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Interaction of poly(*N*-acryloyl-amino acids) with saccharides in aqueous media

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ABSTRACT

The interaction of a series of poly(*N*-acryloyl-amino acids) (pAXaa) with saccharides has been investigated by ^{1}H NMR. ^{1}H NMR for methyl-β-D-galactopyranoside (MβGal) in the presence of pAXaa indicated that hydrophobic interaction or hydrogen bonding was not considerable in the interaction of the polymers with MβGal in aqueous media whereas CH–π interaction was relatively important. ^{1}H NMR for several saccharides in the presence of poly(*N*-acryloyltryptophan) (pATrp) indicated that pATrp interacted more strongly with the β-anomers than with the α-anomers presumably because of the triple CH–π interactions of the three axial protons in the β-anomers. In the interaction of pATrp with MβGal, MβGal interacted with two or more Trp residues because Trp residues were localized on the polymer chain.

1. Introduction

Saccharides are one of important classes of compounds in biological systems, not only because saccharides are the energy source of living organisms but also because saccharides are the recognition sites on peptides, cells, and viruses [1,2]. In biological systems, saccharides interact with proteins, i.e., enzymes, antibodies, and lectins, to form complexes, resulting in expression of various functions [1,2]. Detail studies on binding sites for saccharides have indicated that complexes of saccharides with proteins are formed via hydrogen bonding, hydrophobic interaction, and CH–π interaction [3]. Among these interactions, CH–π interaction has been studied actively in recent years and these studies have indicated that CH–π interaction is important in the complexation of saccharides with proteins [4–8].

Recently, we have synthesized (co)polymers of *N*-(meth)-acryloyl-amino acids and studied their solution properties and their interaction with other chemical species [9–11]. Our preliminary ^{1}H NMR study exhibited that a relatively large upfield shift upon mixing poly(*N*-acryloyltryptophan) (pATrp) with methyl-β-D-galactopyranoside (MβGal), indicative of a considerable interaction of pATrp with MβGal. This preliminary result motivated us to study the interaction of a series of poly(*N*-acryloyl-amino acids) (pAXaa) with saccharides not only because there have been only a few examples on the interaction of synthetic water soluble polymers with saccharides [12] but also because detail understanding on the interaction of pAXaa with saccharides may allow ones to develop new types of separation materials or chemical sensors for saccharides. In this study, we have thus investigated the interaction of a series of pAXaa shown in Scheme 1 with several saccharides as studied by ^{1}H NMR and elucidated that the interaction of saccharides with pAXaa of aromatic amino acids was relatively strong, indicative of the importance of CH–π interaction.

2. Experimental

2.1. Materials

DL-Aspartic acid, DL-asparagine, DL-tyrosine, DL-phenylalanine, DL-tryptophan, and acryloyl chloride were purchased from TCI [13]. D-Galactose (Gal) and D-maltose (Mal) were purchased from Sigma–Aldrich. Sucrose (Suc) and treharose (Tre) were purchased from Wako. Methyl-β-D-galactopyranoside (MβGal) was purchased from NBS Biologicals. D-Fucose (Fuc) was purchased from Kanto Chemical. These reagents were used as received. 2,2′-Azobis(isobutyronitrile) (AIBN) was purchased from Wako and purified by recrystallization using ethanol. *N*,*N*-Dimethylformamide (DMF) used as solvent for polymerization was distilled under reduced pressure. Water was purified with a Millipore Milli-Q system. Other reagents were used without further purification.

Sodium salt of DL-tryptophan (Trp), used as a model compound in this study, was prepared by neutralization with an equimolar amount of NaOH and then recovered by freeze–drying.

2.2. Monomers

N-Acryloylaspartic acid (AAsp), *N*-acryloylasparagine (pAAsn), *N*-acryloylphenylalanine (APhe), *N*-acryloyltyrosine (ATyr), and

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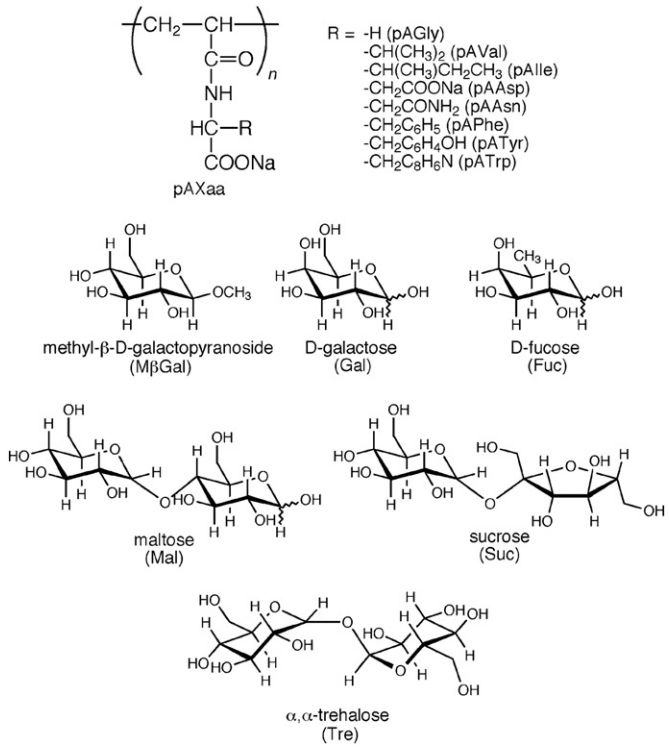

Scheme 1. Chemical structures of the polymers and saccharides used in this study.

N-acryloyltryptophan (ATrp) were prepared from acryloyl chloride and the corresponding amino acids, respectively, according to the procedure of Kulkarni and Morawetz [14]. AAsp, AAsn, APhe, ATyr, and ATrp were purified by recrystallization using ethyl acetate or a mixed solvent of ethyl acetate and hexane.

2.3. Polymers

An *N*-acryloyl-amino acid (i.e., AAsp, AAsn, APhe, ATyr, or ATrp) and AIBN were dissolved in DMF in a flask equipped with a three-way stop-cock under an argon atmosphere. The sealed flask was immersed in an oil bath thermostated at 70 °C. After 24 h, the reaction mixture was poured into a large excess of ethyl acetate to precipitate polymer. The polymer obtained was purified by reprecipitation from methanol into a large excess of ethyl acetate or water and dissolved in an aqueous solution of NaOH. The aqueous solution was dialyzed against water for a week. The polymer (i.e., pAAsp, pAAsn, pAPhe, pATyr, or pATrp) was recovered by freeze–drying.

Poly(*N*-acryloylglycine) (pAGly), poly(*N*-acryloylvaline) (pAVal), and poly(*N*-acryloylisoleusine) (pAIle) were the same as those used in our previous work [11].

The weight-average molecular weights (M_w) and the ratio of z-average to weight-average molecular weight (M_z/M_w) for the polymers used were (1.51–9.69) $\times 10^4$ and 1.28–2.78, respectively (see Table S1 in Electronic Supplementary Material).

2.4. Measurements

One-dimensional ^{1}H NMR spectra were measured on a JEOL JNM LA500 spectrometer in D_2O at 30 °C using sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) as an internal standard (0 ppm). Two-dimensional nuclear Overhauser effect spectroscopy (NOESY) measurements were carried out with a Varian Unity INOVA 600 spectrometer in D_2O. Mixing time before the acquisition of free induction decay was carefully varied and fixed at 150 ms to obtain a genuine NOE and to avoid the effect of spin diffusion. In this study, the concentrations of saccharides were fixed at 1 mM, and the monomer unit concentrations of pAXaa were fixed at 30 and 5 mM for the one-dimensional ^{1}H NMR and NOESY measurements, respectively.

3. Results and discussion

Fig. 1 shows expanded ^{1}H NMR spectra for the C3 and C5 protons in MβGal in the absence and presence of pAXaa (30 mM monomer units). In the presence of pAGly, the ^{1}H NMR spectrum for MβGal does not exhibit any significant changes (Fig. 1b), indicating that pAGly does not interact or interacts only weakly with MβGal. In the presence of pAVal or pAIle, which is expected to show hydrophobic interaction, the ^{1}H NMR spectrum for MβGal exhibits no significant shifts (Fig. 1c and d), indicating that hydrophobic interaction does not contribute significantly in the interaction of these polymers with MβGal. In the presence of pAAsp or pAAsn, which is considered to act as hydrogen bonding sites, the ^{1}H NMR spectrum for MβGal does not indicate significant shifts, neither (Fig. 1e and f). This observation indicates that hydrogen bonding is not considerable in the interaction of these polymers with MβGal because both the components are strongly hydrated in aqueous media. In the presence of pAPhe, pATyr, or pATrp, on the other hand, the ^{1}H NMR spectrum for MβGal exhibits upfield shifted signals because of the ring current of the aromatic amino acid residues (Fig. 1g–i), indicative of the interaction of these polymers with MβGal through the CH–π interaction [15]. It should be noted here that the largest shifts

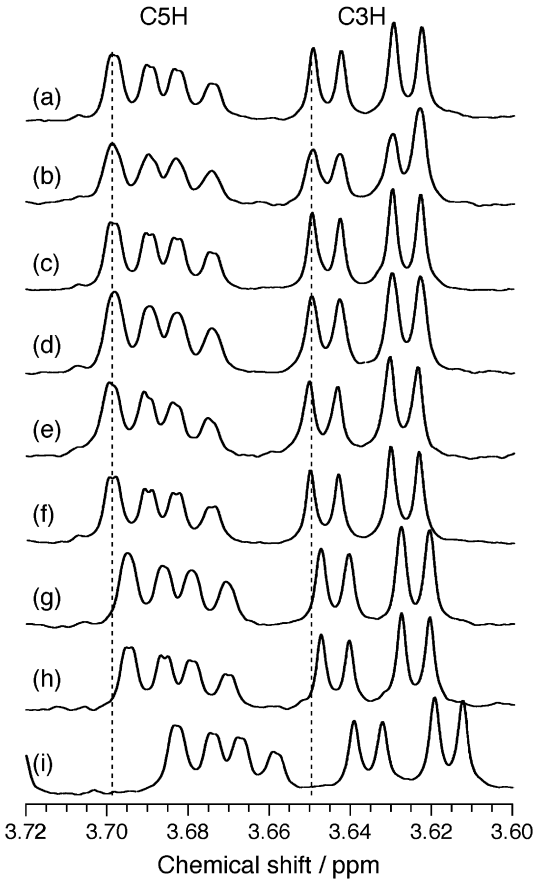

Fig. 1. Expanded ^{1}H NMR spectra for 1 mM MβGal in the absence (a) and presence of pAGly (b), pAVal (c), pAIle (d), pAAsp (e), pAAsn (f), pAPhe (g), pATyr (h), and pATrp (i) (30 mM monomer units) in D_2O at 30 °C.

Table 1
Values of upfield shift ($\Delta\delta_{C1H}$) for the C1 proton in saccharides upon addition of 30 mM pATrp.

Saccharide	$\Delta\delta_{C1H}$/Hz	
	α-anomer	β-anomer
MβGal	–	5.49
Gal	0.38	2.97
Fuc	0.46	4.43
Mal	0.92	3.20
Suc	1.30	–
Tre	0.84	–

were observed in the presence of pATrp. This may be because the indolyl ring, the largest aromatic ring, causes the strongest CH–π interaction and the strongest effect of the ring current. NOESY experiments confirmed these observations: NOESY data did not exhibit significant correlation signals for a mixture of pAGly, pAVal, pAIle, pAAsp, or pAAsn with MβGal, whereas NOESY data showed weak but clear correlation signals for a mixtures of pAPhe, pATyr, or pATrp with MβGal (see Fig. S1 in Electronic Supplementary Material).

Since pATrp caused the largest upfield shift in ^{1}H NMR for MβGal, the interactions of pATrp with five other saccharides (i.e., Gal, Fuc, Tre, Suc, and Mal) were also investigated by ^{1}H NMR spectroscopy. ^{1}H NMR spectra indicated upfield shifted signals of all the saccharides examined in the presence of pATrp (30 mM Trp units), indicative of interaction. From the spectra, peak shifts for the C1 protons ($\Delta\delta_{C1H}$) were determined as listed in Table 1. Table 1 also includes $\Delta\delta_{C1H}$ for MβGal. The data in Table 1 indicate that $\Delta\delta_{C1H}$ values for the β-anomers (2.97–5.49 Hz) are significantly larger than those for the α-anomers (0.38–1.30 Hz), indicating that pATrp interacts with the β-anomers more strongly than with the α anomers. This may be because all the β-anomers examined bear three axial protons and the indolyl ring in pATrp interacts with the three axial protons

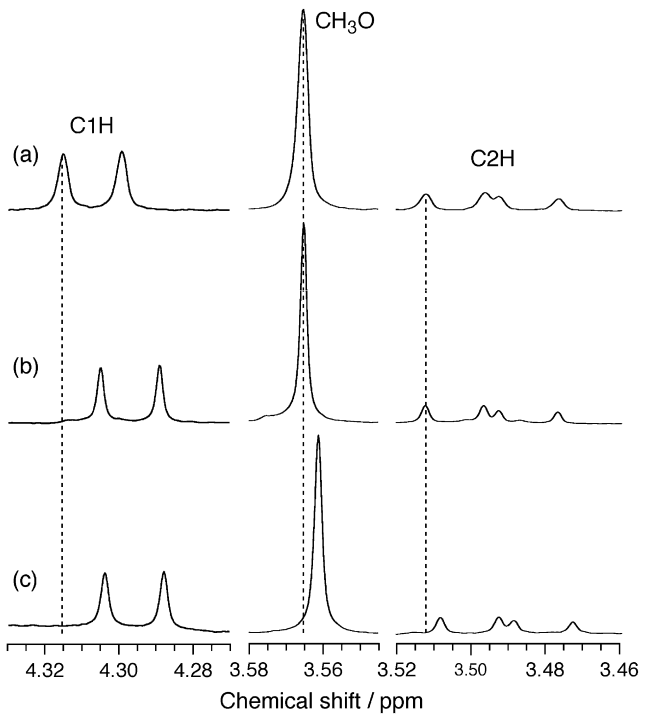

Fig. 2. Expanded ^{1}H NMR spectra for MβGal in the absence (a) and presence of Trp (b) and pATrp (c) (30 mM Trp units) in D_2O at 30 °C.

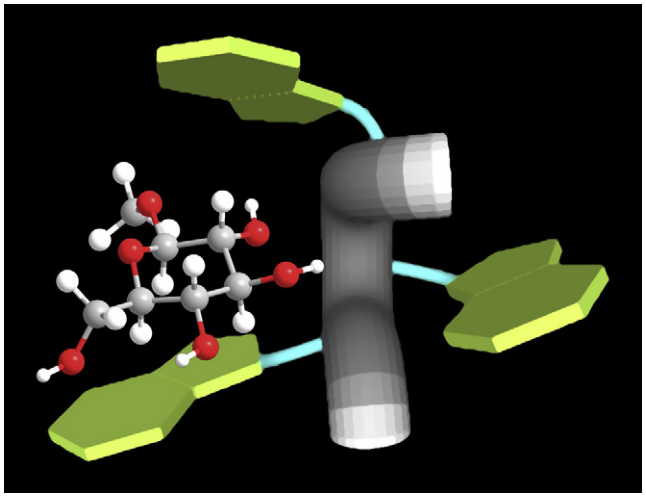

Fig. 3. A conceptual illustration for the interaction of pMTrp with MβGal.

through the triple CH–π interactions. The noteworthy is that MβGal exhibits the largest $\Delta\delta_{C1H}$ among the saccharides examined. This is because the methyl group also interacts with a neighboring Trp residue in pATrp as discussed later.

Interaction of a reference system containing Trp and MβGal was also investigated by ^{1}H NMR. Fig. 2 compares ^{1}H NMR spectra for the C1, C2, and methoxy protons in MβGal in the absence and presence of Trp or pATrp (30 mM Trp units). In both the spectra in the presence of pATrp or Trp, the signal ascribed to the C1 proton exhibits an upfield shift, indicating the interaction of Trp or pATrp with MβGal (Fig. 2b and c). It should be noted here that the signals of the C2 and methoxy protons in MβGal exhibit no significant shifts in the presence of Trp (Fig. 2b) whereas those indicate upfield shifts in the presence of pATrp (Fig. 2c). These spectra indicate that both Trp and pATrp interact with MβGal, but there are some differences in how to interact with MβGal. In the case of Trp, Trp and MβGal form mainly a one-to-one complex at the Trp concentration of 30 mM. On the other hand, in the case of pATrp, two or more Trp residues in pATrp interact with an MβGal molecule because Trp residues are localized on the polymer chain as shown in Fig. 3.

In the case of the protein–saccharide interaction in biological systems, proteins bear well-defined three-dimensional arrangements of amino acid residues appropriate for certain saccharides, and realize specific binding utilizing combinations of different non-covalent bonds, e.g., hydrophobic interaction, hydrogen bonding, and CH–π interaction. In the case of the pAXaa–saccharide interaction, on the other hand, pAXaa polymers used in this study are practically atactic and do not have any defined three-dimensional arrangements, but pAPhe, pATyr, and pATrp interact with saccharides through plural aromatic groups on the polymer chain. If pAXaa copolymers bearing suitable three-dimensional arrangements of amino acid residues are prepared from different AXaa monomers, it may provide a new approach towards artificial lectins.

4. Conclusion

The interaction of pAXaa with saccharides has been investigated by ^{1}H NMR. MβGal did not interact significantly with pAGly, pAVal, pAIle, pAAsp, or pAAsn, indicating that hydrophobic interaction or hydrogen bonding was not considerable in the interaction of pAXaa with MβGal in aqueous media. On the other hand, MβGal interacted with pAPhe, pATyr, or pATrp, indicative of the importance of CH–π interaction. The interaction of pATrp with several saccharides

indicated that pATrp interacted more strongly with the β-anomers than with the α-anomers. These observations indicated that the three axial protons in the β-anomers interacted with the Trp residue in pATrp. In the interaction of pATrp with MβGal, MβGal interacted with two or more Trp residues because Trp residues were localized on the polymer chain. Details on the formation of complexes of pATrp with MβGal are being investigated.

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Appendix. Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.polymer.2009.10.070.

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