

IMMOBILIZATION OF POTENTIALLY BIOACTIVE MOIETIES ONTO POLYETHER WITH POLY(ETHYLENE GLYCOL)-SULFONATE SPACER*

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ABSTRACT

A new reactive graft copolymer, poly(tetramethylene glycol)-graft- ω -propyl sodium sulfonate-poly(ethylene glycol) (PTMG-g-PEG—CH₂CH₂CH₂SO₃⁻Na⁺), was synthesized by the cationic polymerization of α - ω -bifunctional PEG macromonomer (—CH₂CH₂CH₂SO₃Na) and THF. The obtained copolymer exhibits the expected structure as indicated by the result of characterization. Two amino acids (L-arginine, L-tyrosine) were covalently attached to the copolymer after converting the sulfonate group to sulfonyl chloride. So the new reactive graft copolymer (PTMG-g-PEG—CH₂CH₂CH₂SO₃⁻Na⁺) is expected to be very useful in attachment of potentially bioactive moieties to polymer via a hydrophilic PEG spacer.

INTRODUCTION

In the last few decades, many efforts have been made to develop truly biocompatible materials, including those for blood contacting applications. There have been, however, very few promising candidates emerging, among them are materials immobilized by heparin, albumin, prostaglandins, etc.^[1,2]. But these bioactive macromolecules may be degraded under the action of blood protease and can not be used in the long-term implantation.

More recent work was focused on permanently binding biologically active moieties to polymer chains or polymer surface. Jozefowicz^[3] has modified polystyrene

* Supported by the National Natural Science Foundation of China and Huoyindong Education Foundation

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Received April 12, 1996

resins and dextrans by covalently attaching amino acids. The resulting materials contain not only amino acids but also sulfonamide and free sulfonate groups. These functional groups have shown to have "heparin-like" activity—acting as catalyst of the antithrombin III-thrombin inhibiting reaction.

A substantial amount of works have proved that the bioactivity of the covalently-bound bioactive macromolecules would be improved if these molecules were immobilized onto the surface of polymer substrates via a suitable spacer^[4,5]. In the research of immobilizing heparin onto Biomer, Kim has reported that the hydrophilic polyethylene glycol (PEG) spacer increases the heparin bioactivity by extending heparin into "bulk" solution and improving heparin flexibility^[5].

The immobilized bioactive moieties can also be expected to have higher bioactivity by immobilization through a suitable PEG spacer. A novel sulfonate terminated PEG grafted copolymer PTMG-g-PEG-SO₃⁻Na⁺ has been synthesized in our laboratory^[6]. And in this study, synthetic pathway has been developed to attach potentially bioactive moieties to polymer via a hydrophilic PEG-sulfonate spacer.

EXPERIMENTAL

1. Preparation of α - ω -bifunctional PEG macromonomer

Sodium tetrahydrofurfuryl alcoholate (CH₂ONa) was prepared through a reaction of sodium with an excess of tetrahydrofurfuryl alcohol at 50°C and precipitated in diethyl ether. 1,3-Propane sultone was distilled (180°C/30mmHg) before use. All other reagents were anhydrous and subjected to further purification when necessary.

α - ω -Bifunctional PEG macromonomer (CH₂—PEG—CH₂CH₂CH₂SO₃Na) was synthesized by anionic polymerization of ethylene oxide (EO) using CH₂ONa as an initiator, followed by termination of the alkoxide function of monofunctional "living" PEG chain with 1,3-propane sultone.

2. Synthesis of PTMG-g-PEG—CH₂CH₂CH₂SO₃⁻Na⁺

PTMG-g-PEG—CH₂CH₂CH₂SO₃⁻Na⁺ was synthesized by cationic copolymerization of tetrahydrofuran (THF) with α - ω -bifunctional PEG macromonomer. Boron trifluoride diethyl etherate (BF₃.Et₂O), EO, and dichloromethane (CH₂Cl₂) were used as initiator, coinitorator and solvent, respectively. Macromonomer, THF, CH₂Cl₂, BF₃.Et₂O and EO were added sequentially into a reaction vessel under nitrogen atmosphere at -10°C. After 48h incubation at 0°C, the reaction was terminated

by adding 2% NaHCO_3 solution and the reaction mixture was precipitated with a large amount of hot distilled water. The precipitate was repeatedly washed by hot distilled water until total elimination of unreacted macromonomer was achieved. Further purification was performed by means of fractional precipitation technique using *n*-heptane/cyclohexane (75/25) as precipitant/solvent pair.

3. Derivatization of reactive sulfonated polyether

Covalent attachment of amino acids to sulfonated polyether was achieved by converting the sulfonate group to sulfonyl chloride first and then reacting the sulfonyl chloride with the substituent^[7]. Sulfonated polyether (PTMG-g-PEG— $\text{CH}_2\text{CH}_2\text{CH}_2\text{SO}_3^-\text{Na}^+$) was dissolved in freshly distilled DMF at a concentration of 10%. Thionyl chloride in stoichiometric amount relative to sulfonate groups was added to the polymer solution. The system was kept at -5°C in a ice/water/ NaCl bath and stirred under a dry-nitrogen atmosphere for 2h.

During the conversion of the sulfonate to sulfonyl chloride, the amino acid was dissolved or suspended in DMF in a second vessel and stirred under dry nitrogen at 0°C . After 2h, the contents of the first vessel were transferred to the second one, with maintenance of the dry-nitrogen atmosphere. The reaction between the amine and the sulfonyl chloride group was allowed to proceed under dry nitrogen for more than 12h at 0°C . The resulting polymer was precipitated with hot distilled water and washed with hot distilled water more than 10 times.

RESULTS AND DISCUSSION

1. Synthesis and characterization of α - ω -bifunctional PEG macromonomer

The IR spectrum of α - ω -bifunctional PEG macromonomer exhibited the strong absorption of ether linkage at 1120cm^{-1} and that of sodium sulfonate at 1040cm^{-1} and 1190cm^{-1} .

Table 1 Quantitative results of α - ω -bifunctional PEG macromonomer

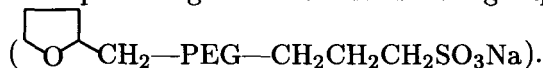
Sample number	By feed	$\overline{M}_n$		By VPO	End group percentage	
		By NMR 1 [#]	By NMR 2 [#]			R-SO ₃ Na
2-16	1475	1559	1455	1518	97.4%	104.3%
2-20	2615	/	/	2591	/	/

NMR-1[#] are $\overline{M}_n$ calculated by the NMR peak area ratio of tetrahydrofurfuryl to ethylene group

NMR-2[#] are $\overline{M}_n$ calculated by the NMR peak area ratio of propyl sulfonic sodium to ethylene group

In the $^1\text{H-NMR}$ spectrum, a strong peak at $\delta = 3.6\text{ppm}$ due to the ethylene group in $-(\text{CH}_2\text{CH}_2\text{O})_n$ appeared. Moreover, the peak of propyl sodium sulfonate at $\delta = 3.14\text{--}3.34\text{ppm}$ and the peak of tetrahydrofurfuryl () at $\delta = 1.3\text{--}1.8\text{ppm}$ were also presented. The values of $\overline{M}_n$ calculated by the ratio of these peak area were found to be quite close to those determined by VPO and predicated theoretically. (See Table 1.)

These above results confirm that the obtained PEG macromonomer has high mole percentage of bifunctional end groups and the following expected structure



2. Synthesis and structural identification of $\text{PTMG-g-PEG---CH}_2\text{CH}_2\text{CH}_2\text{SO}_3^-\text{Na}^+$

$\text{PTMG-g-PEG---CH}_2\text{CH}_2\text{CH}_2\text{SO}_3^-\text{Na}^+$ was synthesized by cationic copolymerization of THF with macromonomer () $\text{CH}_2\text{---PEG---CH}_2\text{CH}_2\text{CH}_2\text{SO}_3\text{Na}$). As a modeling test, α -propyl sodium sulfonate- ω -phenyl PEG, instead of () $\text{CH}_2\text{---PEG---CH}_2\text{CH}_2\text{CH}_2\text{SO}_3\text{Na}$, was added to the same reaction mixture and very little mole percentage of EO ($< 5\%$, perhaps come from the EO co-initiator) was found in resulting materials, which fully proved that the cationic active centre were not transferred by PEG and terminated by the propyl sodium sulfonate. The cationic copolymerization occurs only between the tetrahydrofurfuryl of the α - ω -bifunctional PEG macromonomer and THF. The copolymer was supposed having "comb-like" or "T-like" structure.

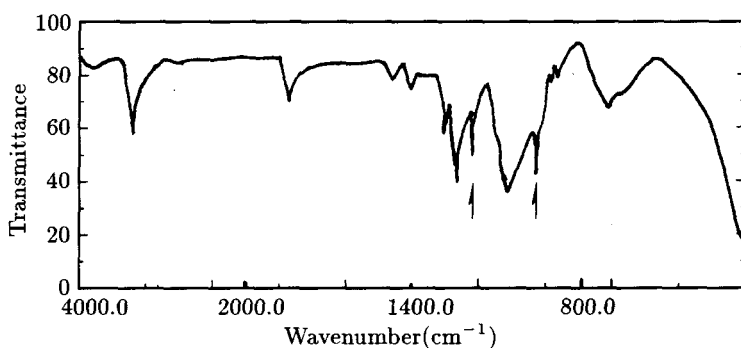


Fig.1 IR spectrum of $\text{PTMG-g-PEG---CH}_2\text{CH}_2\text{CH}_2\text{SO}_3^-\text{Na}^+$

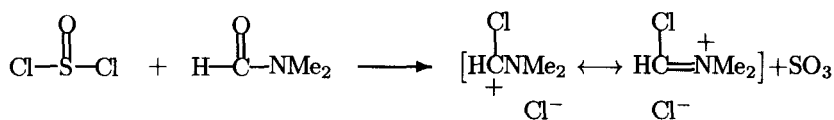
The IR spectrum of the purified $\text{PTMG-g-PEG---CH}_2\text{CH}_2\text{CH}_2\text{SO}_3^-\text{Na}^+$ (Fig.1) exhibited the absorption of ether linkage at 1120 cm^{-1} . Two small peaks at 1040 cm^{-1} and 1205 cm^{-1} were assigned to the propyl sodium sulfonate end group. $^1\text{H-NMR}$

spectrum showed peaks at $\delta = 1.3\text{--}1.8\text{ppm}$ due to the ethylene group in $(\text{---}(\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{O})_n\text{---})$ unit, a peak at $\delta = 3.1\text{--}3.5\text{ppm}$ arising from the methylene group in $(\text{---}(\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{O})_n\text{---})$ unit and a peak at $\delta = 3.5\text{--}3.6\text{ppm}$ contributed by ethylene group in PEG side chain. These characteristic peaks are perfectly consistent with the expected macromolecular structure of the graft copolymer.

3. Derivatization of reactive sulfonated polyether

During the derivatization of reactive sulfonated polyether, when the sulfonate conversion reagent, thionyl chloride was added to the polymer solution, some white solid could be found and then redissolved slowly. Similar experiment phenomenon has been reported by Contreras and Jones^[8] for the formation of carboxylic and sulfonic acid chlorides using phosgene or thionyl chloride in the presence of DMF.

The proposed mechanism for the covalent attachment of amino acid onto sulfonate polymer is shown in Fig.2.



Reactive salt 1

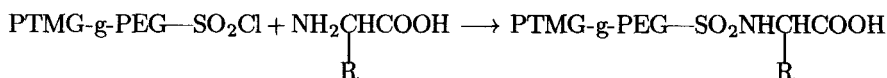
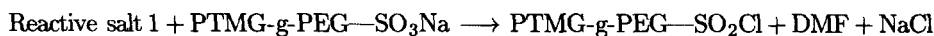


Fig.2 The proposed mechanism for derivatization of reactive sulfonated polyether

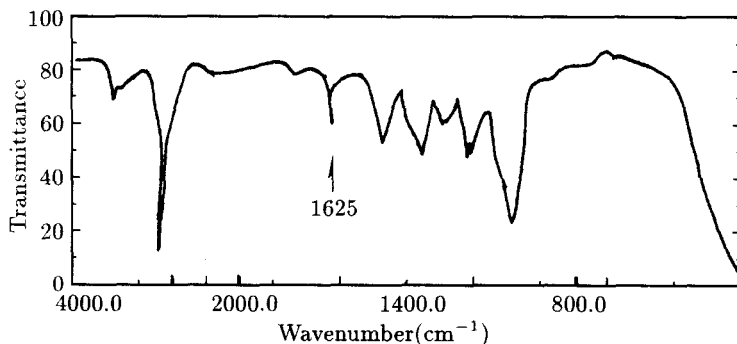


Fig.3 IR spectrum of arginine immobilized product

DMF acts not only as the solvent, but also the catalyst in the reaction. The white solid precipitate which is believed to be the reactive salt-1, can dissolve in DMF and convert the sulfonate group to sulfonyl chloride. Following the conversion, the sulfonyl chloride can react with amine group of amino acid and produce HCl which may degrade polyether. The reaction temperature must be kept below 10°C to minimize the acid-catalyzed hydrolysis.

In the IR spectrum of arginine immobilized product (Fig.3), a peak at 1625 cm^{-1} is assigned to the characteristic absorption of the guanidyl group on the side chain of arginine as suggested by Brash^[7].

The UV spectrum of L-tyrosine immobilized polyether exhibited two characteristic strong absorption at 224nm and 275nm, while the absorption of the sulfonic polyether was very low at the same range. And the absorption of the pure L-tyrosine, which corresponded very well with the absorption of L-tyrosine immobilized polyether (See Fig.4), suggested these peaks at 224nm and 275nm may arise from phenol on the side of L-tyrosine.

It can be concluded that the potentially bioactive moieties—arginine and L-tyrosine have been successfully attached onto the novel sulfonated polyether.

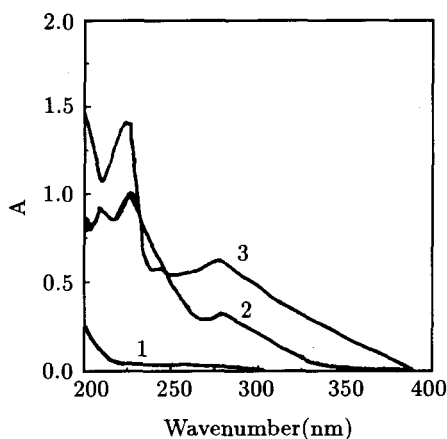


Fig.4 UV spectrum of L-tyrosine covalent product

1. polyether; 2. L-tyrosine covalent product; 3. pure L-tyrosine

CONCLUSION

A new reactive graft copolymer, (PTMG-g-PEG—CH₂CH₂CH₂SO₃⁻Na⁺), was synthesized by the cationic polymerization of α - ω -bifunctional PEG macromonomer ( —CH₂—PEG—CH₂CH₂CH₂SO₃Na) and THF. The obtained copolymer exhibited the expected structure as indicated by the result of characterization. By first converting the sulfonyl group to sulfonyl chloride, two amino acids (L-arginine, L-tyrosine) were successfully covalently attached to the polymer. It is expected that the synthetic pathway will be generally useful in attachment of potentially bioactive moieties to polymer via a hydrophilic PEG spacer.

Keywords:

Potentially bioactive moieties; Polyether; Biocompatible materials

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