Microchemical Journal 90 (2008) 89–92

Contents lists available at ScienceDirect

Microchemical Journal

journal homepage: www.elsevier.com/locate/microc

Selective recognition of Ca^{2+} ions using novel polymeric phenols

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ARTICLE INFO

Article history:
Received 19 March 2008
Accepted 27 March 2008
Available online 10 April 2008

Keywords:
Calcium
Binding site
Selectivity
Phenol
Polymer

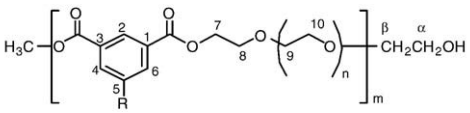
ABSTRACT

The research described here elucidates the fundamental interactions of various cations with phenolic moieties constituting the side chains of novel copolymers. The phenolic group was chosen because similar interactions of the tyrosine (Tyr) moiety in proteins with alkaline earth cations are of particular interest for some biochemical systems where both the alkaline earth cations and the aromatic compounds are abundant. The present study has revealed the preferred binding site for our polymeric systems.

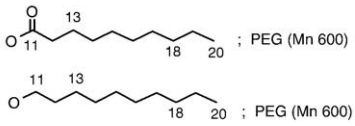
1. Introduction

The interactions of metal ions with organic ligands are of special interest to understand various biochemical processes [1–6]. The research described here elucidates the fundamental interactions of various cations with phenolic moieties constituting the side chains of novel copolymers. The phenolic group was chosen because investigations of the interactions of the tyrosine (Tyr) moiety in proteins with alkaline earth cations have been well emphasized in recent years [7–8]. Another interesting feature of phenolic groups is the presence of multiple side-chain binding sites (the π-site along with the oxygen non-bonded electrons site) [9–11]. Thus it would be interesting to know what the preferred binding sites will be for the metal ions in our polymeric system.

Herein we have studied the interactions of the phenolic moiety with alkali and alkaline earth metal ions using the co-polymers **1a–c** constituted by the phenols and polyethylene glycol (PEG) (Scheme 1). The polymers **1a–c** were synthesized by our recently developed biocatalytic approach [12–16]. The advantage of using the PEG unit is that it confers solubility to these aromatic polyester polymers in aqueous medium.



1a R = OH; PEG (Mn 600)
1b R = OH; PEG (Mn 900)
1c R = OH; PEG (Mn 1500)
2 R = H; PEG (Mn 900)



3 R = ; PEG (Mn 600)

4 R = ; PEG (Mn 600)

2. Materials and methods

2.1. Materials

Novozyme-435 (*Candida antarctica* Lipase B), an immobilized enzyme, was a gift from Novozymes Inc., Denmark, and was dried over P_2O_5 under vacuum prior to use. Poly(ethylene glycol) was dried under vacuum for 24 h before use, and acetone was dried by distillation over fused potassium carbonate. All other chemicals and solvents were of analytical grade and were used as received unless otherwise noted.

2.2. Instrumentation

Gel permeation chromatography (GPC) was used to determine the molecular weights and molecular weight distributions, M_w/M_n of

Abbreviations: Tyr, Tyrosine; DNA, Deoxyribose nucleic acid; RNA, Ribose nucleic acid; PEG, Polyethylene glycol; UV, Ultraviolet; PD, Polydispersity.

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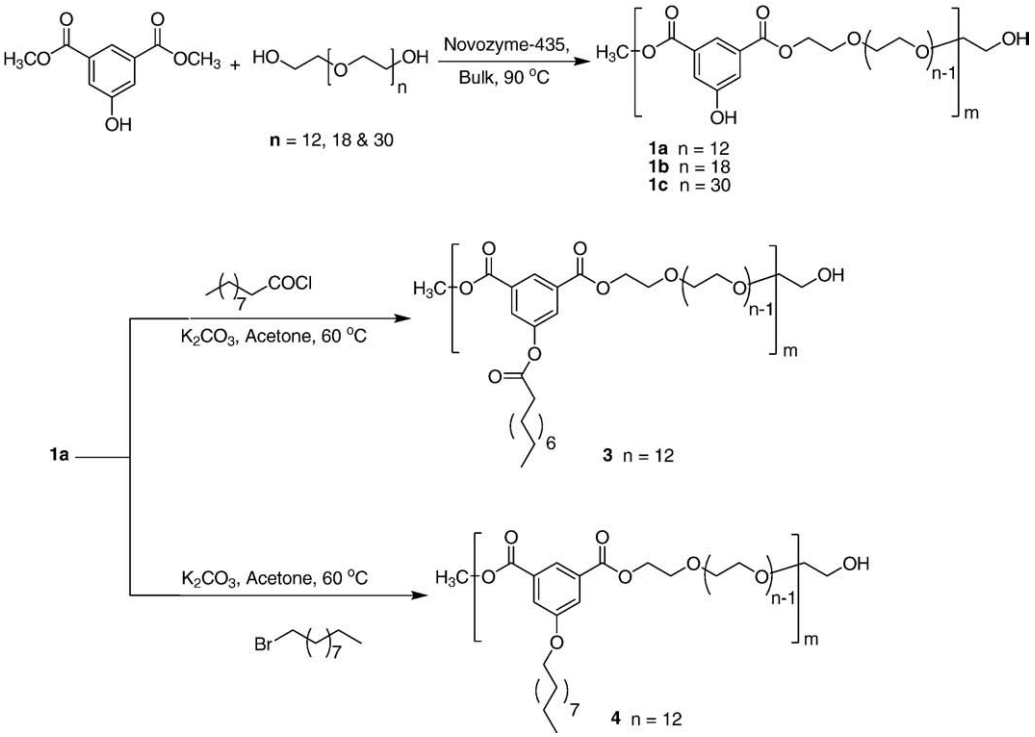
doi:10.1016/j.microc.2008.03.012



Scheme 1. Enzyme catalyzed synthesis of polymers.

polymer samples. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Instrument Inc. DPX 500 spectrometer at 500 and 125 MHz, respectively. Static light scattering data were collected on a laser light scattering photometer (Wyatt Technology DAWN model F) equipped with a 632 nm He–Ne laser as the light source. Dynamic light scattering was performed using a 50 Mw He–Ne Laser, an avalanche photodiode detector BI-APD, a digital time correlator BI-9000 and software from Brookhaven Instruments Corporation, and dynamic light scattering software CONTIN and DOUBLE EXPONENTIAL. UV spectra were recorded on a Perkin Elmer Lambda-9-UV–Vis-Near IR spectrophotometer.

2.3. General method of polymerization [16]

Dimethyl 5-hydroxyisophthalate (1.0 mmol) and PEG (MW 600, 900, 1500, 1.0 mmol) were placed in a round-bottom flask. To this mixture were added 4Å molecular sieves (30 wt.% with respect to PEG) and the enzyme (10 wt.% with respect to monomers), the reaction flask was then placed in a constant temperature oil bath maintained at 90 °C under vacuum. The reaction was allowed to proceed for 48 h, after which it was quenched by adding water. The enzyme was then filtered and any unreacted monomer was removed under vacuum. The filtrate was dialyzed using a dialysis membrane with a molecular weight cut off of 6000 (MWCO 6000). After the completion of dialysis, the product polymer was obtained as a semisolid by freeze-drying (Scheme 1).

2.3.1. Poly[(poly(oxyethylene-600)-oxy-5-hydroxyisophthaloyl] (**1a**) [12,16]

This polymer was obtained by heating dimethyl 5-hydroxyisophthalate (1 mmol, 0.21 g) with PEG 600 (1 mmol, 0.6 g) in the presence of Novozyme-435 (0.08 g) at 90 °C under solvent-free conditions for 48 h under vacuum. It was obtained as a viscous oil after freeze-drying in 90% yield. M_n-(GPC) 8000 Da; PD = 1.8; isolated yield 80%; ^{1}H NMR data ($CDCl_3$): δ 3.60–3.79 (brs, methylene protons of PEG main chain), 3.86 (t, C-8H), 3.96 (s, $COOCH_3$ end group), 4.50 (t, C-7H), 7.75 (s, C-4H and C-6H), and 8.24 (s, C-2H); ^{13}C NMR data ($CDCl_3$): δ 52.74 (−OCH_3 end group), 62.07 (C-α), 64.74 (C-7), 69.44 (C-8), 70.35–71.18 (repeating PEG units' carbons), 72.90 (C-β), 121.43, 122.53, 131.18, 157.57, 166.11, 166.79 (COO−).

2.3.2. Poly[(poly(oxyethylene-900)-oxy-5-hydroxyisophthaloyl] (**1b**). [12,16]

This polymer was obtained by heating dimethyl 5-hydroxyisophthalate (1 mmol, 0.21 g) with PEG 900 (1 mmol, 0.9 g) in the presence of Novozyme-435 (0.11 g) at 90 °C under solvent-free conditions for 48 h under vacuum. It was obtained as a viscous oil after freeze-drying in 93% yield. ^{1}H NMR data ($CDCl_3$): δ 3.63–3.81 (brs, methylene protons of PEG main chain), 3.82 (t, C-8H), 3.92 (s, $COOCH_3$ end group), 4.46 (t, C-7H), 7.69 (s, C-4H and C-6H), and 8.15 (s, C-2H); ^{13}C NMR data ($CDCl_3$): δ 52.73 (−OCH_3 end group), 62.07 (C-α), 64.72 (C-7), 69.43 (C-8), 70.23–70.98 (repeating PEG units' carbons), 72.89 (C-β), 121.43, 122.51, 131.99, 157.56, 166.38 (COO−).

2.3.3. Poly[(poly(oxyethylene-1500)-oxy-5-hydroxyisophthaloyl] (**1c**). [12,16]

This polymer was obtained by heating dimethyl 5-hydroxyisophthalate (1 mmol, 0.21 g) with PEG 1500 (1 mmol, 1.5 g) in the presence of Novozyme-435 (0.17 g) at 90 °C under solvent-free conditions for 48 h under vacuum. It was obtained as a viscous oil after freeze-drying in 90% yield. ^{1}H NMR data ($CDCl_3$): δ 3.61–3.79 (brs, methylene protons of PEG main chain), 3.86 (t, C-8H), 3.96 (s, $COOCH_3$ end group), 4.51 (t, C-7H), 7.75 (s, C-4H and C-6H), and 8.24 (s, C-2H); ^{13}C NMR data ($CDCl_3$): δ 52.69 (−OCH_3 end group), 62.02 (C-α), 64.70 (C-7), 69.43 (C-8), 70.46–71.23 (repeating PEG units' carbons), 72.91 (C-β), 121.48, 122.38, 131.95, 157.62, 166.07 (COO−).

2.3.4. Poly[(poly(oxyethylene-900)-oxy-isophthaloyl] (**2**)

^{1}H NMR data ($CDCl_3$): δ 3.56–3.69 (brs, methylene protons of PEG main chain), 3.82 (t, C-8H), 4.48 (t, C-7H), 7.51 (t, C-5H), 8.21 (d, 2H, C-4H and C-6H), and 8.67 (s, 1H, C-2H); ^{13}C NMR data ($CDCl_3$): δ 62.02 (C-α), 64.77 (C-7), 69.47 (C-8), 70.67–71.03 (repeating PEG units' carbons), 72.93 (C-β), 128.94, 130.97, 131.27, 134.29, 166.02 (COO−).

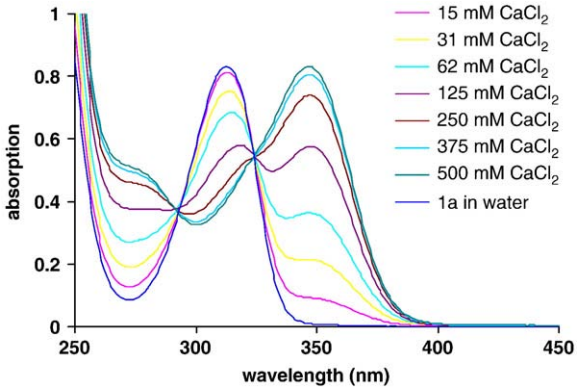


Fig. 1. UV absorption spectra of polymer **1a** at various $CaCl_2$ concentrations in water.

*2.3.5. Coupling of Alkyl chain with poly[(poly(oxyethylene-600)oxy-5-hydroxyisophthaloyl] (***1a***)*

Equimolar quantities of **1a** and decanoyl chloride/bromodecane were dissolved in anhydrous acetone, and to the resultant solution was added an equimolar amount of anhydrous potassium carbonate. The reaction mixture was stirred at room temperature (for decanoyl chloride) or refluxed (bromodecane), and progress of the reaction was monitored by TLC using ethyl acetate in petroleum ether (30%). On completion of the reaction, potassium carbonate was removed by filtration, and the solvent was removed under vacuum to give the alkylated product.

*2.3.6. Poly[(polyoxyethylene-600)-oxy-5-decanoyloxyisophthaloyl] (***3***)*

^{1}H NMR data ($CDCl_3$): δ 0.86–0.92 (bs, C-20H), 1.27–1.38 (m, C-14H to C-19H), 1.78–1.80 (m, C-13H), 2.56–2.62 (m, C-12H), 3.61–3.67 (brs, CH_2 protons of PEG main chain), 3.81 (t, C-8H), 3.96 (s,–$COOCH_3$ end group), 4.50 (t, C-7H), 7.96 (m, C-4H and C-6H), and 8.60 (s, C-2H); ^{13}C NMR data ($CDCl_3$): δ 14.50 (C-20), 22.71, 25.18, 29.24, 29.48, 29.63, 32.23, 34.61, (C-12 to C-19), 52.69 (–OCH_3 end group), 52.94 ($OCOCH_2$), 62.11 (C-α), 65.01 (C-7), 69.43 (C-8), 70.76-70.95 (repeating PEG units' carbons), 72.89 (C-β), 127.71, 127.78, 132.32, 151.11, 165.25 & 174.21 (COO–).

*2.3.7. Poly[(polyoxyethylene-600)-oxy-5-decyloxyisophthaloyl] (***4***) [12]*

^{1}H NMR data ($CDCl_3$): δ 0.88–0.92 (bs, C-20H), 1.27–1.38 (m, C-13H to C-19H), 1.75–1.85 (m, C-12H), 3.65–3.67 (brs, CH_2 protons of PEG main chain), 3.76 (t, C-8H), 3.95 (s,-$COOCH_3$ end group), 4.06 (t, C-11H), 4.51 (t, C-7H), 7.70 (m, C-4H and C-6H), and 8.16 (s, C-2H); isolated yield 85%. ^{13}C NMR data ($CDCl_3$): δ 14.52 (C-20), 23.05–32.26 (C-12 to C-19), 52.69 (–OCH_3 end group), 61.76 (C-α), 64.81 (C-7), 69.46 (C-8 and C-11), 70.38–70.95 (repeating PEG units' carbons), 72.87 (C-β), 120.04, 123.32, 131.99, 159.53, 166.07 (COO–).

2.4. Method for studying ionic interactions with the polymer

The stock solution (A) of the polymer was made by dissolving approximately 100 mg polymer in 100 ml Tris–HCl buffer (50 mM, pH 9.0) and the exact concentration of the solution was calculated by recording its UV absorption and comparing the data with a calibration curve. The calibration curve was obtained from a plot of absorbance vs concentration of the polymer solution at 313 nm. The stock solution (B) of the salt (usually 500 mM, 10 ml) was prepared by dissolving a requisite amount of the salt in buffer. The UV spectra of the polymer with and without salt were recorded by taking a fixed amount of the polymer solution (Stock 'A') and varying amounts of salt solution (Stock 'B'), the total volume was then made up to 1.0 ml by adding the requisite amount of buffer. The solution was then transferred to a UV cuvette and the absorption spectrum recorded on a Perkin Elmer UV spectrophotometer. The final concentration of the polymer solution in a typical experiment was kept around 3 μM.

3. Results and discussion

Considering the different spectroscopic tools available to study the polymer–cation interactions, we decided to use UV spectrophotometry as the polymer has strong absorption (at 313 nm) in the UV region (Fig. 1). By keeping the concentration of the polymer constant and adding varying amounts of the alkaline earth metal salts, the changes in the UV spectrum (if any) could be measured. On addition of calcium chloride we observed that the λ_{max} shifted towards higher wavelength (to 347 nm) (Fig. 1). The intensity of the peak at 347 nm started increasing at the expense of the absorption at 313 nm (Fig. 1) with a gradual increase in the concentration of calcium chloride, thus indicating that calcium ions were interacting with our polymeric system.

The polymers **1b** and **1c**, obtained by using PEG 900 and PEG 1500, respectively instead of PEG600 also exhibit the same phenomenon.

The nature of these cationic interactions (i.e. aromatic or phenolic) have been identified. We observed that if the phenolic group is replaced by hydrogen (polymer **2**) or protected as an ester or ether (polymer **3** or **4**), no change in the UV absorption maxima of the polymer occurred on addition of $CaCl_2$. This indicates that the observed shifts in the UV spectrum are due to the interaction of the metal ion with the phenolic groups rather than due to any cation–π interactions. Phenols, due to their acidic nature, can also exist as phenoxide ions and thus we determined the pK_a of our polymeric system and found it to be 9.0, which was measured spectrophotometrically by UV absorption (monitored at 313 nm) at different pH values in Tris–HCl buffer. We compared the effect of NaCl, KCl and $CaCl_2$ on the UV absorption spectrum of our polymeric material at its pK_a value and noticed that calcium ions interact more strongly with our polymeric system as compared to sodium (Na^+) and potassium (K^+) ions (Fig. 2). However, no significant interactions were observed with $AlCl_3$, $BaCl_2$, $MgCl_2$, and LiCl.

The effect of increasing the calcium chloride concentration in the polymeric solution in buffer at pH 9.0 also showed a similar behavior as observed in water (Fig. 3). Calcium bromide and calcium iodide also interact with the polymeric system in an identical manner indicating that effect is a result of a cationic interaction and is not influence of significantly by the anion. Increasing the temperature has a significant adverse effect on the Ca^{2+}–polymer interactions, i.e. with a rise in temperature of about 20 °C, the UV spectrum starts reverting back to its original shape.

To further support the interaction of the phenolic group with calcium ions, we studied the effect of addition of calcium chloride on chemical shift values of the aromatic carbon atoms in the ^{13}C NMR spectrum of the polymeric system **1a**. We observed an upfield

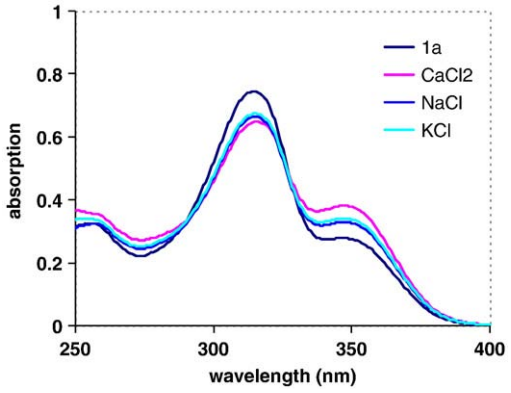


Fig. 2. Effect of various salts on UV absorption spectrum of polymer **1a** in buffer at pH 9.0.

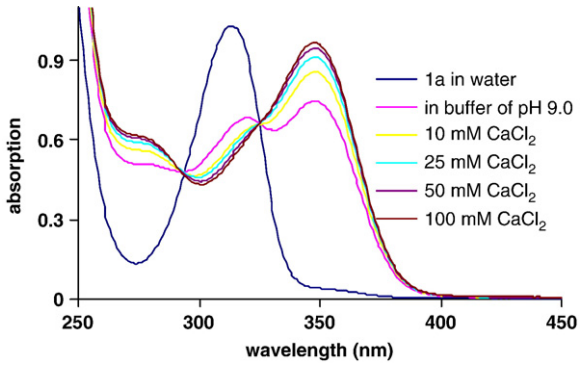


Fig. 3. Effect of varying calcium ion concentration on UV absorption spectrum of polymer **1a** in buffer at pH 9.0.

incremental value of the aromatic carbons *ortho* to the phenolic group on addition of calcium chloride, i.e. the C-4 and C-6 moved upfield ($\Delta\delta$ − 1.12ppm). However, the carbon bearing phenolic group, i.e. the C-5 moved downfield ($\Delta\delta$ + 1.16ppm). We suggest that this may be due to the ionization of the phenolic groups caused by interaction with calcium ions resulting in a downfield shift of C-5 and an upfield shift of the neighboring C-4 and C-6 carbons.

4. Conclusion

In summary, we have shown selective interactions between calcium ions and a co-polymer of phenol and PEG in aqueous media. Our study has helped to find out the site of interaction for metal ions with aromatic–phenolic systems, which is further supported by the fact that the polymer **2** that lacks the phenolic group does not exhibit any interaction with any of these salts. We believe that these results would be of relevance in biological systems and may have far reaching implications in the fields of materials science, biology and sensors.

Acknowledgement

Award of Overseas Associateship to SKS from the Department of Biotechnology, Government of India is gratefully acknowledged.

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