SAXS studies on added-salt species dependence of conformation of fully-charged poly(L-glutamate) in aqueous solutions

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Abstract. The effect of counter-ion species on the local conformation of fully-charged poly(L-glutamate) in added-salt aqueous solutions were studied by small-angle X-ray scattering (SAXS). In the case of presence of monovalent counter-ion, the scattering behaviour could be explained by taking into consideration the excluded volume effect and intermolecular-interface interaction on the scattering function of wormlike chain. On the other hand, since SAXS profile of the solution in the presence of divalent counter-ion well fitted to the scattering function of dendrimer, it suggested that the conformation of polymer chain in such solution would be more compact conformation than that of presence of monovalent ion.

1. Introduction

Some of polypeptide chains exhibit a transition between helical state and random-coiled state, and the transition is useful employed as a model for the phenomena of folding or denaturation of proteins. Poly(L- or D-glutamic acid) (PLGA or PDGA) shows such a conformational transition when the pH of the solution is changed. Many studies have been directed to the analysis of the structure in each conformational state: By intrinsic viscosity and light scattering, Holtzer et al. [1] have shown that the conformation of fully-charged poly(sodium L-glutamate) PLGA-Na is represented by random coil with unperturbed effective bond length of ca. 8 Å. Muroga et al.[2] have estimated the averaged helical-sequence length of PDGA-Na in the helical- and helix/coil transition-states by small-angle X-ray scattering (SAXS).

In general, counter-ions as well as bi-ions[3] affect the conformation of charged PLGA. The bond formed between carboxylate-ion and counter-ion bears both features of ionic- and covalent-natures and their fraction would depend on the ionisation energy of the counter-ion. Accordingly, the conformation of PLGA-salt should also depend on the species of counter-ions, but the counter-ion species dependence of the conformation has not been fully clarified yet.

In the present work, our attention is directed to the local conformation of fully-charged PLGA in random-coiled state in added-salt aqueous solution, and the counter-ion species dependence of the conformation is studied with respect to Na⁺, Li⁺, (CH₃)₄N⁺(TMA⁺), Mg²⁺, Ca²⁺ and Ba²⁺, where these ions are known to have different ionisation energy. The conformation
was clarified by analyzing SAXS curve in a high scattering-vector range, where the data would reflect a part of a polymer chain and the analytical result was expected to be insensitive to the molecular-weight distribution of the sample and free from possible excluded volume effect.

2. Experimental Section

2.1. Samples and Preparations

PLGA-Na of $M_\text{w} = 1.78 \times 10^4$(Sample-I) and $5.48 \times 10^4$(Sample-II) (Sigma-Aldich Co.) were converted to the acid form PLGA by passing them through a column filled with a mixed-bed of ion-exchange resins, Amberlite IR-120B and IR-400. The potentiometric titration of PLGA was carried out in 0.05M NaOH aqueous solution with 0.2M NaCl under the atmosphere of N$_2$ gas at room temperature with Accumet Model-15 pH meter(Fisher Scientific Co.). The degree of neutralisation of carboxylic-group $\alpha$ for the sample employed in SAXS was adjusted with respective hydroxide on the basis of the titration data. The concentration $C_\text{p}$ of added salts, NaCl, LiCl, (CH$_3$)$_2$NCl(TMACl), MgCl$_2$, CaCl$_2$ and BaCl$_2$, was 0.02 or 0.2 mol/L and the polymer concentration $C_\text{p}$ ranged from $2.5 \times 10^{-3}$ to $1.0 \times 10^{-2}$ g/mL (Table1).

2.2. SAXS measurements

SAXS measurements were performed at the Photon Factory of High Energy Accelerator Research Organization, Tsukuba, Japan. The wavelength of incident X-ray $\lambda$ was 1.488Å. The scattered X-ray was detected by a one-dimensional position sensitive proportional detector(PSPC) with 512 channels. The absolute value of scattering vector $|q|/(q = (4\pi/\lambda)\sin(\theta/2))$, where $\theta$ is a scattering angle) per channel was evaluated with diffraction from a reference sample, chicken tendon collagen. The sample cell, made of stainless steel, had quartz windows of 20µm thickness whose intervals were 1mm. The excess scattered intensity $I(q)$ of the sample solution over the solvent was obtained after transmission corrections for solutions and solvents. The details of the apparatus and the measurement are described elsewhere[4].

3. Results and Discussion

Figure 1(a) shows the scattering profile for PLGA-Na with $C_\text{p} = 5.0 \times 10^{-3}$ g/mL (O ) and $1.0 \times 10^{-2}$ g/mL (×) in 0.2M NaCl aqueous solution, as an example, plotted in the form of $I(q)$ vs $q$. As is shown in Figure 1(b), if $I(q)$ is reduced to $I(q)/C_\text{p}$, the two scattering profiles satisfactorily coincided on a single curve over $q$ ranging from 0.015 to 0.35 Å. Therefore, it was safely assumed that scattering data for all samples with $C_\text{p}$ of $2.5 \times 10^{-3}$ to $1.0 \times 10^{-2}$ g/mL would reflect only intramolecular-interferences, at least, in that $q$ range.

In general, $I(q)$ of a rodlike polymer chain is given by[5]:

$$I(q) \propto I_{\text{thin}}(q)I_{\text{cs}}(q)$$  \hspace{1cm} (1)

where $I_{\text{thin}}(q)$ is scattering intensity of a hypothetical chain with no cross-section and $I_{\text{cs}}(q)$ is the cross-sectional factor. $I_{\text{thin}}(q)$ is proportional to $1/q$ for a needle-like rod and $I_{\text{cs}}(q)$ is proportional to $\exp(-q^2/R_{\text{cs}}^2)$ for a chain with mean-squared radius of a cross-section $<R_{\text{cs}}^2>$. In a $q$ range of:

$$\frac{1}{L_p^2} < q^2 < \frac{1}{<R_{\text{cs}}^2>},$$  \hspace{1cm} (2)

where $L_p$ is persistence length, the local structure of a polymer chain could be regarded as a rigid-rod. Thus, when $I(q)$ is plotted in the form of $\ln(I(q)/q)$ vs $q^2$, one can draw a straight line in a $q$ range of Eq.(2), the slope of which, $-\text{slope}$, is related to $<R_{\text{cs}}^2>$ through the relation of $<R_{\text{cs}}^2> = 2 \cdot (-\text{slope})$.

In Figure 2 is shown an example for the plot of $\ln(I(q)/q)$ vs $q^2$ for PLGA-salts in the presence of (a) Na$^+$ and (b) Mg$^{++}$. $<R_{\text{cs}}^2>^{1/2}$ for all samples thus evaluated is listed in Table 1. For
Figure 1. SAXS profile of Sample-I of $\alpha = 0.85$ in 0.2M NaCl. (a): Plot of $I(q)$ vs. $q$ for ($\bigcirc$) $C_p = 5.0 \times 10^{-3}$ g/mL and ($\times$) $C_p = 1.0 \times 10^{-2}$ g/mL. (b): Plot of $I(q)/C_p$ vs $q$.

all counter-ion species, $< R_{cs}^2 >^{1/2}$ is 4.0 ± 0.5 Å, which is slightly smaller than that expected for PLGA-Na if its side chain takes a fully-stretched form, 5.2 – 5.6 Å[6].

The Kratky plot $I(q)q^2$ vs $q$ is shown for all samples in Figure 3. From the appearance of their profiles, they seem to be classified into three categories: Category (I) for Li$^+$ (a), Na$^+$ (b), TMA$^+$ (c), and Mg$^{++}$ (d), where the profile is represented by a smoothly-increasing curve when $q$ is increased; Category (II) for Ca$^{++}$ (e), where it is represented by a horizontal curve in a higher $q$ range; Category (III) for Ba$^{++}$ (f), where it is represented by a curve with a bump in a small $q$ range. The features in Category (I), (II) and (III) are observed in the Kratky plot of a wormlike chain, Debye function for Gaussian chain and gel-like structure having high segment-density, respectively.

The profiles (a)-(d) in Figure 3 were compared with scattering function of wormlike chain
Figure 2. Plot of ln(I(q)q) vs. $q^2$ for Sample-I. ●: Sample-I in NaCl, $\alpha = 0.85$, $C_s = 0.02$M, $C_p = 1.0 \times 10^{-2}$ g/mL. ○: Sample-II in MgCl$_2$, $\alpha = 0.77$, $C_s = 0.02$M, $C_p = 2.5 \times 10^{-3}$ g/mL.

Table 1. Root mean-square radius of cross-section $< R_{cs}^2 >^{1/2}$ for PLGA in random-coil region.

| Sample | Added salt | $\alpha$ | $C_s$ (mol/L) | $C_p$ (g/mL) | $< R_{cs}^2 >^{1/2}$ (Å) |
|---------|------------|----------|--------------|--------------|-------------------|
| Sample-I | LiCl       | 0.84     | 0.2          | 0.010        | 4.0±0.5          |
| Sample-I | NaCl       | 0.85     | 0.2          | 0.010        | 4.0±0.5          |
| Sample-I | NaCl       | 0.85     | 0.2          | 0.010        | 4.0±0.5          |
| Sample-II| NaCl       | 0.85     | 0.02         | 0.005        | 4.0±0.5          |
| Sample-I | TMACl      | 0.84     | 0.2          | 0.010        | 4.0±0.5          |
| Sample-II| MgCl$_2$   | 0.77     | 0.02         | 0.0025       | 4.0±0.5          |
| Sample-II| CaCl$_2$   | 0.86     | 0.02         | 0.0050       | 4.0±0.5          |
| Sample-II| CaCl$_2$   | 0.90     | 0.02         | 0.0050       | 4.0±0.5          |
| Sample-II| CaCl$_2$   | 0.85     | 0.02         | 0.0025       | 4.0±0.5          |
| Sample-II| BaCl$_2$   | 0.83     | 0.02         | 0.0025       | 4.0±0.5          |
| Sample-II| BaCl$_2$   | 0.86     | 0.02         | 0.0050       | 4.0±0.5          |
| Sample-II| BaCl$_2$   | 0.90     | 0.02         | 0.0050       | 4.0±0.5          |
Figure 3. Kratky plot of PLGA aqueous solution in the presence of several added salt species without cross-section correction. (a) •: Sample-I in LiCl, $\alpha=0.84$, $C_s = 0.2M$, $C_p = 0.01$ g/mL, (b) ■: Sample-I in NaCl, $\alpha=0.85$, $C_s = 0.2M$, $C_p = 0.01$ g/mL, (c) ▲: Sample-I in TMA.Cl, $\alpha=0.85$, $C_s = 0.2M$, $C_p = 0.01$ g/mL, (d) ▲: Sample-II in MgCl$_2$, $\alpha=0.77$, $C_s = 0.02M$, $C_p = 2.5 \times 10^{-3}$ g/mL, (e) ◦: Sample-II in CaCl$_2$, $\alpha=0.84$, $C_s = 0.02M$, $C_p = 2.5 \times 10^{-3}$ g/mL, (f) ●: Sample-II in BaCl$_2$, $\alpha=0.83$, $C_s = 0.02M$, $C_p = 2.5 \times 10^{-3}$ g/mL. (e) ◦: Sample-II in CaCl$_2$, $\alpha=0.84$, $C_s = 0.02M$, $C_p = 2.5 \times 10^{-3}$ g/mL.

$P(q)$$^{[7][8]}$:

$$P(q) = 2u^{-2} \left[ \exp(-u) - 1 + u \right] + \frac{4}{15L_r} + \frac{7}{15L_r u} - \left( \frac{11}{15L_r} + \frac{7}{15L_r u} \right) \times \exp(-u)$$  \hspace{1cm} (3)$$

where $u$ and $L_r$ are defined by the contour length of a polymer chain $L$ and the persistence length $L_p$ as

$$u = \frac{L L_p q^2}{3}$$  \hspace{1cm} (4)$$

$$L_r = \frac{L}{2L_p}$$  \hspace{1cm} (5)$$
Figure 4. Comparison between $I(q)q^2$ and theoretical scattering curves (solid curve). Observed curves: (a) •: Sample-I in LiCl, $\alpha=0.84$, $C_s=0.2M$, $C_p=0.01$ g/mL, (b) □: Sample-I in NaCl, $\alpha=0.85$, $C_s=0.2M$, $C_p=0.01$ g/mL, (c) ▲: Sample-I in TMACl, $\alpha=0.85$, $C_s=0.2M$, $C_p=0.01$ g/mL, (d) ▲: Sample-II in MgCl$_2$, $\alpha=0.77$, $C_s=0.02M$, $C_p=2.5 \times 10^{-3}$ g/mL, (e) ○: Sample-II in CaCl$_2$, $\alpha=0.84$, $C_s=0.02M$, $C_p=2.5 \times 10^{-3}$ g/mL, (f) ●: Sample-II in BaCl$_2$, $\alpha=0.83$, $C_s=0.02M$, $C_p=2.5 \times 10^{-3}$ g/mL.

Eq.(3) is valid under the conditions,

$$L_t > 10 \quad \text{and} \quad (2L_pq)^2 < 10$$  \hspace{1cm} (6)

A solid curve in (a)-(d) of Figure 4 is a theoretical curve computed with Eq.(3). It is seen that the observed data in a higher $q$ range is well mimicked by the theoretical curve, although the data in a lower $q$ range deviates downward from the curve. When a slight effect of excluded-volume effect[7] and intermolecular-interferences[9][10] are taken into account, however, these deviations were significantly improved, as is shown by a solid curve in Figure 4. The persistence length $L_p$ thus evaluated is 5Å for PLGA-Li, 6Å for PLGA-Na, 6Å for PLGA-Mg and 8Å for PLGA-TMA, respectively (Table 2). Such a difference in $L_p$ among PLGA-salts having different counter-ions might suggest that bulkiness of counter-ion condensed around the polymer chain seriously affects the stiffness of the chain. In Figure 4(e), the observed data are well compared with the theoretical scattering function for wormlike chain, with $L_p$ of 6 Å.
Table 2. The persistence length $L_p$ estimated by the scattering function of wormlike chain with the excluded volume effect and the intermolecular interaction.

| Solvent       | $C_s$ (mol/L) | $L_p$ (Å) |
|---------------|---------------|-----------|
| LiCl aq.      | 0.2           | 5.0       |
| NaCl aq.      | 0.2           | 6.0       |
| TMACl aq.     | 0.2           | 8.0       |
| MgCl$_2$ aq.  | 0.02          | 6.0       |
| CaCl$_2$ aq.  | 0.02          | 6.0       |

The Kratky plot of PLGA-Ba was analysed with $P(q)$ for regular-network polymer, a so-called dendrimer [11]:

$$S(q) = N_b \left[ S_{sb}^S(q) + S_{sb}^I(q) + S_{sb}^a(q) \right] + N_b(N_b - 1)S_{ib}(q)$$  \hspace{1cm} (7)

where $S_{sb}^S(q)$ is one intrabranch self correlation part, $S_{sb}^I(q)$ one intrabranch cross-correlation part between blocks that originate from same, $S_{sb}^a(q)$ one intrabranch cross-correlation part form different stems, $S_{ib}(q)$ one intrabranch correlations part, and these correlation parts are described in terms of segment length $b$, $N$ generations of monomeric blocks, $f + 1$ functionality parameter, $N_b$ number of branches and $n$ number of monomers forming Gaussian links with segments. The solid curves in Figure 4(f) are theoretical curves calculated by Eq. (7). The curve shows a good fit to the data when the parameters of $b = 10$, $N = 4$, $N_b = 2$, $f = 2$, $n = 10$ are chosen. This result would suggest that a gel-like network structure is developed in PLGA-Ba.

In general, the bond formed between carboxylate-ion and counter-ion bears both ionic- and covalent-bonding natures and their relative weights would depend on the ionisation energy of the counter-ion. That is, the ionic-feature of the bond would be more accentuated as the ionisation energy of counter-ion becomes lower. In the case of divalent counter-ions, the increase of the ionic-feature of the bond would lead to the inter- or intra-molecular bridging of the divalent-ion to two carboxylate-ions and, as a result, to the formation of the network chain. The present result that the conformation of PLGA-salts would become more compact and gel-like in the order of Ba$^{2+}$ > Ca$^{2+}$ > Mg$^{2+}$ is compatible with the fact that the first ionisation energy is in the order of Ba$^{2+}$ (5.2 eV) < Ca$^{2+}$ (6.1 eV) < Mg$^{2+}$ (7.6 eV).

4. Conclusions
The local conformation so fully charged PLGA in the presence of counter-ion was studied by small-angle X-ray scattering. In the presence of Li$^+$, Na$^+$, TMA$^+$, Mg$^{2+}$ and Ca$^{2+}$, their SAXS curves were well explained the scattering function of the wormlike chain by taking into account the excluded volume effect and intermolecular interferences. In the presence of Ba$^{2+}$, however, the SAXS curve was only explained by the scattering function of dendrimer.

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