be pictured as a long cylinder, was studied by Riley and Oster,[3] who found also an ordered structure for its concentrated solutions.

We thought it highly interesting to investigate the solute distribution in dilute solutions because it is the basic factor determining solution properties. Furthermore, it was inferred that, when the particles were electrically charged, more regular ordering in solutions might be maintained than for neutral species. Thus we wished to carry out a small-angle X-ray study of dilute solutions of electrically charge particles.[1a] Literature survey shows that some degrees of ordering persist even at fairly low concentrations for particles such as simple ions, macroions, ionic surfactant micelles, and polymer latex particles. For example, the following experimental results were interpreted to be at least partly due to the ordered distribution of the respective solute particles: (1) the low intensity of the scattered light from salt-free polyelectrolyte solutions,[4] (2) the dependence of the mean activity coefficients of simple electrolytes[5,6] and polyelectrolytes[7] on the cube root of concentration, (3) a small-angle X-ray scattering of simple electrolyte solutions,[8,9] (4) the catastrophic change of the diffusion coefficient of macroions with the salt concentration observed by dynamic light scattering,[10,11] (5) a small-angle neutron scattering of polyelectrolyte solutions,[12,13] (6) the magnetic birefringence of solutions of the polypeptide, the nucleic acid fragments, and the rod-like virus,[14] (7) an X-ray scattering of ionic micelle solutions,[15,16] and (8) a direct visual observation of polymer latex particles in solution.[17]

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[1] (a) Preliminary communication: Ise, N.; Okubo, T.; Hiragi, Y.; Kawai, H.; Hashimoto, T.; Fujimura, M.; Nakajima, A.; Hayashi, H. *J. Am. Chem. Soc.* **1979**, *101*, 5836–5837. Presented partly at the US-Japan Joint Seminar on the Electrolytic Aspects of Biopolymers and Biological Systems, Honolulu, June 1979, and at the 40th Fall Meeting of the Chemical Society of Japan, October 1979, Fukuoka, Japan. (b) For a review on the distribution of electrically charged solutes in dilute solutions see: Ise, N.; Okubo, T. *Acc. Chem. Res.*; **1980**, *13*, 303–309. (c) Institute for Chemical Research, Kyoto University.

[2] Bernal, J. D.; Fankuchen, I. *J. Gen. Physiol.* **1941**, *25*, 111–165.

[3] Riley, D. P.; Oster, G. *Discuss. Faraday Soc.* **1951**, *11*, 107–116.

[4] For a convenient review, see: Stacey, K. A. "Light-Scattering in Physical Chemistry"; Butterworths: London, 1959, Chapter 6.

[5] Frank, H. S.; Thompson, P. T. *J. Chem. Phys.* **1959**, *31*, 1086–1095.

[6] Desnoyers, J. E.; Conway, B. E. *J. Phys. Chem.* **1964**, *68*, 2305–2311.

[7] Ise, N.; Okubo, T. *J. Phys. Chem.* **1966**, *70*, 1930–1935, 2400–2405; **1967**, *71*, 1287–1290, 1886–1890 (correction 4588); **1968**, *72* 1370–1373. Asai, K.; Takaya, K.; Ise, N. *Ibid.* **1969**, *73*, 4071–4076. Okubo, T.; Ise, N.; Matsui, F. *J. Am. Chem. Soc.* **1967**, *89*, 3697–3703. Matsui, F.; Ise, N.; Okubo, T. *Polym. J.* **1970**, *1*, 64–70. A review by Ise, N. *Adv. Polym. Sci.* **1971**, *7*, 536–593.

[8] Bahe, L. W. *J. Phys. Chem.* **1972**, *76*, 1062–1071.

[9] Bahe, L. W.; Parker, D. *J. Am. Chem. Soc.* **1975**, *97*, 5664–5670.

[10] Lee, W. I.; Schurr, J. M. *J. Polym. Sci., Polym. Phys. Ed.* **1975**, *13*, 873–888.

[11] Lin, S. C.; Lee, W. I.; Schurr, J. M. *Biopolymers* **1978**, *17*, 1041–1064.

[12] Moan, M. *J. Appl. Crystallogr.* **1978**, *11*, 519–523.

[13] Rinaudo, M.; Domard, A. *Polym. Lett.* **1977**, *15*, 411–415.

[14] Maret, G.; Torbet, J.; Senechal, E.; Domard, A.; Rinaudo, M.; Milas, H. Proceedings of the 31st Symposium European Chemical Society, September 1978, Fontevaud.

[15] Mattoon, R. W.; Stearns, R. S.; Harkins, W. D. *J. Chem. Phys.* **1948**, *16*, 644–658.

[16] Brady, G. W. *J. Chem. Phys.* **1951**, *19*, 1547–1550.

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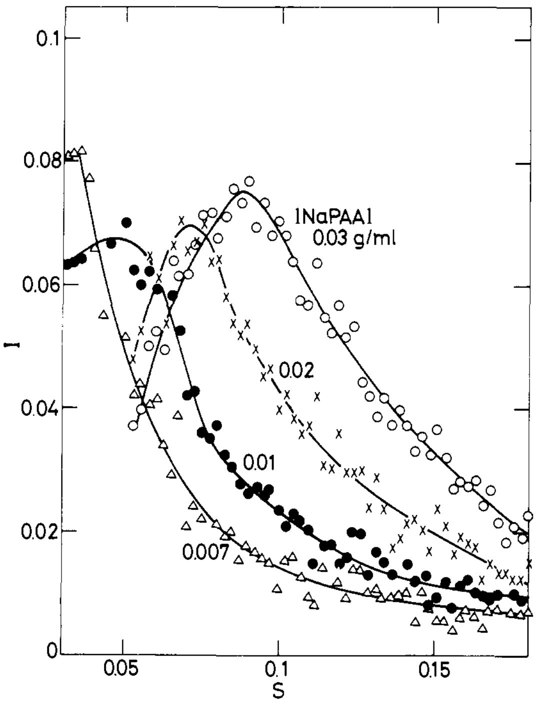

Figure 1. Scattered intensity (I) vs. scattering vector (s) plots for Na-PAA at 25 °C: influence of polymer concentration at $\alpha = 0.65$ and $P_w = 1020$.

In the course of our X-ray study, Dušek et al. reported similar work on polyelectrolyte solutions, using the same technique.[18] Their study and our own clearly showed a distinct peak in the scattering curves, which is ascribable to an interference caused by the ordering of macroions in dilute solutions. In the present article, we describe our small-angle X-ray scattering (SAXS) study on dilute solutions of sodium polyacrylates in detail.

Experimental Section

Small Angle X-ray Scattering Measurements. The X-ray source used in the present measurements was a Rigaku RU A (60 kV, 200 mA), and the scattered intensity of the Cu Kα X-ray was measured by using a position-sensitive proportional counter (PSPC). A detailed description of the instruments was given in previous publications.[19,20] All measurements were made with a point-focusing camera system. The solution was placed in a small compartment between two parallel mica films (1 mm apart). The scattering intensities from both solution and solvent were corrected for their respective absorptions. The measurements were done in a temperature-controlled room at 22, 25, or 26 ± 0.5 °C. However, the temperature of the cell compartment was not regulated in the present experiments. The experimental error in the scattering intensity and the Bragg distance (described below) was estimated to be within ±5% from repeated runs.

Materials. Polyacrylic acid (PAA, Wako Chemical Co., Osaka) was carefully fractionated into nine fractions by 1,4-dioxane and heptane as was described earlier[21] and purified by repeated precipitation, dialysis, and ion exchange by using columns of ion-exchange resins (Amberlite IR-120B and IR-45). Completeness of the conversion from the salt form to the acid one by the ion exchange was checked by the atomic absorption spectra. Finally the samples were freeze-dried. The weight-average degree of polymerization (P_w) of the polyacrylic acid samples was determined in dioxane solution by using a low angle light scattering photometer (Type KMX-6, Chromatix, Mountain View, Calif.). According to the sedimentation patterns (Beckmann, analytical ultracentrifuge, Type E), the P_w/P_N value was below 1.5 (P_N = number-average degree of polymerization). As will be shown below, the X-ray diffraction is sensitive to the ionic strength and furthermore, the forces responsible for the ordering of macroions were believed to be electrostatic. Thus, the purification of water was done with great care; distillation of ion-exchanged water was carried out twice under nitrogen atmosphere by using a quartz vessel. The specific conductance of the purified water was as low as 2×10^{-7} Ω^{-1} cm^{-1} just after purification. The impurity level of the water (Na^+, K^+, Ag^+, Hg^{2+}) was found to be hardly detectable by the atomic absorption spectra. A slight deterioration of the water was inevitable during solution preparation, but the purity was believed to be satisfactory to give a reliable and reproducible diffraction pattern, though we are trying to establish procedures to further reduce the influence of such a deterioration.

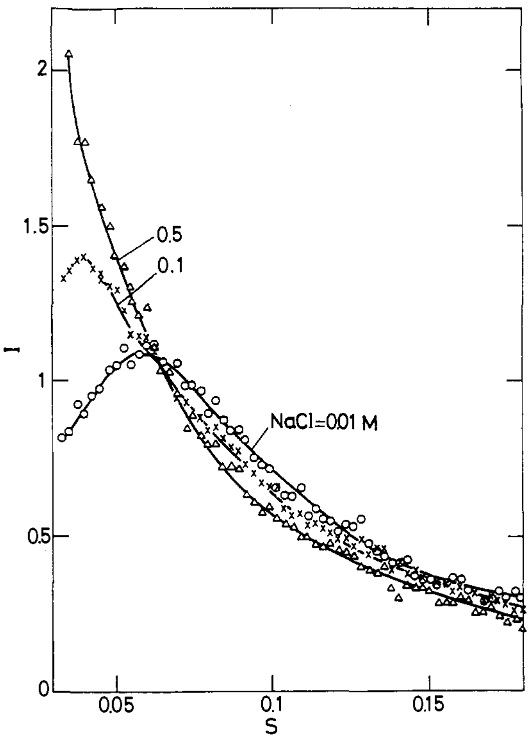

Figure 2. Scattered intensity (I) vs. scattering vector (s) plots for Na-PAA at 22 °C: influence of NaCl added at $\alpha = 0.5$, $P_w = 1470$, and [NaPAA] = 0.02 g/mL.

Results

A. Polymer Concentration Dependence. Typical scattering intensity (I)–scattering vector (s) curves at various polymer concentrations of sodium polyacrylate (NaPAA, degree of neutralization (α) = 0.65) are shown in Figure 1. The vector, s, is given by the equation $|s| = 4\pi (\sin \theta)/\lambda$, where λ and 2θ are the wavelength of the X-ray used and the scattering angle, respectively. In the concentration range of 0.03–0.01 g/mL, a distinct peak was observed. The position of the peak (s_m) shifted toward a lower angle with decreasing polymer concentration. At 0.007 g/mL, the peak could not be detected with our technique. The dimension of the repeating unit of the ordered structure ($2D_{\text{exptl}}$) was calculated by the Bragg equation ($2D_{\text{exptl}} = 2\pi/s_m$) and shown in Table I. It should be noted that $2D_{\text{exptl}}$ increased with decreasing polymer concentration (experiments 2–4 or 16–18). This concentration dependence is in accord with those reported by Riley and Oster[2] for proteins and by Dušek et al.[18] for polyelectrolytes, using X-ray analysis, and by Moan also for polyelectrolytes, using neutron scattering.[12]

B. Salt Concentration Dependence. Figure 2 shows the scattering curves at three different concentrations of NaCl added. Clearly, the distinct scattering peak was observed at lower salt concentrations. The peak could not be obtained, however, in the presence of a large amount of salt (0.5 M). Being in complete qualitative agreement with independent X-ray[18] and neutron

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(17) Kose, A.; Ozaki, M.; Takano, K.; Kobayashi, Y.; Hachisu, S. *J. Colloid Interface Sci.* **1973**, *44*, 330–338.

(18) Pleštil, J.; Mikeš, J.; Dušek, K. *Acta Polym.* **1979**, *30*, 29–32.

(19) Shibayama, M.; Fujimura, M.; Saijo, K.; Suehiro, S.; Hashimoto, T.; Kawai, H. Presented in part at the 27th Symposium on Polymers, 1978, Kyoto (Polymer Preprints, Japan, **1978**, *27*, 1652–1655).

(20) Hashimoto, T.; Suehiro, S.; Shibayama, M.; Saijo, K.; Kawai, H. *Polym. J.*, and Fujimura, M.; Hashimoto, T.; Kawai, H. *Polym. J.*, in press.

(21) Newman, S.; Krigbaum, W. R.; Laugier, C.; Flory, P. J. *J. Polym. Sci.* **1954**, *14*, 451–462.

(22) The values of $2D_0$ were obtained by the relation, $2D_0 = (P_w w/cN_A)^{1/3}$, where c is the polyelectrolyte concentration in g/mL, w denotes the weight of the monomer unit, and N_A is the Avogadro number.

Table I. SAXS Data of NaPAA in Dilute Solutions[a]

expt	α	c, g/mL	temp, °C	P_w	[NaCl], M	s_m, Å^{-1}	$2D_{exptl}$, Å	$2D_0$, Å	$2D_{theor}$, Å
1	0.8	0.02	22	1470	0	0.071	88	222	62
2	0.65	0.03	25	1020	0	0.089	71	169	49
3		0.02	25	1020	0	0.068	92	194	60
4		0.01	25	1020	0	0.050	126	244	85
5		0.02	26	1470	0.01	0.058	108		60
6		0.02	26	1470	0.04	(0.043)	(146)		60
7		0.02	26	1470	0.1	(0.034)	(185)		60
8		0.02	26	1470	0.3	none			60
9	0.5	0.0221	25	1020	0	0.071	88	184	56
10		0.02	22	1470	0.01	0.059	106	216	59
11		0.02	22	1470	0.1	(0.040)	(157)		59
12		0.02	22	1470	0.5	none			59
13		0.02	26	760	0	0.070	90	173	59
14		0.02	26	760 + 5000	0	0.064	98		59
15		0.02	26	5000	0	0.060	105	326	59
16	0.4	0.03	25	1020	0	0.076	83	166	48
17		0.02	25	1020	0	0.068	93	190	58
18		0.013	25	1020	0	0.059	106	219	72
19	0.3	0.02	25	1020	0	0.064	98	188	58
20	0.2	0.02	25	1020	0	0.056	111	186	57
21	0.1	0.02	25	1020	0	(0.049)	(129)	184	56
22	0	0.02	22	1470	0	none			
23	-0.2[b]	0.02	22	1470	0	none			

[a] The values in the parentheses were obtained from a comparatively vague peak in the scattering curves, and their experimental errors would exceed ±5%. $2D_{exptl}$: the Bragg distance. $2D_0$: intermacroion distance calculated for simple cubic distribution. $2D_{theor}$: intermacroion distance calculated by the Lifson–Katchalsky theory for infinitely long rod-like macroions. α: degree of neutralization. c: polymer concentration. P_w: weight-average degree of polymerization. s_m: scattering vector at the peak position. [b] The solution contains PAA of acid form and HCl of 0.2 equiv to the polymer.

scattering[12,23] measurements, this fact suggests that the ordered structure in solutions is formed by the action of electrostatic forces. Table I shows that $2D_{exptl}$ **increases** (not decreases) with the addition of NaCl, if the small differences in the degree of polymerization and in temperature are ignored (experiments 5–8 or 10–12). The increase in $2D_{exptl}$ with the salt addition implies what was shielded by the salt must be mainly an "attractive" interaction between macroions. If the repulsive force is the factor responsible for the ordering, $2D_{exptl}$ should decrease with addition of salt, which is not the case.

We recall that the addition of simple salts caused diminution of the Bragg distance for the solution of tobacco mossaic virus.[2] In this case the solution was so concentrated that the interparticle force must have a repulsive character which can be weakened by the simple salts, bringing about a decrease in the Bragg distance. Also, we might have to take into account the difference in the rigidities of the virus and polyacrylate ions. The latter may coil up by salt addition, which changes also the potential field around themselves.

C. Dependence on Degree of Neutralization. The scattering curves at various degrees of neutralization (α) are given in Figure 3. When PAA was not neutralized, no peak was detectable (experiment 22), and when α was raised, a clear peak appeared. This observation is in agreement with that reported by Dušek et al.[18] Though omitted in Figure 3, we could observe a scattering peak even at $\alpha = 0.1$ (see Table I). These facts confirm the above-mentioned interpretation that electrostatic interaction is responsible for the ordered structure and also show that relatively small amounts of electric charges on macroions suffice to cause the ordering. Table I indicates that, at a given polymer concentration, the augmentation in α (an increase in the number of the charge) caused a decrease in $2D_{exptl}$ (experiments 3, 17, 19–21). We note that this tendency was observed also for the polymethacrylate solution by Dušek et al.[18] Because the increase in α causes an increase in the number of the charge, $2D_{exptl}$ should increase with increasing α if a repulsive force is responsible for the ordering. This is not the case, however, implying that the "attraction" between macroions is important.

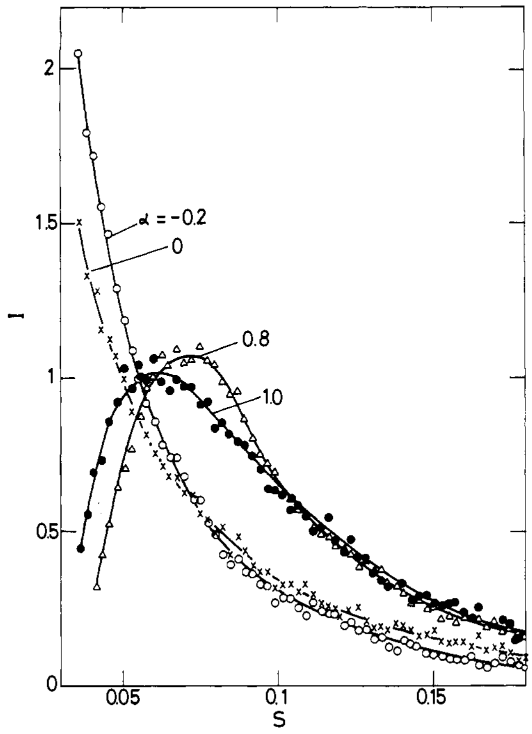

Figure 3. Scattered intensity (I) vs. scattering vector ($\mathbf{s}$) plots for NaPAA at 22 °C: influence of degree of neutralization (α) at P_w = 1470 and [NaPAA] = 0.02 g/mL.

D. Dependence on Degree of Polymerization. Figure 4 shows the scattering curves of two PAA fractions (degree of polymerization (P_w) = 760 and 5000). In both cases, a clear peak was observable and the s_m value depended on P_w as is seen from the figure and Table I. When P_w increased, $2D_{exptl}$ increased. When an equal volume of the solutions of the two fractions was mixed, a single peak was observed, and the s_m value fell between those of the two original fractions (experiments 13–15). We note that Rinaudo et al.[13] reported the insensitivity of the neutron scattering peak toward the degree of polymerization for polyglutamates (PGA). The discrepancy with our results appears to be due to

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[23] Nierlich, M.; Williams, C. E.; Boue, F.; Cotton, J. P.; Daoud, M.; Farnoux, B.; Jannink, G.; Picot, C.; Moan, M.; Wolff, C.; Rinaudo, M.; de Gennes, P. G. *J. Phys. (Paris)* **1979**, *40*, 701–704.

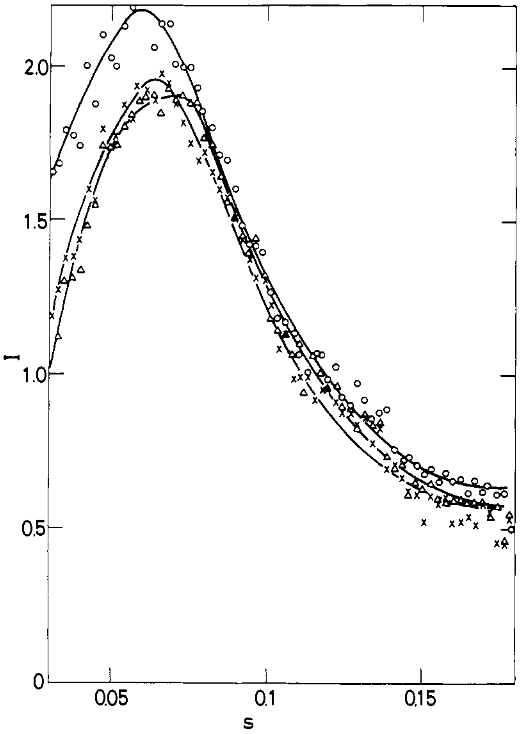

Figure 4. Scattered intensity (I) vs. scattering vector (s) plots for NaPAA at 26 °C: influence of degree of polymerization (P_w) ([NaPAA] = 0.02 g/mL, α = 0.5): O, P_w = 5000; ×, 5000 + 760; △, 760.

the low degrees of polymerization of PGA (23–560).

Discussion

A. Intermolecular Ordering or Intramolecular Ordering? Although the possibility of intramacroion ordering cannot be completely ruled out, we strongly believe that the observed peaks are due to intermacroion ordering for the following reasons. First, $2D_{\text{exptl}}$ is fairly large, and, second, mixing of two fractions gave a single peak (not two). If the peak is due to intramacroion ordering, we might expect two independent peaks after the fractions are mixed. This is not the case, however. Furthermore, the addition of NaCl increased $2D_{\text{exptl}}$, as mentioned above. The macroions are usually believed to shrink up when the salt is added to the solution. Thus, if $2D_{\text{exptl}}$ is related to the intramolecular ordering, it might be expected to become smaller with increasing salt concentration. The results show, on the contrary, that $2D_{\text{exptl}}$ increased with salt concentration. Similarly, the dependence of $2D_{\text{exptl}}$ on the degree of neutralization may be interpreted to disfavor the intramacroion ordering. It is generally accepted that the macroion chain would be expanded with increasing α. Thus if $2D_{\text{exptl}}$ is related to the intramacroion ordering, this quantity may be expected to increase with increasing α. These considerations may suggest that $2D_{\text{exptl}}$ represents the distance between the macroions which are distributed equidistantly. This conclusion, we note, is consistent with the suggestions of an ordered distribution of simple ions,[5,8,9] ionic surfactant micelles,[15,16] polymer latex particles,[17] proteins,[3] and viruses[2] in solutions.

B. "Attraction" between Macroions through the Intermediary of Gegenions and Two-State Structure of the Solution. In Table I is given the interparticle distance ($2D_0$) calculated with the assumption of simple cubic distribution of the macroions in solution.[22] It is most noteworthy that $2D_0$ is always larger than $2D_{\text{exptl}}$. We further note that this relation holds not only for our system but also for other cases studied by X-ray scattering[18] and by neutron scattering,[23] though the authors of this work did not pay attention to the cases in ref 18 and 23. For example, the values of $2D_0$ of sodium polystyrenesulfonate ($P_w/P_N < 1.2$) are 288, 160, and 110 Å at concentrations of 0.5×10^{-2}, 2.91×10^{-2}, and 9.09×10^{-2} g/mL whereas the $2D_{\text{exptl}}$ values calculated from the peak of the neutron scattering intensity are 190, 92, and 50 Å.[23] Thus, the relation $2D_0 > 2D_{\text{exptl}}$ appears to be generally valid in dilute solutions of polyelectrolytes. Then, it is most difficult to escape the conclusion that (1) an "attractive" interaction between the macroions is, though unexpectedly, operative and (2) the region, in which the macroions distribute in a regular manner with a distant $2D_{\text{exptl}}$, does not spread all over the solution but is localized. The second point leads us to the concept of the two-state structure. In order to facilitate understanding, we give a highly simplified scheme in Figure 5. In the drawing of the scheme, the macroion was pictured as a circle but this does not necessarily mean that all the macroions can be represented by rigid spheres. Naturally, the macroion would be expanded by intramolecular repulsive forces between ionized groups. However we have to take into account the mutual interaction with other macroions at concentrations being discussed in this paper, which might be expected to bring about shrinking of the charged chain. Thus, in this paper, we treat flexible macroions conveniently as rather permeable spherical coils of larger dimensions than those of the coil of uncharged chains, realizing that with decreasing concentration and degree of polymerization this will become increasingly inaccurate. In addition, the fact that a large portion of gegenions condense inside the macroion domain is not shown in Figure 5. Furthermore, we note that the scheme is drawn on the basis of highly reliable information on the solute distribution obtained by Hachisu et al.[17] for polymer latex solutions. These authors confirmed **visually** that dilute solutions of electrically charged latex particles contained both ordered and disordered regions, and the two regions were in dynamic equilibria. Thus, in Figure 5, a large number of macroions are shown to be in the ordered state together with their gegenions, whereas the rest are in the disordered state. At present, we are not finally sure about this simplification for polyelectrolyte solutions; in other words, it might be possible that *all* macroions exist only in the ordered state. In this respect, further quantitative analysis is definitely necessary.

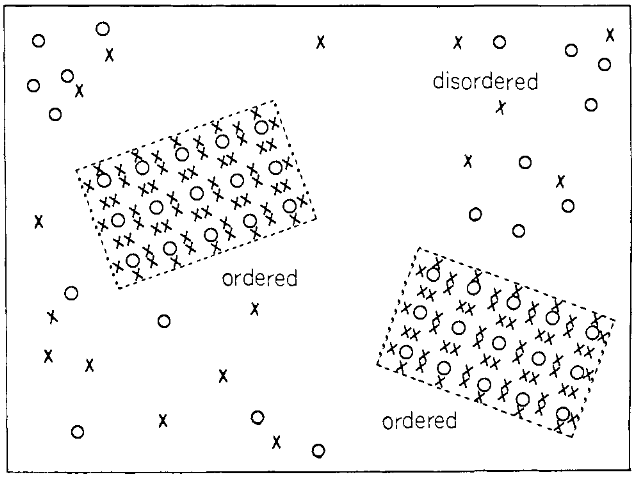

Figure 5. Simplified scheme of two-state structure of dilute solutions of polyelectrolytes. For simplicity, the macroion is represented by O and the gegenion by ×.

It should be particularly important to note the following. We are not claiming that the repulsion between the macroions does not exist in our system. It of course exists, but it is important only in the vicinity of the macroions. At larger distance from the macroion, it begins to "attract" other macroions as a result of a truly attractive interaction of these macroions with the gegenions which sit in the space between the macroions. The repulsion between the macroions is thus balanced with the intermacroion "attraction" at a distance which is $2D_{\text{exptl}}$. In other words, if the "attraction" does not exist and if only the repulsion is operative, $2D_{\text{exptl}}$ may be equal to $2D_0$. On the other hand, if only the "attraction" is important, $2D_{\text{exptl}}$ must be close to zero (provided that the thermal agitation is small).

It might be of some help to refer the readers to another similar, well-known situation, namely, the water structure. In the case of liquid water, the hydrogen bonds between water molecules lead to the formation of an iceberg (ordered) structure, which is in

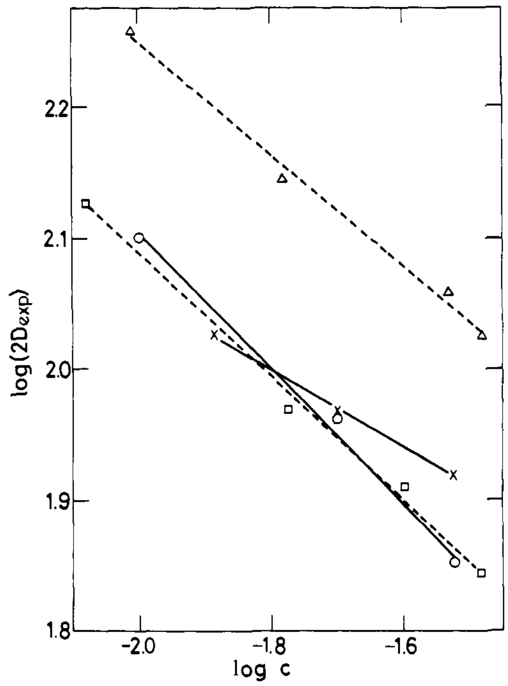

Figure 6. log ($2D_{\text{exptl}}$) vs. log c plots for NaPAA and NaPMA: (○) NaPAA, $\alpha = 0.65$, slope = −0.51; (×) NaPAA, $\alpha = 0.4$, slope = −0.29; (□) NaPMA,[18] $\alpha = 1$, slope = −0.46; (△) NaPMA,[18] $\alpha = 0.2$, slope = −0.43.

equilibrium with the free water molecules (in the disordered phase). Though the driving forces are quite different, it seems quite reasonable that the two-state structure exists in polyelectrolyte solutions. Our argument may be refuted on the basis of the dynamic character of the iceberg structure. At present, we have no experimental evidence indicating the time fluctuation of the ordered state for polyelectrolyte systems. However, the polymer latex solution again shows unequivocally that the ordered and disordered states fluctuate with time. Though the pictures given in ref 17 do not show the fluctuation of the ordered state, the authors described the situation as follows: "At one moment an ordered portion disintegrated into disorder and in the next moment an ordered aggregate appeared at another place". Furthermore, the motion picture shows the fluctuation most vividly.[24]

A brief comment is necessary on the concentration dependence of $2D_{\text{exptl}}$ in relation to the two-state structure. It was reported by Bernal and Fankuchen[2] and Riley and Oster[3] that the $2D_{\text{exptl}}$ value decreased linearly with the cube root for human hemoglobin and with the square root of concentration for the tobacco mosaic virus, serum albumin, and DNA. The cube-root and square-root dependences were related to the three- and two-dimensional regularities of the solute distribution, respectively. This reasoning is justified for the highly concentrated solution or gel state because the ordered structure occupies practically the entire volume of the system, in other words, because they had a one-state structure. (Actually under their condition $2D_{\text{exptl}} \simeq 2D_0$.) Recently, a similar kind of reasoning was extended to dilute solutions of polymethacrylate[12,18] and polyglutamate,[13] and the square-root dependence was found to be valid. On the basis of this concentration dependence, it was commonly claimed that the Lifson–Katchalsky theory (to be discussed below) was correct. In this respect, our own data are plotted against the logarithm of the (total) polymer concentration in Figure 6. For $\alpha = 0.65$ and 0.4 the slopes were −0.51 and −0.29, respectively. For comparison, $2D_{\text{exptl}}$ values of sodium polymethacrylate reported by Dušek et al.[18] are shown and the slope was −0.46 for $\alpha = 1$ and −0.43 for $\alpha = 0.2$. It is tempting to suggest that the macroions distribute in a two-dimensional regularity at high α (with a slope of −0.5) whereas the distribution is rather close to three-dimensional regularity at low α. However, as was emphasized above, we have the two-state structure; the sum of the volume of the ordered structure amounts only to about 0.125 of the total solution volume,[25] because $2D_{\text{exptl}}$ is approximately half of $2D_0$ (see Table I). Under such a circumstance, it seems difficult to discuss the mode of the distribution and the geometry of the macroions from the plot of $2D_{\text{exptl}}$ against the analytical polymer concentration.

C. Comparison with Rod-Like Models. About 25 years ago, an ordered distribution of the macroions was taken into consideration in a theory of Lifson and Katchalsky,[26] in which the macroions were assumed to be fully stretched and distributed parallel and equidistantly in the entire volume of solution. It is worthwhile to compare our present results with the theory. In this respect, an interparticle distance was calculated by this theory and given in Table I (see $2D_{\text{theor}}$ values[27]). We note that $2D_{\text{exptl}}$ is roughly of the same order of magnitude as $2D_{\text{theor}}$. The better agreement may be claimed at a high degree of neutralization. Thus, it might be the case that the macroions take a stretched conformation when they are highly charged as the theory assumed. However, one difficulty exists in this argument. It is the dependence of $2D_{\text{exptl}}$ or $2D_{\text{theor}}$ on the degree of polymerization (P_{w}). As is seen from Table I, $2D_{\text{exptl}}$ varied with P_{w} whereas $2D_{\text{theor}}$ does not at all because infinitely long rods were assumed for the macroions in the theory.[28] This tells us that an extension of the Lifson–Katchalsky theory is necessary before the justification of its use in the present case can be definitely claimed.

It is also worth considering the hexagonal packing of fully stretched (infinitely long) rod-like macroions in dilute solutions. In this treatment, no dead space is allowed between the macroions (unlike the Lifson–Katchalsky theory), so that the intermacroion distance $2D_{\text{hex}}$ [$(W/N_{\text{A}}lc \sin 60)^{1/2}$] was smaller than $2D_{\text{theor}}$ and $2D_{\text{exptl}}$. For example, $2D_{\text{hex}}$ values were 59 and 47 Å for experiments 1 and 2, respectively. Even if we regard the difference between $2D_{\text{exptl}}$ and $2D_{\text{hex}}$ as insignificant, we should have a one-state structure, in which the hexagonal array of the rod-like macroions covers the whole solution. In other words, a great number of fully stretched macroions of a definite degree of polymerization must be lined up to form a (infinitely) long rod, which then builds up the hexagonal array with other rods. This situation appears to us most implausible. If this one-state structure were real, we may expect optical anisotropy of such solutions. To our knowledge, this kind of observation has not been reported. Furthermore, it is to be noted, the observed P_{w} dependence of $2D_{\text{exptl}}$ cannot be accounted for in terms of the hexagonal packing of infinitely long rods.

Concluding Remarks

We described our experimental data and interpretation on sodium polyacrylate in the preceding sections. We note that qualitatively similar results were obtained recently for poly-L-lysine on the dependences of $2D_{\text{exptl}}$ on polymer concentration, salt concentration, and degree of polymerization, which will be described in a forthcoming paper.[29] Also for poly-L-lysines of high molecular weight, $2D_{\text{exptl}}$ was smaller than $2D_0$ in the same concentration range in this paper.[30] Systematic studies are being

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(24) Hachisu, S., private communication.

(25) In this estimation, all of the macroions are assumed to be in the ordered phase. If this is not, as is depicted in Figure 5, the ordered structure must occupy a smaller volume than 0.125 of the solution volume.

(26) Lifson, S.; Katchalsky, A. *J. Polym. Sci.* **1954**, *13*, 43–55.

(27) The theoretical distance, $2D_{\text{theor}}$, was obtained from $2D_{\text{theor}} = 2(w/\pi N_{\text{A}}lc)^{1/2}$, where l is the length of the monomer unit, and the other notations were described in ref 22.

(28) This argument may be criticized for the reason that the degree of neutralization is too low to apply the theory. According to our recent unpublished data on fully protonated poly-L-lysine,[29] however, $2D_{\text{exptl}}$ also increased with P_{w}.

(29) Ise, N.; Okubo, T.; Yamamoto, K.; Kawai, H.; Hashimoto, T.; Fujimura, M.; Hiragi, Y., in preparation.

(30) As was already mentioned in the text, our interpretation is that the macroions attract each other through the intermediary of oppositely charged gegenions. For detail, the readers are referred to our recent review article,[1b] in which the stability of ionic crystals and ionic aggregates and the unusual concentration dependence of the activity of the macroion species and ionic detergents were discussed with reference to such net attractive forces between similarly charged ionic species through the intermediary of gegenions.

carried out in our laboratory on other polyelectrolytes and electrically charged particles in general in order to establish a unified interpretation on the structure of dilute solutions of these solute particles.[31]

Finally we note that the experimental data of the small-angle X-ray scattering (except the molecular weight dependence) can also be described by an isotropic model proposed by de Gennes et al.[34] A detailed check of this model on macroion systems will be reported later, although it obviously cannot be applied to other charged systems.

Acknowledgment. H.K. acknowledges a grant administered by the Ministry of Education, Japan, for the construction of the SAXS apparatus (243021). The sedimentation measurements were carried out with the kind help from Professor H. Inagaki and Dr. T. Fukuda, Kyoto University, to whom our thanks are due.

Note Added in Proof: Almost complete insensitivity of the position of the peak of the neutron scattering toward salt concentration was reported for a polystyrenesulfonate by Nierlich et al.[23] This is in contradiction with our X-ray scattering data (Figure 2 and Table I). However, the French group observed recently the same salt concentration dependence for the polystyrenesulfonate as ours.

Rinaudo and Milas kindly drew our attention to their recent observation on xanthane showing again insensitivity of the neutron scattering peak toward degree of polymerization, which does not agree with our results described in the present paper. Both their results and ours can be reasonable, however, because xanthane molecules probably assume comparatively stretched conformation as a result of the chain stiffness so that chain-end effect in this case might be much less significant than in loosely coiled macroions (like polyacrylate).

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(31) In a recent paper (*Makromol. Chem.* **1977**, *178*, 2429–2353), Dolar et al. have thrown strong doubt on the presence of the lattice structure in polyelectrolyte solutions suggested earlier by us.[7] The SAXS and SANS studies described in this article provide a clear answer to their questioning, indicating that their conclusion is at fault. Furthermore, it is to be noted that the cube-root dependence of the mean activity coefficients has not been claimed by us to hold *always* for all polyelectrolytes, as Dolar et al. seem to believe. As a matter of fact, for example, we observed marked deviation from this dependence for polyglutamate (PGA) and DNA.[32,33] Nonetheless, the neutron scattering shows the presence of an ordering in PGA solution.[13] The cube-root dependence may suggest the presence of an ordered structure, but converses are not always true. Thus, even if one accepts the finding by Dolar et al. that the cube-root relation does not apply to cadmium polystyrenesulfonate, this does not imply the absence of the ordered structure for this particular polymer. The interpretation advanced by Dolar et al. should thus be discarded.

(32) Ise, N.; Okubo, T. *Macromolecules* **1969**, *2*, 401–407.

(33) Okubo, T.; Ise, N. *Macromolecules* **1969**, *2*, 407–411.

(34) de Gennes, P. G.; Pincus, P.; Velasco, R. M. *J. Phys.* (*Paris*) **1976**, *37*, 1461–1472.

(35) Nierlich, M.; Jannink, G., private communication.

(36) Rinaudo, M.; Milas, M., private communication.

Proximity of Metal Ions and Hydrocarbon Side Chains of Chelated α-Amino Acids and Peptides[1]

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Abstract: In order to elucidate the factors responsible for neutral, unbound side-chain conformations in chelates of α-amino acids and peptides, we evaluated rotamer populations about the α–β bond by vicinal proton coupling constant analysis in well-defined complexes of tetragonal, diamagnetic Pd(II). In a dipeptide, for example, the backbone chelates the Pd(II) in two five-membered rings by amino and ionized amide nitrogen and carboxylate oxygen donor atoms. For aromatic phenylalanine or tyrosine side chains in the carboxylate terminal residue of a dipeptide, the mole percentage of the rotamer that directs the side chain over the metal ion rises to about 60% from about 20% in unbound ligand. A similar population increase is also observed in tripeptide complexes of both Pd(II) and diamagnetic Ni(II). Increases in the two rotamers that direct aliphatic side chains toward the metal ion are also observed in complexes of amino acids and peptides of valine and isoleucine. The mole percentages in the complexes are little changed by 80% Me_2SO solvent in place of water. Since several di- and tripeptides contain only one side chain, hydrophobic interactions between side chains cannot be responsible for favoring placement of side chains over the metal ion. There is some precedent for direct metal ion–aromatic side chain interactions in crystal structures. Direct, weak metal ion–aliphatic side chain interactions may be of sufficient energy to affect relative rotamer populations. Alternatively, aliphatic and also aromatic side chains may simply prefer space over a metal ion to a greater interaction with solvent.

What is the disposition of the side chains in chelated α-amino acids and peptides? To what extent do across chelate ring side chain–side chain interactions determine side-chain conformation? Do other kinds of interactions also influence conformation? This paper reports results for hydrocarbon side-chain conformations as deduced from ^1H NMR vicinal coupling constants. Even in the absence of side chain–side chain interactions in the chelated ligands, hydrocarbon side chains are found to favor the conformation that projects them toward the metal ion.

In solution, a lesser difference between the logarithms of the first and second stability constants for Cu^{2+} and phenylalanine compared to alanine (1.3 vs. 1.0), for example, has been interpreted as due to an unusually large second stability constant in the bis(phenylalanine) complex.[3] This result suggests a modest degree of favorable interaction between the two aromatic rings in the bis complex. Some additional stability in the mixed complex of tryptophan and adenosine triphosphate has been assigned to interactions between the two kinds of aromatic rings.[4,5] Favorable side-chain interactions between aromatic bipyridyl or 1,10-phenanthroline and aliphatic amino acid side chains have also been

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[1] This research was supported by a grant from the National Science Foundation. P.I.V. gratefully acknowledges a fellowship from the Royal Norwegian Council for Scientific and Industrial Research.

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(3) Martin, R. B. *Met. Ions Biol. Syst.* **1979**, *9*, 1–39.

(4) Sigel, H.; Naumann, C. F. *J. Am. Chem. Soc.* **1976**, *98*, 730.

(5) Mitchell, P. R.; Prijs, B.; Sigel, H. *Helv. Chim. Acta* **1979**, *62*, 1723.

0002-7863/80/1502-7906$01.00/0