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Conformational effects on cationization of poly(ethylene glycol) by alkali metal ions in matrix-assisted laser desorption/ionization time-of-flight mass spectrometry

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Abstract

Conformational effects of polymer chains on matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOFMS) were studied by using an equimolar mixture of uniform poly(ethylene glycol)s (PEGs) and by molecular dynamics simulations. Uniform PEGs with degrees of polymerization $n = 8$–39 were separated from commercial PEG samples by preparative supercritical fluid chromatography. MALDI-TOFMS spectra of an equimolar mixture of the uniform PEGs in aqueous ethanol were measured by adding a mixture of 2,5-dihydroxybenzoic acid (as a matrix reagent) and five alkali metal chlorides (LiCl, NaCl, KCl, RbCl, and CsCl). After optimization of the matrix concentration and laser power, five types of adduct cationized by Li^+, Na^+, K^+, Rb^+, and Cs^+ could be identified simultaneously in the same spectrum. In the lower molecular-mass region around 10^3, the spectral intensity increase rapidly with increasing molecular mass of PEG; this rapid increase in the spectral intensity started at a lower molecular mass for smaller adduct cations. Molecular dynamics simulations were used to calculated the affinity of PEG for the adduct cations. These experimental and simulated results showed that the observed spectral intensities in MALDI-TOFMS were markedly affected by the species of adduct cations and the degree of polymerization of the PEG, and that they were dependent on the stability of the PEG–cation complex.

Keywords: MALDI-TOFMS; Molecular dynamics; Poly(ethylene glycol); Uniform oligomer; Alkali metal ion

1. Introduction

The technique of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOFMS) [1,2] has led to major developments in the analysis of the composition and structure of synthetic polymers [3,4]; however, the precision in quantitative analysis is not completely understood because of the presence of a number of nonquantitative effects, such as a dependence on laser power [5–7], detector saturation [8–10], sample preparation method [11–14], and the physical properties of the object polymer. Some of these effects can be avoided by careful manipulation.

One of the most serious and unavoidable effects is the conformational effect of the polymer and additive cation, which originates from their chemical structures. For poly(ethylene terephthalate) (PET) oligomer with a degree of polymerization index of $n = 3$, two isomers – an open form and a closed form – were observed and predicted from theory by Gidden et al. [15]. They reported that the populations of the open and closed isomer forms were markedly dependent on the laser power and that the mass spectrum of a PET oligomer sample was strongly affected by the nature of the cationizing alkali metal ion. Ehlera et al. [16] applied density functional calculations to obtain the binding energy for metal cation–oligomer complexes of *n*-alkanes and

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of poly(ethylene glycol)s (PEGs). Reinhold et al. [17] showed by experiments and ab initio quantum calculations that PEG oligomers cannot be detected when they consist of fewer than five monomer units.

PEG is one of the most interesting polymers for studying the relationship between the conformation of a polymer and its MALDI spectrum, because it can form a helical structure that has a similar diameter to that of alkali metal ions. Von Helden et al. [18] studied the structure of the complex of PEG with sodium ion at various temperatures by molecular mechanics methods and by using a MALDI source coupled to an ion-chromatography instrument. Togashi and Kobayashi [19] analyzed the observed relative intensities of PEG monododecyl ether ($n = 1$–8) cationized by Li^+, Na^+, and K^+ by using a one-dimensional random-walk model for the migration of a metal ion in the PEG chain. The larger metal ions were able to move within the PEG chain more easily than could the smaller ones, which implied that the binding energy between the metal ion and an oxygen atom in the PEG chain decreases in the order $Li^+ > Na^+ > K^+$.

These results suggest that PEGs with higher degrees of polymerization are more stable in the form of adducts cationized by larger ions. The same phenomenon has actually been observed for other kinds of polymer, such as poly(methyl methacrylate) [20,21] and PET [22], i.e., the presence of larger alkali metal cations produces shifts in the molecular-mass distribution to the higher molecular-mass region. However, important results were reported by Wang and co-workers [23,24] that appear at first sight to be inconsistent with the above conclusion. Cesiated PEG ion intensities were much stronger than those of the corresponding lithiated PEG ions when indolacrylic acid was used as the matrix. In addition, like poly(propylene glycol) displayed different selectivities for Cs^+ and Li^+ with different matrices.

We think the reason for these inconsistencies lies in a failure to distinguish between solubility and affinity in relation to polymers and cations. If the object polymer has a molecular-mass distribution, it is not easy to distinguish between the various causes of increases in spectral intensities. One possible cause of such an increase is a change in the solubility of the polymers, cations, and matrix reagents; another is a change in the stability of complexes of polymers and cations at different degrees of polymerization (n). In this study, we tried to resolve this confusion by using an equimolar mixture of uniform PEG oligomers.

Uniform oligomers are special polymers that essentially have no molecular-mass distribution and are useful in evaluating the quantitativeness of MALDI-TOFMS [25–27]. We separated uniform PEGs with degrees of polymerization of $n = 8$–39 from commercial PEG samples by preparative supercritical fluid chromatography (SFC) [28]. First, the optimum matrix concentration for avoiding a dependence of the MALDI spectra on the laser power was determined. Then, by using a fitting function we determined characteristic n values for five cations (Li^+, Na^+, K^+, Rb^+, and Cs^+) at which the spectral intensity I begins to increase rapidly. Furthermore, we applied molecular dynamics (MD) simulations to determine the affinities between oligomers with different ns and three cations: Li^+, Na^+, and K^+.

2. Experimental

2.1. Preparation of an equimolar mixture of PEGs

Uniform PEGs were separated from commercial samples of PEG 400, PEG 1000, and PEG 1540 [Wako Pure Chemical Industries Ltd., Osaka, Japan, $HO(CH_2CH_2O)_nH$] by preparative SFC (JASCO Co., Tokyo, Japan, SUPER-201) with a Superpak SIL60-5 silica gel column (JASCO Co., Tokyo, Japan). An evaporative light-scattering detector (Alltech Associates, IL, USA; ELSD MKIII) was operated at a drift-tube temperature of 90 °C. A modifier gradient was used to separate the uniform PEGs. Details of the separation conditions have been given previously [6,28].

The mole fraction of each uniform PEG in an equimolar PEG mixture in extra-pure water was accurately determined by means of total organic carbon (TOC) measurements. In comparison with weighing each PEG by using a microbalance, this method has the advantage of being free from errors caused by residual water in the PEGs. TOC measurements were carried out with a SHIMADZU TOC-500 apparatus with potassium biphthalate [Tomiyama Pure Chemical Industries, Tokyo, Japan; $C_6H_4(COOK)(COOH)$] as the calibration reagent. The equimolar mixture consisted of eight uniform PEGs with degrees of polymerization $n = 8$, 12, 17, 21, 26, 30, 34, and 39. After the TOC measurements had been made, the aqueous mixture of equimolar PEGs was dried under a vacuum: the total weight of the equimolar mixture of eight uniform oligomers was 8 μg.

2.2. Measurement of MALDI-TOFMS

2,5-Dihydroxybenzoic acid (DHBA) used as a matrix was purchased from the Aldrich Chemical Co. (Milwaukee, WI, USA). LiCl, NaCl, KCl, RbCl, CsCl, and ethanol were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Extra-pure water was made by the Milli-Q system (Nihon Millipore KK, Tokyo, Japan).

Mass spectral data were acquired by using a reflectron MALDI-TOF mass spectrometer (Shimadzu/Kratos Kompact-MALDI III) with a nitrogen laser operating at a wavelength of 337 nm. The laser power was regulated with a neutral-density filter over a logarithmic power scale. For ion extraction, the static acceleration voltage was set at 20 kV. The data were accumulated over 400 laser shots. The laser was automatically fired at 400 aim points on an individual sample spot across the entire spot at same intervals. This aiming method can produce good repeatability compared to the common method of choosing sweet spots by human eyes. For each laser power, we recorded three spectra from three different spots, and then averaged the area of each peak over the three spectra.

For the measurement of MALDI-TOFMS, 0.15 mg of DHBA and 16 μl of a mixed solvent of ethanol and water (9:1, v/v)

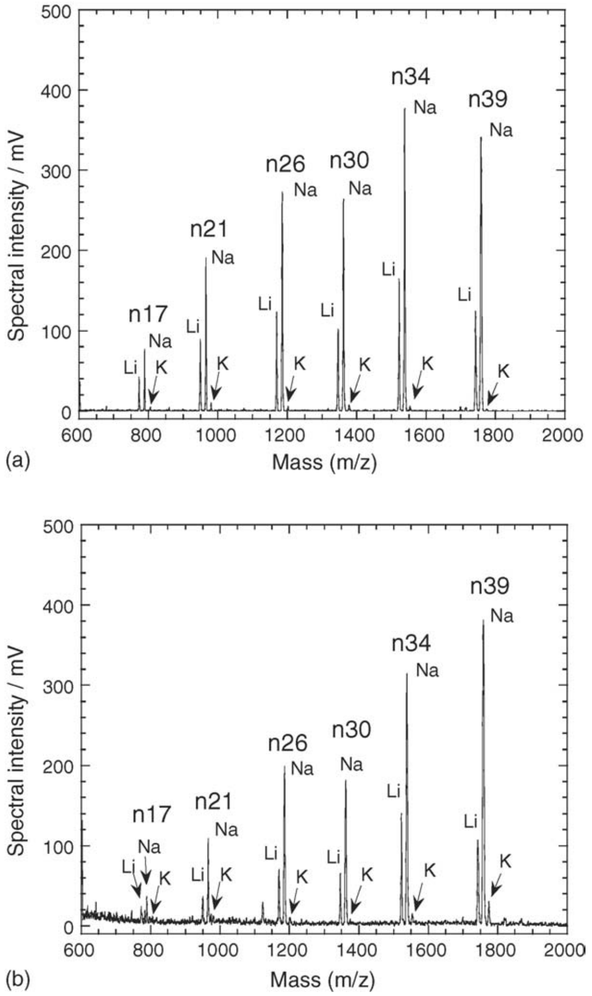

Fig. 1. Typical MALDI-TOFMS spectra of the equimolar mixture of uniform PEGs ($n = 8, 12, 17, 21, 26, 30, 34$, and 39) mixed with five equimolar salts (LiCl, NaCl, KCl, RbCl, and CsCl) and 0.10 mg of DHBA (*lower* matrix concentration). LPs are 17.1 a.u. (a) and 31.6 a.u. (b), respectively. The unit of LP is defined in the text. The peaks labeled Li, Na, K correspond to $[\mathrm{PEG}n + \mathrm{Li}]^+$, $[\mathrm{PEG}n + \mathrm{Na}]^+$, and $[\mathrm{PEG}n + \mathrm{K}]^+$, respectively.

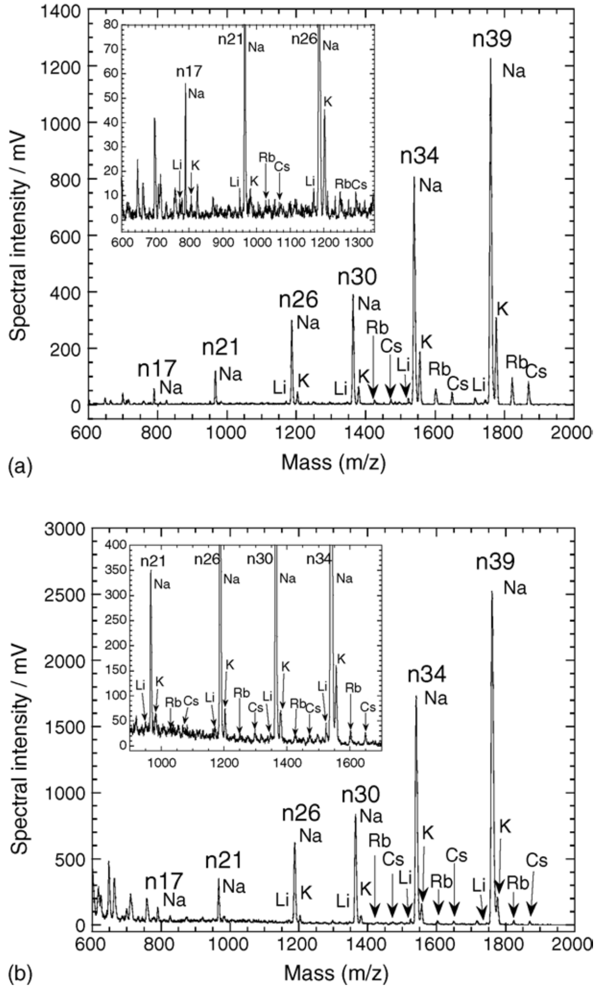

Fig. 2. Typical MALDI-TOFMS spectra of an equimolar mixture of uniform PEGs ($n = 8, 12, 17, 21, 26, 30, 34$, and 39) mixed with equimolar amounts of five salts (LiCl, NaCl, KCl, RbCl, and CsCl) and 0.15 mg of DHBA (*suitable* matrix concentration). LPs are 17.1 a.u. (a) and 31.6 a.u. (b), respectively. The unit of LP is defined in the text. The insets show enlargements of the spectrum in the ranges 600–1350 *m*/*z* (a) and 900–1700 *m*/*z* (b). The peaks labeled Li, Na, K, Rb, and Cs correspond to $[\mathrm{PEG}n + \mathrm{Li}]^+$, $[\mathrm{PEG}n + \mathrm{Na}]^+$, $[\mathrm{PEG}n + \mathrm{K}]^+$, $[\mathrm{PEG}n + \mathrm{Rb}]^+$, and $[\mathrm{PEG}n + \mathrm{Cs}]^+$, respectively.

containing 38 μg of an equimolar mixture of LiCl, NaCl, KCl, RbCl, and CsCl were added to 8 μg of the equimolar mixture of eight uniform PEGs. For the MALDI measurements, a 1-μl aliquot of solution was placed on each spot of a sample slide and dried in the air. Standard sample slides with 20 spots for the Kompact-series instruments were used.

The laser power (LP) is indicated by relative values on a linear power scale. An LP value of 1 a.u. corresponds to about 2 μJ. The observed threshold LP at which a spectrum could first be detected was about 10 a.u. We employed LPs in the range 12.6–31.6 a.u. to obtain adequate peak intensities for quantitative analysis. The peak intensity was observed by the output voltage of the detector, which is represented by spectral intensity (mV) in Figs. 1 and 2. In Figs. 3 and 4, it is shown by the area under each peak as a percentage of the total peak areas. In general, integrated peak intensity on a mass scale instead of a time scale cannot give the correct averaged mass and distribution of the polymer [29–31]. However, here we made the equimolar mixture of uniform oligomers by SFC and TOC analysis, and the increments of spectral intensities with increasing the values of *n*s were so large that remarkable differences were not observed if either integrated peak intensity or signal intensity was used.

2.3. Molecular dynamics simulations

MD simulations were undertaken in the NVT ensemble by using the Discover program 2002 1, a module of Materials Studio (Version 2.2, Accelrys Inc., USA). A polymer consistent force field (PCFF) was used to identify the stable structures of PEG complexes of different *n*s with three different cations: Li^+, Na^+, and K^+. The temperature was assumed to be 298 K. The dynamic time step was 0.1 fs. The number of MD steps was 500,000, which corresponds to 0.5 ns. Two series of MD simulations were carried out; the first was for complexes of

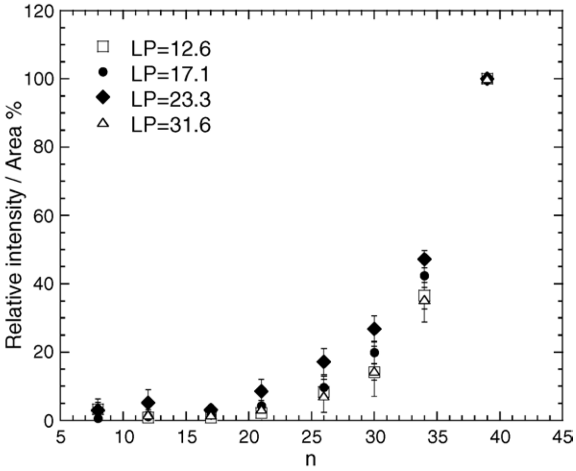

Fig. 3. LP dependence of relative spectral intensities cationized by Na$^+$ as a function of degree of polymerization n for the higher (suitable) matrix concentration. LPs are 12.6 a.u. (□), 17.2 a.u. (●), 23.3 a.u. (♦), and 31.6 a.u. (△). The unit of LP and length are defined in the text. Error bars denote the standard deviations of three repeated measurements.

PEG with the cations and the second was for separate structures of PEG and the cations. In the first case, a cation with +1 charge, i.e., Li$^+$, Na$^+$, or K$^+$, was set near to a PEG molecule with $n = 12$, 17, 21, 26, 30, 34, or 39 as an initial structure. The energy of the PEG complex with the cation was defined as the total energy, comprising the kinetic and potential energies, after 500 ps dynamic run time. In the second case, a cation with +1 charge was set separated from a PEG molecule at a distance 100 times larger than that in the first case. The affinity of the cation to the PEG molecule was determined as the difference between the energy of the complex structure and that of the separated polymer and cation. All MD simulations and analyses were made on a HP Workstation XW8000/CT with a Xeon 3.06-GHz dual processor.

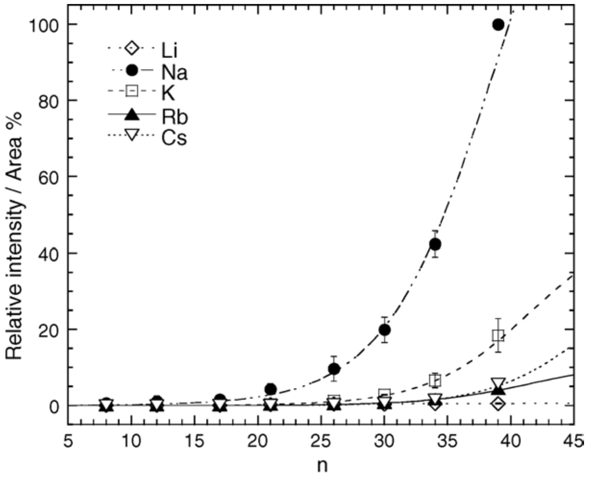

Fig. 4. Relationships between relative spectral intensities and degree of polymerization n for the equimolar mixture of uniform PEGs cationized by five cations at LP = 17.1 a.u. The unit of LP is defined in the text. Cations are Li$^+$ (◊), Na$^+$ (●), K$^+$ (□), Rb$^+$ (▲), and Cs$^+$ (▽). Lines are the least-squares curves calculated by Eq. (1). Error bars denote the standard deviations of three repeated measurements.

3. Results and discussion

3.1. Optimization of the matrix concentration and laser power

In our previous study using tetrahydrofuran as a solvent [6], LP had a considerable effect on the observed spectra, but the concentration of the matrix did not have a marked effect on them. In the present experiment in which a mixed solvent of ethanol and water was used as the solvent, we began by investigating the effects of the LP and the matrix concentration.

Before turning to our experimental results, let us summarize the observations; lower concentration of matrix gave rise to an LP dependence in the observed spectra, as shown in Fig. 1; however, higher concentration of matrix did not lead to an LP-dependence, as shown in Figs. 2 and 3. Fig. 1 shows spectra of an equimolar mixture of the uniform PEGs ($n = 8$, 12, 17, 21, 26, 30, 34, and 39) mixed with an equimolar mixture of five salts (LiCl, NaCl, KCl, RbCl, and CsCl) in the *lower* matrix concentration at two different laser powers. In this case, 0.10 mg of DHBA, which is two-thirds of the concentration described in Section 2, was added to the solution of the equimolar PEG mixture and an equimolar mixture of the five salts. The spectrum shown in Fig. 1a, measured at LP = 17.1 a.u., is similar to previous spectra obtained with tetrahydrofuran as the solvent. In our previous study [6], we observed that a molecular mass dependence of spectral intensity occurs not only at lower values of ns but also at higher values of ns. The LP dependence was quite strong at this *lower* matrix concentration. Fig. 1b illustrates a different spectrum measured at a higher LP (31.6 a.u.) Here, a decrease in the spectral intensity at higher values of ns was not observed. In addition to the strong LP dependence observed in both Fig. 1a and b, only the adducts of Li$^+$, Na$^+$, or K$^+$ were found: adducts cationized by Rb$^+$ or Cs$^+$ were not observed.

Fig. 2 shows typical spectra of the same equimolar mixture of the uniform PEGs mixed with the equimolar mixture of five salts at the *higher* matrix concentration for two different laser powers. In this case, 0.15 mg of DHBA was added to the solution of the equimolar PEG mixture and an equimolar mixture of the five salts. In general, LP can have a considerable effect on the shape of such spectra; however, as shown in Figs. 2a and b and 3, the observed spectra in this case did not change markedly with the LP. In Fig. 3, spectral intensities are converted to relative intensities, where the maximum intensities are designated as 100 a.u.

For lower matrix concentrations, as shown in Fig. 1, higher LPs gave a spectrum that was similar to those observed at higher matrix concentrations, as shown in Fig. 2. In the case of Fig. 1, the matrix concentration is not sufficient to permit the detection of the five kinds of adduct cation at appropriate spectral intensities. These findings indicate that the optimum matrix concentration is that which was used in generating the spectra shown in Fig. 2. If the matrix concentration is lower than that shown in Fig. 2, the LP has significant effect on the spectral profile, as shown in Fig. 1. On the contrary, if the concentration is higher than that shown in Fig. 2, no significant effect is observed, except that noise levels at lower ns increase substantially.

3.2. The relationship between molecular mass of PEG and species of adduct cations

As clearly shown in Fig. 2, with a *suitable* matrix concentration, five kinds of adduct cationized by Li^+, Na^+, K^+, Rb^+, and Cs^+ could be identified simultaneously in the same spectrum for each *n*s, in contrast to Fig. 1 where only three kinds of adduct cationized by Li^+, Na^+, and K^+ could be observed. For all adducts of PEGs and cations, lower values of *n* for PEG produced lower spectral intensities. Note that the spectral intensity of adducts cationized by Na^+ was detected the most strongly among the adducts cationized by the five different salts, but this cannot lead us to the conclusion that the PEG–Na^+ complex is the most stable among the five complexes. Other important factors, such as the solubility of the PEG, salts, and matrix in the solvent, must be taken into account in addition to the stability of the complex.

In Fig. 3, the repeatability of normalized spectral intensity is represented by the standard deviation of three measurements made by using the same sample at different spots. Samples prepared in MALDI matrices often exhibit a poor homogeneity, which results in poor repeatability at different spots [11–14]. In our experiments, however, by using aiming methods instead of choosing sweet spots, and by spotting an aliquot solution containing polymer and matrix instead of separate spotting of polymer and matrix like a sandwich, the normalized spectral intensities at all values of LP were so repeatable that the *n*-dependence of spectral intensities of an adduct cationized by a certain salt could be compared with those of other adducts cationized by other salts. In this section, we investigate the relationship between the *n*-dependences of spectral intensity and the species of adduct cation by using a characteristic fitting function between *n* and the spectral intensity *I* to distinguish between the stability of the complex and the solubilities of the PEG, salts, and matrix in the solvent.

To extract the factor relating to the stability of PEG–cation complexes, we first establish the relationship between *n* and the spectral intensity *I* by means of the following phenomenological function for each of the adductive cations:

$$I = \frac{n_3}{1 + e^{-n_2(x-n_1)}} \tag{1}$$

where n_1 is the critical degree of polymerization at which the spectral intensity begins to increase, n_2 a fitting parameter of the equation, and n_3 is the maximum relative intensity (%) for each spectrum. These values were obtained by least-squares fitting of Eq. (1) to experimental data for each value of LP and for each adduct cation. The intensity curve is presumed to be asymptotic to n_3 and has an inflection point at $x = n_1$, $I = n_3/2$. As shown in our previous study [6] and Fig. 1a, spectral intensities at molecular masses greater than about 10^3 slowly decrease with increasing mass. Therefore, in this case, spectral intensities are assumed to have a maximum value near n_3, beyond the n_1-mer. A number of quantitative models and energetic formulae of ionization in MALDI have been proposed [32–35]; however, in this fitting to the formula, the fitting function is insufficiently sensitive to lead to such a conclusion. Other formulae could also be used to deduce the relationship between the radius of cations and the characteristic *n* for the rapid increase in spectral intensities.

Some examples of fitted curves are shown in Fig. 4 for five species of cation at LP = 17.1 a.u. At any other values of LP, the fitting curves are so close to the experimental intensities that they can give reasonable estimates of the values of n_1–n_3. The parameters obtained are plotted against the radii of cations in Fig. 5 for n_1 (a), n_2 (b), and n_3 (c) at different LPs and for different cations. The radius of a certain cation is taken as the van der Waals radius at a coordination number of six, i.e., Li 0.90 Å, Na 1.16 Å, K 1.52 Å, Rb 1.66 Å, and Cs 1.81 Å [36,37]. The fitting parameter n_2 shown in Fig. 5b is almost constant and is independent of the cation radius. This indicates that the curve fittings of the experimental data by Eq. (1) did not markedly change their slope for different values of n_1 and n_3.

The maximum intensities n_3 on each spectrum are shown in Fig. 5c. The highest value of n_3 corresponds to the adduct cationized by Na^+ and the lowest corresponds to that cationized by Li^+. This seems to be the inverse of the relationship for the solubilities of the five salts in water. The molar solubilities of the salts are LiCl 3.01, NaCl 0.68, KCl 0.76, RbCl 1.15, CsCl 1.61 mol/(100 g of water) at 100 °C, and LiCl 1.58, NaCl 0.61, KCl 0.37, RbCl 0.64, CsCl 0.96 mol/(100 g of water) at 0 °C [38]. That is, the molar solubilities of LiCl, NaCl, KCl, RbCl, and CsCl at above room temperature are in the order LiCl > CsCl > RbCl > (KCl or NaCl). It is suggested that a higher solubility of a salt in water could lead to a lower affinity between PEG and the cation, because the cations will be more stable when complexed with water. The salts are hardly dissolved in pure ethanol except for Li, but all salts have good solubility 90:10 ethanol–water mixture. In the mixture of ethanol and water, the solubility of the salts is attributed to the dissolution of the salts into water. Once the salts formed hydrous structures, hydrated cations are not easily decomposed even under vacuum. The hydration of the salts is speculated as to inhibit the formation of PEG and naked cation complex. In addition, as shown in Fig. 1a and b for a lower matrix concentration, the spectral intensities of Li^+ are larger than those in Fig. 2. If the lower concentration of matrix leads to a lower temperature for the laser desorption/ionization process, the increase in intensities of Li^+ can be attributed to the great change in the solubility of Li^+ in water at lower temperatures. For example, the molar solubilities of LiCl at 0 and 100 °C are 1.58 and 3.01 mol/(100 g of water), respectively, whereas those of NaCl are 0.61 and 0.68 mol/(100 g of water), respectively. The difference between the solubility of LiCl at low temperatures and that at high temperatures is much larger than the corresponding values for NaCl, KCl, and CsCl. Note that several studies on the stability of the PEG–cation complexes have been made by using the maximum intensities (corresponding in this case to n_3), but the stability of PEG–cation complexes reflect the values of n_1, as described below, rather than n_3.

The characteristic degree of polymerization n_1 in Fig. 5a increased with increasing radius of the adduct cation. This finding means that the spectral intensity begins to increase at around the characteristic degree of polymerization n_1 for each cation.

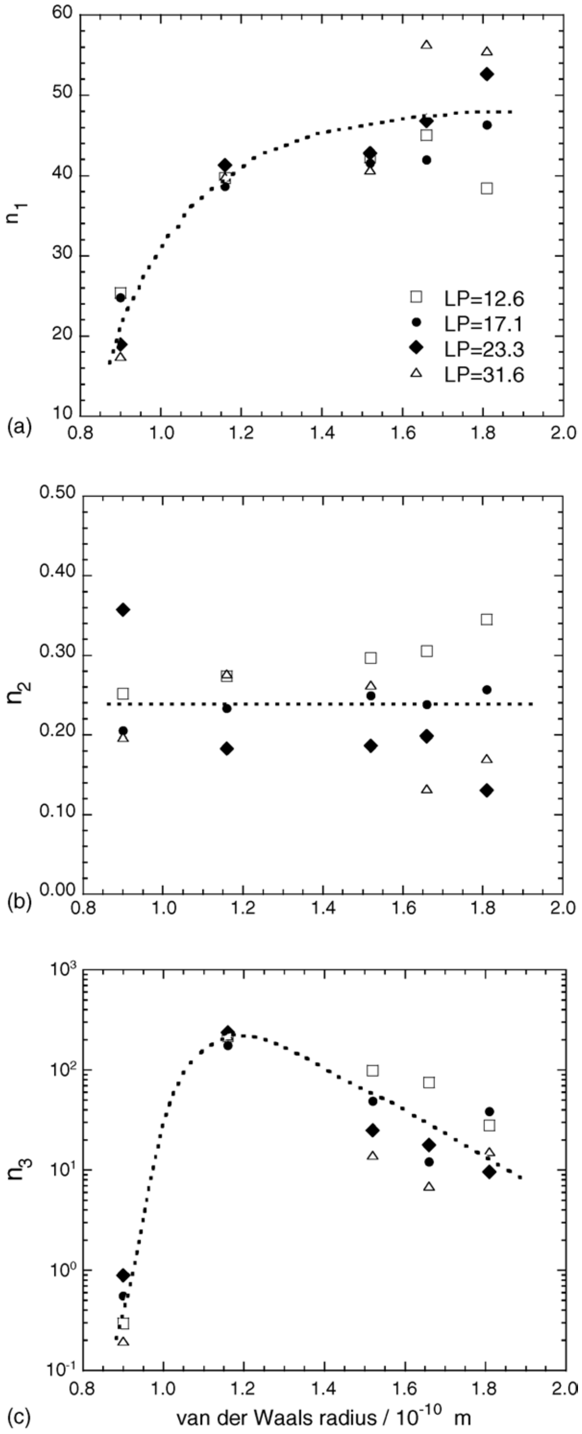

Fig. 5. Plots of the characteristic degree of polymerization n_1 (a), fitting parameter n_2 (b), and maximum intensities n_3 (c) against the van der Waals radius of cations at different LPs. LPs are 12.6 a.u. (□), 17.2 a.u. (●), 23.3 a.u. (♦), and 31.6 a.u. (△). Values of n_1–n_3 were obtained by the least-squares fittings of experimental data to Eq. (1). Dotted lines are guides for the eye.

As suggested by some authors [18,19,39], smaller cations give more-stable adducts for shorter PEG chain lengths than do larger cations. In other words, the larger cations need longer PEG chains to form stable complexes. This phenomenon is observed in the shift of n_1 to a higher value with increasing radius of the cation. In the next section, we verify the change in affinity for a number of PEG–cation pairs for various *n*s by means of MD simulations.

3.3. Molecular dynamics simulations for affinity of PEG–cation

Typical examples of the lowest-energy models of PEG–cation complexes are illustrated in Fig. 6. The top three models are, from left to right, complex models of $[\mathrm{PEG}n12 + \mathrm{Li}]^+$, $[\mathrm{PEG}n12 + \mathrm{Na}]^+$, and $[\mathrm{PEG}n12 + \mathrm{K}]^+$. Similarly, the bottom three models are, from left to right, complex models of $[\mathrm{PEG}n39 + \mathrm{Li}]^+$, $[\mathrm{PEG}n39 + \mathrm{Na}]^+$, and $[\mathrm{PEG}n39 + \mathrm{K}]^+$. In all the models, the cations tend to be enclosed by the oxygen atoms present in PEG. A perfect helical structure was not observed in this simulation, but the PEG chains move quite flexibly. Local helical structures appear and adduct cations are incorporated into these structures. The sizes of the cyclic structures of the PEG chains around the cation increase with increasing radius of the cation. In other words, larger cations need longer chains in order to form complexes. These local structures are not expected to exist for other polymers, such as polystyrene, and in fact no such unusual conformational effects were observed in our previous work [5] on polystyrene uniform oligomer. The characteristic local structure of the PEG–cation complex is observed to be stable for more than several nanoseconds. Initial structures were assigned arbitrarily; however, after less than 100 ps, the structures were optimized and no marked changes in energies were observed until 500 ps, the final state of the simulations. The fluctuation is represented by the standard deviation of averaged total energies shown in Fig. 7.

For three kinds of cation, Fig. 7 shows the affinity of the cation to PEG. The affinity was determined as the difference between the energy of a complex structure and the energy of the separated structures of a PEG and a cation. As shown in Fig. 7, the affinity increases with increasing *n*. This calculated result agrees with the experimentally determined increase in spectral intensity with increasing *n*, as shown in Figs. 2 and 3.

In Fig. 7, a change between Na^+ and K^+ did not produce a marked difference. This trend is similar to the observation shown in Fig. 5a, where the values of n_1 for Na^+ and for K^+ are almost same. In addition, the decline in the rate of increase of the affinity of Li^+ at larger values of *n*s is considered to correspond to the observation that a small value of n_1 is obtained for the adduct cationized by Li^+, as shown in Fig. 5a. That is, the energy advantage of forming the PEG–cation complex decreases with increasing *n*s for a small cation, such as Li^+. A similar tendency was reported by Togashi and Kobayashi [19] for PEG oligomers in an ethanolic solution of DHBA. This trend in MD simulations is consistent with the observation that the value of n_1 for Li^+ is much smaller than that for other cations, as shown in Fig. 5a. Note that this does not mean that changing the cation has no effect on MALDI spectra. The different additive cations produce changes in solubilities between polymer, salts, and matrix, and the corresponding spectral intensities change as shown in Figs. 2 and 4.

In our simulation, no rigid helical structure of the PEG chain was observed. However, it is generally known that PEG can

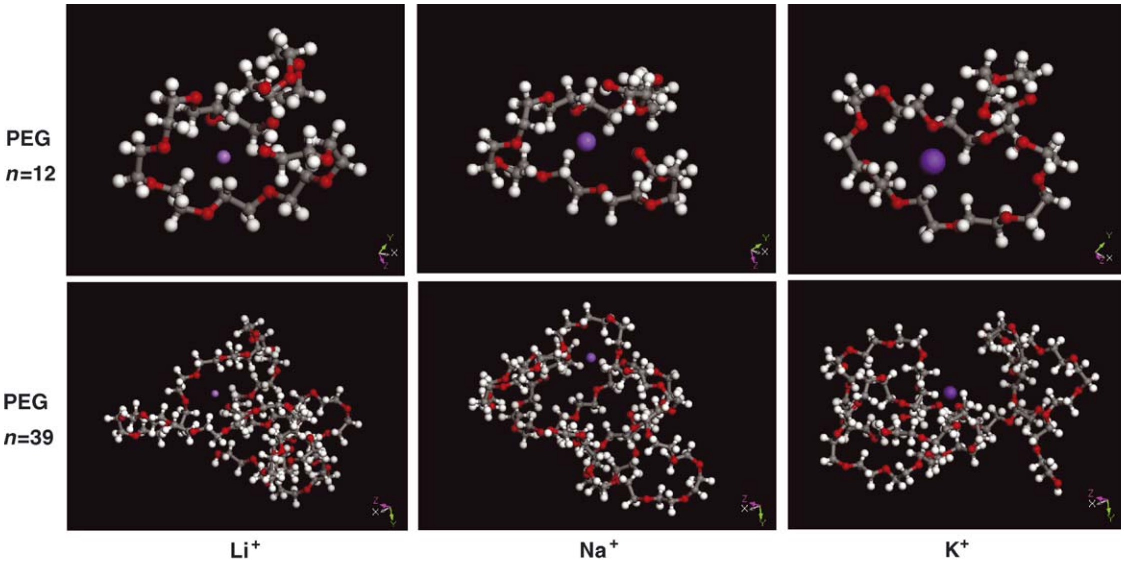

Fig. 6. Typical structures of PEG–cation complexes for a PEG oligomer with $n = 12$ or 39 and cations of Li^+, Na^+, or K^+. The top three models are, from left to right, complex models of $[PEGn12 + Li]^+$, $[PEGn12 + Na]^+$, and $[PEGn12 + K]^+$. The bottom three models are, from left to right, complex models of $[PEGn39 + Li]^+$, $[PEGn39 + Na]^+$, and $[PEGn39 + K]^+$.

form a transient helical structure with a diameter comparable to that of alkali metal ions. In contrast to PEG, polystyrene did not show the *n*-dependence based on conformational effects in our previous study [5] using polystyrene uniform oligomers, because polystyrene is not a crystalline polymer and does not have a characteristic structure. In the case of polystyrene, the cation prefers to interact with two aromatic rings as reported by Scrivens et al. [40], but this phenomenon is observed at any range of mass and the critical degree of polymerization does not appear. In the case of both PEG and polystyrene, the influence of LP can be neglected by optimizing the matrix concentration and selecting a suitable LP. It should be pointed out again that the conformational effect of PEG is fundamental and cannot easily be avoided in quantitative analysis by MALDI-TOFMS, whereas the effects of matrix concentration and LP can be eliminated.

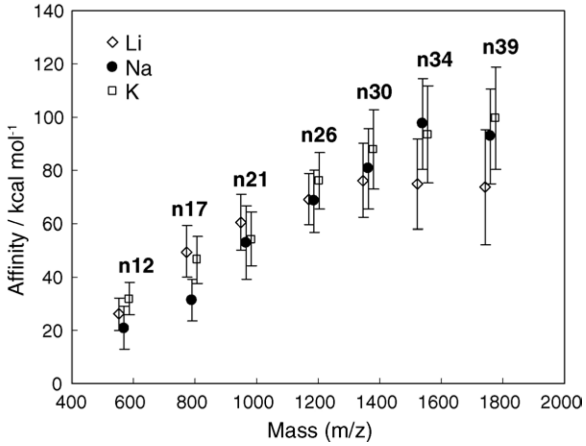

Fig. 7. Plots of the calculated affinity of PEG–cation complexes with different *n*s and three kinds of cations: Li^+ (◇), Na^+ (●), and K^+ (□) from molecular dynamics simulations. Error bars denote the standard deviations of fluctuations of averaged total energy.

4. Conclusions

Uniform PEGs of different degree of polymerization were subjected to MALDI-TOFMS to investigate the relationship between the molecular-mass dependence of the MALDI signal intensity and radius of the adduct cation. We separated uniform PEGs with degrees of polymerization from $n = 8$ to 39 by preparative supercritical fluid chromatography of commercial PEG samples. A great dependence of the MALDI spectral intensities on the value of *n* was observed in the spectra of an equimolar-PEG mixture prepared in aqueous ethanol by adding a mixture of 2,5-dihydroxybenzoic acid as a matrix reagent and five alkali metal chlorides (LiCl, NaCl, KCl, RbCl, and CsCl). We succeeded in observing all of five adducts cationized by Li^+, Na^+, K^+, Rb^+, and Cs^+ in the same spectrum simultaneously.

Our main findings are as follows:

(1) By using an equimolar mixture of uniform PEG oligomers, spectral intensities can be quantitatively analyzed for different degree of polymerization *n*.
(2) To distinguish the effect of affinity from that of solubility, we used a fitting function to evaluate n_1 and n_3. The affinity of PEG and cations is reflected in n_1, which corresponds to the value of n at which the spectral intensity increases rapidly. The solubility is correlated to n_3.
(3) MD simulation supported the tendency shown by n_1 in the case of the affinity. The shift of n_1 to a higher value on increasing the radius of the cation can be simulated by the observation that larger cations need a longer PEG chain to form a stable complex.

In the case of the polymer which has characteristic structures, such as a helical structure for PEG, the conformational effect of the polymer is fundamental and cannot be avoided in quantitative analysis by MALDI-TOFMS.

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