Functionalization of Polyethylene Oxide with 4-Amino-N-(2-pyrimidinyl) Benzene Sulfonamide at One End

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ABSTRACT: Polyethylene oxide (PEO) with 4-amino-*N*-(2-pyrimidinyl) benzene sulfonamide (APBS; PEO_{as}) at one end was used as a carrier polymer for attachment (via the hydroxyl end group) of drugs such as mustard. For this purpose, the amine group of PEO_{as} was protected first by the formation of a Schiff's base; then protected PEO_{as}—Br and PEO_{as}—NH₂ were prepared. In order to attach *N*,*N*-dichloroethyl amine (DCEA) to the PEO_{as}—NH₂, DCEA hydrogen chloride salt was reacted with maleic anhydride first, then the resulting *N*,*N*-dichloroethyl maleinamic acid (DCEMA) could react with protected PEO_{as}—NH₂ continuously in the presence of dicyclohexylcarbodiimide (DCC). However, DCEMA is unstable; the chloroethyl group was rearranged to a corresponding ternary ammonium salt with a three-membered ring (DCEMA_r). Therefore, the protected PEO_{as}—NH₂ was coupled with the DCEMA_r in the presence of DCC; then it attacked the nucleophilic center of the aniline group of the APBS end of the PEO_{as} after deprotection. Thus, a PEO with higher molecular weight and wider distribution was formed due to the intermolecular addition of PEO_{as}. © 1999 John Wiley & Sons, Inc. J Appl Polym Sci 73: 1379–1385, 1999

Key words: polyethylene oxide; 4-amino-N-(2-pyrimidinyl) benzene sulfonamide; functionalization

INTRODUCTION

Recently the research on polymeric drugs with antitumor activity^{1,2} has aroused extensive attention and interest all over the world because of the low toxicity and long duration of drug activity³ of polymeric drugs compared to others. In addition, the polymers with abundant chain configurations and conformations can also supply ideal materials and reaction sites for the design and synthesis of drugs. Some difficulties in the preparation of small drugs can be resolved when polymer materials are used.⁴ It is well known that the pharmacological properties of the drugs are dependent on the structure and conformation itself: the drugs with the same structure and different conformation would show different properties. The variation of the structure, even though the addition or subtraction of a small group or only the change of the substitution place of the group, would completely change the pharmacological activity of the original drugs.

For instance, sulfadiazine is a well-known antibiotic drug. As early as the 1950s, however, it was found that sulfadiazine and its homologies could be selectively concentrated in malignant tissue of rats^{5,6}; the concentration ratio of the tumor to the liver was about 3:1 to 4:1. These findings are of considerable interest for scientists because tumors generally take up injected compounds to a lesser extent than the liver. Although sulfadiazine is not an antitumor agent, an attempt was

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Contract grant sponsors: Natural Science Foundation of China; Doctor Training Foundation, State Education Ministry of China.

Journal of Applied Polymer Science, Vol. 73, 1379-1385 (1999)

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made to exploit its ability to concentrate in tumor cells by designing a cytotoxic antitumor drug on the sulfadiazine for targeting the delivery drug to the tumor. Sulfadiazine-mustard with the following structure was prepared by Abel et al.⁷:



Unfortunately, it lost the ability to be taken up by those tumors that concentrate sulfadiazine; the distribution of this drug in the tumors was much less than that in the liver as common antitumor agents did. Because of the failure of the modification of the small antitumor drug, we tried to fasten the sulfadiazine and antitumor drug on both ends of polyethylene oxide (PEO) with a suitable molecular weight utilizing a considerable variety of conformations and high molecular weight polymers to screen or weaken the strong interaction of sulfadiazine and mustard when they reacted directly. This kind of polymeric drug would have both the pharmacological activity of the antitumor drug and the ability of sulfadiazine to concentrate in tumor cells.

We report the preparation of PEO with sulfadiazine at one end and amine hydroxyl at the other end by ring-opening polymerization of ethylene oxide initiated by protected sodium aminoethanol^{8,9} and sodium 4-amino-N-(2-pyrimidinyl) benzene sulfonamide (APBS).¹⁰ This article focuses on the functional reactions of PEO with APBS (PEO_{as}).

EXPERIMENTAL

Materials

Sulfadiazine (Shanghai Twelfth Pharmaceutical Factory) was purified by a mixed solvent of dimethyl sulfoxide and alcohol (9/1 v/v; decomposition temperature of the purified product 250–253°C). Diethanolamine (Shanghai Third Reagent Factory) was dried by CaH₂ and distilled under reduced pressure at 154–156°C/10 mmHg. Maleic anhydride was purified twice by recrystallization with toluene (mp of purified product 54°C. Benzaldehyde (Jiang Shu Yi Xing Auxiliary Factory, AR) was dried about 2 days and then distilled under reduced pressure, and the fraction of 96°C/5 mmHg was collected (refractive index n^{25}_{D} 1.5427). Hexamethylenediamine (Shanghai Ting Xin Chemical Factory) was purified by sub-

limation in N_2 (mp 41–42°C). Phosphorus tribromide (Merck) was used as received without further purification; dicyclohexylcarbodiimide (DCC) was also used as received (Shanghai She Shan Chemical Factory). All other solvents were derived with ordinary purification.

Synthesis of PEO with APBS and Hydroxyl End Groups (PEO_{as}—OH)

PEO_{as}—OH with a molecular weight of 1800 was prepared by ring-opening polymerization of ethylene oxide initiated by sodium APBS,¹⁰ which we briefly describe as follows:



Protection of APBS and Bromination of PEO_{as}—OH



Two hundred milliliters of methanol containing 10 mL (ca. 0.099 mol) of benzaldehyde was introduced into a 500-mL three-necked flask fitted with a funnel, magnetic stirrer, and condenser; then 150 mL of methanol dissolved in 25 g (0.013 mol) of PEO_{as}—OH was added dropwise with stirring under N₂ in 30 min and kept in this state for 24 h at room temperature. The reaction mixture was dried overnight by the addition of 20 g of anhydrous sodium sulfate and then filtrated; the filtrate was concentrated to half of its original volume and precipitated in ether. The Schiff's base protected PEO-(1) could be purified by dissolution or precipitation with chloroform or ether at a yield of 76%.

Thirty-three grams (ca. 0.019 mol) of PEO-(1) was dissolved in 200 mL of chloroform. It was then placed in a 500-mL flask with a funnel, magnetic stirrer, and condenser; 3 mL of triethyl

amine (0.02 mol) was added. Two milliliters (0.02 mol) of phosphorus tribromide in 50 mL of chloroform was introduced dropwise with stirring in 30 min under N_2 at room temperature, then it was reacted for 1 h at 50°C. After filtration the filtrate was distilled to about two-thirds of its original volume, then it was precipitated in ether.

The PEO-(2) could be also purified by the dissolution or precipitation procedure with chloroform or ether at a yield of 65%.

Preparation of Protected PEO_{as} with Hexamethylenediamine End Group [PEO-(3)]



Seventeen grams of PEO(2) (ca. 0.01 mol) was dissolved in 150 mL of absolute alcohol and added to a 250-mL flask with a stirrer and condenser in which 11.6 g (0.1 mol) of hexamethylenediamine was introduced with stirring. The reaction was conducted at 70°C for 18 h under N₂, then it was precipitated with ether. The purification procedure is the same as above with a yield of 71%. IR: 3349 and 3273 cm⁻¹ (—NH₂); ¹H-NMR: 2.42 ppm (s, 2H, —NH₂).

Reaction of *N*,*N*-Dichloroethyl Amine (DCEA) Hydrogen Chloride Salt with Maleic Anhydride



The reaction of DCEA hydrogen chloride salt with maleic anhydride was divided into two steps: the DCEA hydrogen chloride salt was synthesized first, then it was reacted with maleic anhydride. The former was prepared according to the literature¹¹ in a yield of 80% (mp 208–210°C).

Nine grams (0.05 mol) of DCEA hydrogen chloride salt was put into a 250-mL flask in which 50 mL of ether was added with stirring; then 6.9 mL (0.05 mol) of triethyl amine in 40 mL of ether was added dropwise over 45 min. The reaction was carried out for 6 h at room temperature; then it was filtered. The filtrate was distilled to remove the ether, then 20 mL of chloroform was added to the residue. After that 5 g (0.05 mol) of maleic anhydride in 20 mL of chloroform was introduced dropwise over 20 min, and the reaction was conducted at room temperature for 2 h. The flask stood overnight, and the product was separated as a crystal in a yield of 64% (mp $98-102^{\circ}$ C).

Reaction of PEO-(3) with Product

To a 250-mL flask were added 9.5 g of polymer-(3) (0.005 mol) in 50 mL of dimethyl formamide (DMF); then 1.4 mL of triethyl amine was introduced with stirring and 2.4 g (0.01 mol) of the above product and 2 g (0.01 mol) of DCC were added. The reaction was carried out at room temperature for 18 h, then it was precipitated with ether. The product [PE0-(4)] could be purified with the dissolution or precipitation procedure in benzene or ether in a yield of 98%.

Deprotection of Amino Group of PEO_{as}

PEO-(4) (8.5 g) was dissolved in 50 mL of mixed solvents of acetic acid and methanol (v/v: 1 : 1) and acidolysed for 24 h at room temperature with stirring; then it was precipitated with ether. The object product could also be purified by the abovementioned procedure with a yield of 74%. IR: 3423 and 3345 cm⁻¹ (—NH₂ conjugated with the benzene ring) and 1642 cm⁻¹ for the CH=N that disappeared; ¹H-NMR: 3.21 ppm (s, 2H, —NH₂ conjugated with the benzene ring) and 8.34 ppm for the —CH=N— that disappeared.

Measurements and Instruments

IR spectra were recorded on a Magna-550 FTIR spectrometer. ¹H-NMR spectra were scanned with a Bruker MSL-300 spectrometer with tetramethyl silance as the internal standard and $CDCl_3$ as the solvent. The molecular weights and molecular weight distributions of the polymers were derived from a Shimadzu LC-3A gel permeation chromatograph (GPC) with a microcomputer. The measurement conditions were described in a previous publication.¹⁰



Figure 1 IR spectra of protected PEO_{as} —OH (a) before and (b) after bromination.

RESULTS AND DISCUSSION

Effect of Bromination on Protected APBS of PEO_{as}

In our synthesis strategy Schiff's base protected PEO_{as}—Br was prepared by the reaction of phosphorus tribromide with protected PEO_{as}—OH. It was reported, however, that the Schiff's base group would be broken by the attack of the halogen-substituted hydrocarbon in the presence of trace water (the Decker–Forster reaction) to form a secondary amine, and the activity of halogen-substituted hydrocarbon is dependent on the length of the hydrocarbon.^{12,13} In order to smoothly carry out the functionalization of PEO_{as}, it was necessary to confirm whether the Schiff's base protected group of PEO_{as} was reserved or lost in the bromination with phosphorus tribromide. Figure 1 shows the IR spectra of protected PEO_{as} before and after bromination with PBr_3 ; the 530 cm⁻¹ attributed to the $-CH_2$ -Br appeared and the 3449 cm⁻¹ denoted for the —OH disappeared after bromination. We also found that the grey white precipitate of AgBr occurred after the bromated $\ensuremath{\text{PEO}_{\mathrm{as}}}\xspace$ was tested by the Veibel method.¹⁴ If the absorbance intensity of the $-CH_2CH_2O-$ of PEO_{as} at 1149 cm⁻¹ was used as the internal standard, the intensity of 1642 cm⁻¹ was used to measure the variation of the -CH=N- group. Then we found that the absorbance strength ratios of both of them were nearly



Figure 2 GPC measurement of protected PEO_{as}—OH (a) before and (b) after bromination.

the same before and after bromination. The further evidence is given in Figure 2. If the brominated PEO_{as} could attack the Schiff's base end group of

protected PEO_{as}, the reaction would be carried out with the following process and a PEO with a higher molecular weight would be obtained:



However, the GPC measurement told us the molecular weight distribution of protected PEO_{as} was nearly the same before and after bromination. Therefore, in the bromination of the protected PEO_{as} —OH for our system, the Schiff's base protection is reliable.

Rearrangement of Chloroethyl in Reaction of DCEA Hydrogen Chloride Salt with Maleic Anhydride

Figure 3 shows the ¹H-NMR spectrum of fresh product from the reaction of DCEA hydrogen chloride salt with maleic anhydride in chloroform. Two groups of symmetrical triple peaks at 3.89–3.93 and 3.67–3.72 ppm appeared with the coupling constant (*J*) of 7 Hz attributed to two methylenes of chloroethyl. At 3.81 ppm there is only a single peak; its area compared to the former is about 2 : 1; with the hydrogen proton of a double bond at 6.37 (d, 1H, =CH— connected with amide) and 6.75 (d, 1H, =CH— connected with carboxyl) it is about 4:1. Thus, the four hydrogen protons represented by the single peak of 3.81 ppm were in a similar chemical environment.

Further, when the same product was dissolved in DMF and then two or three drops of silver nitrate aqueous solution was added, a white precipitate that did not dissolve in nitric acid was formed. This fact strongly confirmed that chlorine ions existed in this system. Therefore, it could be concluded that the product [N,N-dichloroethyl maleinamic acid (DCEMA)] formed by the reaction of DCEA with maleic anhydride was unstable and one of the chloroethyls of DCEA might be rearranged with the following equation to form the ternary ammonium salt with a three-membered ring (DCEMA_r).



Formation of PEO with Higher Molecular Weight

The reaction of protected PEO-(3) with the DEC- MA_r and then deprotection is conducted in the presence of DCC according to the following equations:



Figure 3 ¹H-NMR spectrum of the product by the reaction of DCEA hydrogen chloride salt with maleic anhydride.



However, it was found that the molecular weight of the product before and after deprotection (Fig. 4) is quite different. Before the deprotection, the molecular weight and molecular weight distribution of the product were about 1800 and 1.06, respectively, which is nearly the same as the original PEO_{as} —OH. However, when the Schiff's base was broken, a product with higher molecular weight and wider



Figure 4 GPC measurement of polymer(4) (a) before and (b) after deprotection of the Schiff's base end group.

molecular weight distribution was formed. It is well known that the PEO_{as} — NH_2 protected with the Schiff's base did not react with the carboxyl group of DCEMA, in the presence of DCC, so the molecular weight of the product did not change in these conditions. If the Schiff's base group was lost, the ternary ammonium salt with a three-membered ring could make an attack on the nucleophilic center of the aniline group of the APBS end via the intermolecular reaction of PEO_{as}; then the molecular weight of the product might be increased with the following equations:



Obviously the molecular weight distribution of the latter was much wider than that of PEO_{as} . It was found that if the product was placed in a dryer for 1 week at room temperature, then it was nearly insoluble in the chloroform or water. This

may have been caused by the rearrangement of another chloroethyl of the end group of PEO_{as} , so the crosslinking net would be formed.

CONCLUSION

PEO with APBS at one end and DCEMA at another end was prepared via the functionalization of the hydroxyl end group of PEO. However, the DCEMA was unstable; the chloroethyl group might be rearranged to the corresponding ternary ammonium salt with a three-membered ring to make the molecular weight of the functionalized PEO increase and the molecular weight distribution broaden.

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