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Synthesis of methoxypoly(oxyethylene)propionic acid

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Summary

We have investigated the reaction chemistry of methoxypoly(oxyethylene)propionitrile, **I**, to synthesize methoxypoly(oxyethylene)propionic acid, **II**. We have found that **II** can be prepared by converting **I** first to the corresponding amide. Subsequent hydrolysis of the amide then yields **II**.

Introduction

Poly(ethylene glycol) (PEG) and its monomethyl ether, mPEG, are well known to be nontoxic, biocompatible, and soluble in water and many organic solvents (1,2). Consequently, PEG and its derivatives are being utilized in many biotechnical and biomedical applications (1-6).

PEG attachment to biomolecules and surfaces requires the use of PEG derivatives, so preparation of new PEG derivatives has become central to many studies on PEG applications (7,8). The methods for PEG functionalization and its covalent attachment to proteins and surfaces have recently been reviewed (1a,9-11).

Active esters such as succinimidyl esters of various carboxylated PEGs are widely used as reagents for modification of proteins and other substrates (1a, 12,13). Consequently, a variety of carboxyl-PEG with different properties has been developed.

Oxidation of the hydroxyl groups of PEG to carboxyl groups by chemical (12) and microbial (14) methods has been carried out. However, strong oxidizing agents, e.g., permanganate, can cause chain cleavage to occur (10). Modification of PEG with α-haloacetic acid or its esters offers a simple method to place the same oxyacetate groups at terminals of the polymer (15,16). The PEG-succinimidyl oxyacetate, **IV** (Scheme 1), has been used to modify a number of enzymes and human hemoglobin (1a, 12,17). The enzymes modified using **IV** preserved their specific activities and the leaving group, N-hydroxysuccinimide, had no adverse effects on the PEG-polypeptide conjugates. However, polymer **IV** was found to be very reactive due to the presence of oxygen in one-carbon proximity to the carbonyl of **IV**. The high reactivity of **IV** would result in the higher probabilty of side reactions and loss of selectivity.

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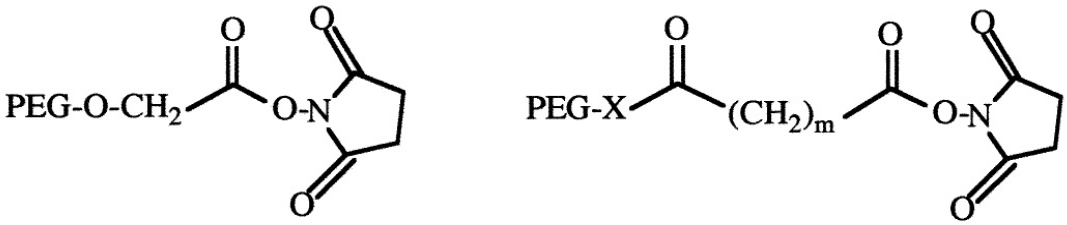
Reactions of a variety of anhydrides with PEG have been utilized to introduce carboxylic acid groups at the terminals of the polymer. The anhydrides used include succinic and glutaric (1a, 18-20). Diacid chlorides or dicarboxylic acids can also be reacted with mPEG to introduce a carboxlic acid group at the end of the polymer chain (21,22). In the case of the diacids a coupling agent such as dicyclohexylcarbodiimide is used. PEG derivatives such as PEG-succinimidyl succinate, **Va**, and PEG-succinimidyl glutarate, **Vb**, (Scheme 1) are less reactive than **1V**, but both suffer from the hydrolytic cleavage under physiological conditions (1a, 18-20). Replacement of the aliphatic ester in **Va** and **Vb** by an amide linkage (**Vc**) improved the stability of the resulting PEG-conjugate, but the synthesis of **Vc** starting from PEG involved 3 to 4 synthetic steps (12, 17).



IV

Va m = 2, X = O
Vb m = 3, X = O
Vc m = 2, X = NH

Scheme 1. Succinimidyl esters of PEG

Thus it is clear that PEG-succinimidyl propionate, prepared by carbodiimide-mediated esterification of poly(oxyethylene)propionic acid, should be less reactive than **IV** and more stable than **Va-c**. The present work describes the synthesis and characterization of methoxypoly(oxyethylene)propionic acid, **II**, from mPEG. We have undertaken this study because we were unable to synthesize **II** from the reaction of ethyl 3-bromopropionate and mPEG-alkoxide, a reported procedure (23).

Other methods to introduce carboxyl groups onto PEG include, reaction of PEG with ethyl isocyanatoacetate followed by hydrolysis, and reaction of amino acids with the 4-nitrophenyl chloroformate of PEG (24-26).

Experimental

Materials and instruments

Methoxypoly(ethylene glycol) (MW 2000) (mPEG), DMSO-d_6, and acrylonitrile were purchased from Aldrich. Acrylonitrile was distilled under nitrogen and stored in a refrigerator in the dark. Proton NMR spectroscopy was performed with a Varian Gemini 200 MHz or a Bruker 200 MHz instrument. The NMR solvent was DMSO-d_6 in all cases. Chemical shift are referenced to internal TMS and reported in ppm. Gel permeation chromatography (GPC) was performed on a Waters 501 liquid chromatograph system equipped with a differential refractometer and a Waters Ultrahydrogel 250 column. Aqueous phosphate buffer (5 mM, pH = 7.2) served as the mobile phase.

*Synthesis of methoxypoly(oxyethylene)propionitrile, **I***

mPEG-2000 (10.0 g, 5 mmol) was dissolved in water (15 mL) containing potassium hydroxide (0.2 g, 3.6 mmol). The solution was cooled to -5°- 0°C, and acrylonitrile

(1.33 g, 25 mmol) was added over ten minutes. The mixture was stirred for 2.5 hours at -5°- 0°C and then kept in a refrigerator overnight. The mixture was neutralized with hydrochloric acid (3M) and extracted with two portions of methylene chloride (100 mL each). The combined methylene chloride solution was washed with brine and dried over anhydrous magnesium sulfate. The volume of methylene chloride solution was reduced to 60 mL and the dried polymer in methylene chloride was then added dropwise to dry, cold diethyl ether (400 mL). The precipitated product was collected and dried in *vacuo* overnight; yield 8.93 g (87%). The degree of substitution for the product ranged from 97-100%, with the remaining being unreacted mPEG, as determined by NMR. ^{1}HNMR: δ 2.75 (t, 2H, -OCH$_2$C*H*$_2$CN), 3.25 (s, 3H, C*H*$_3$O-), 3.51 (s, polymer backbone). The GPC retention volume of **I** was the same as mPEG.

*Base hydrolysis of **I***

I (3 g, 1.46 mmol) was dissolved in water (100 mL) containing potassium hydroxide (5 g, 89 mmol) and the mixture was refluxed for four hours. The reaction flask was then placed in an ice bath and the reaction mixture was neutralized with hydrochloric acid (3M). Extraction, precipitation and drying as for **I** yielded 2.20 grams of product. The product was determined by ^{1}HNMR to be approximately 96% mPEG and 4% of **II**, the desired product. Base hydrolysis of **I** (using the same quantities as above) at ambient temperature for 96 hours resulted in 2.60 grams of an approximately equal molar mixture of mPEG and **II**, based on ^{1}HNMR.

*Synthesis of methoxypoly(oxyethylene)propionamide, **III**, using H_2O_2 and base*

I (5 g, 2.40 mmol) was dissolved in water (30 mL) containing potassium hydroxide (0.1 g, 1.8 mmol). The mixture was placed in an ice water bath, and to this solution H_2O_2 (30%) (1.5 mL was added dropwise over ten minutes. The exothermic reaction was stirred at room temperature overnight. The mixture was then neutralized with hydrochloric acid (3M) and the product was extracted, precipitated and dried as for **I**; yield 4.34 g (85%). ^{1}HNMR: δ 2.30 (t, 2H, -OCH$_2$C*H*$_2$CONH$_2$), 3.25 (s, 3H, C*H*$_3$O-), 3.51 (s, polymer backbone), 6.79 and 7.28 (br d, 2H, -CON*H*$_2$). GPC analysis showed that no PEG chain cleavage occurred during the above reaction.

*Synthesis of **III** using concentrated hydrochloric acid*

I (5 g, 2.40 mmol) was dissolved in concentrated hydrochloric acid (25 mL).The resulting solution was stirred for 44 hours at room temperature. Water (250 mL) was added to the reaction mixture and the reaction product was extracted, precipitated and dried as for **I**; yield 4.39 g (86%). The ^{1}HNMR of the product indicated quantitative conversion of **I** to **III** (85%) and **II** (15 %). ^{1}HNMR of the product in addition to the peaks reported for **III**, prepared by the H_2O_2 and base method, also contained a triplet at 2.42 due to the α protons of **II**. GPC analysis showed that almost no PEG chain cleavage occurred during the above reaction.

*Synthesis of methoxypoly(oxyethylene)propionic acid, **II**, from direct acidic hydrolysis of **I***

I (5 g, 2.40 mmol) was dissolved in concentrated hydrochloric acid (25 mL). The resulting solution was stirred at room temperature. Analysis of a small portion of the reaction mixture after 92 hours resulted in **II** (22%), **III** (68%), mPEG and products

resulting from chain cleavage of mPEG (10%). After 168 hours the reaction mixture was worked up as explained for the synthesis of **III** from **I** using hydrochloric acid. Yield 4.22 g. The ^{1}HNMR of the product indicated conversion of **I** to **II** (46%) with the remaining being mPEG or products resulting from the chain cleavage of the polymer backbone. GPC analysis of the product indicated that substantial PEG chain cleavage had occurred during the above reaction.

*Synthesis of **II** from **III***

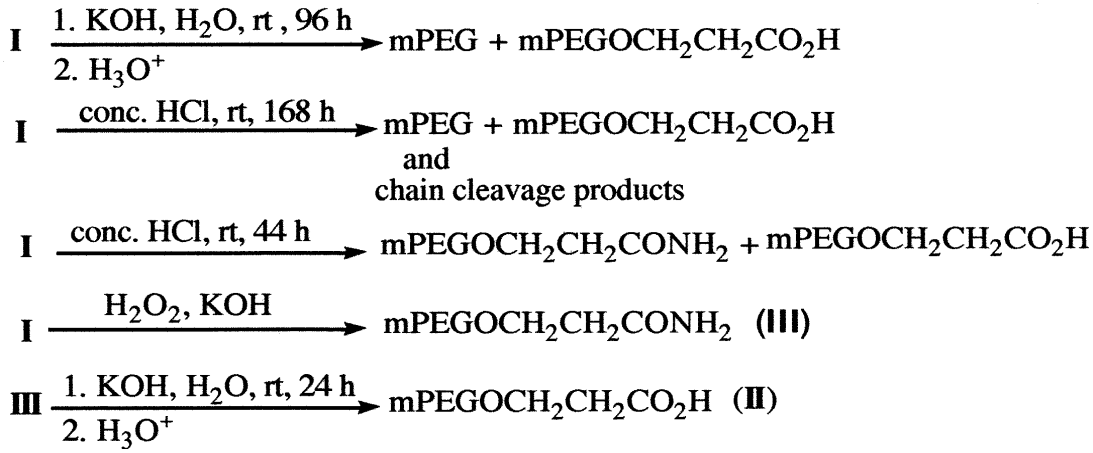
III (3 g, 1.43 mmol) was dissolved in water (100 mL) containing potassium hydroxide (5 g, 89 mmol) and the mixture was stirred for 24 hours. The reaction flask was then placed in an ice bath and the reaction mixture was neutralized with hydrochloric acid (3M). Extraction, precipitation and drying as for **I** resulted to the desired product; yield 2.55 g (86%). ^{1}HNMR: δ2.42 (t, 2H, -OCH$_2$C*H*$_2$CO$_2$H), 3.24 (s, 3H, C*H*$_3$O-), 3.51 (s, polymer backbone). The peak due to COOH was not observable. GPC analysis showed that no PEG chain cleavage occurred during the above reaction. The degree of substitution as determined by ^{1}HNMR was about 97-100% with the rest being MPEG as indicated by thin layer chromatography. The content of mPEG in the product is the same as the percentage of mPEG in **I** used for the synthesis. Thin layer chromatography experiments were carried out on silica gel plates and a mixture of isopropanol: aqueous ammonia (30%): water =10:2:1, was used as the developing solvent. The R_f values for mPEG and **II** were 0.67 and 0.48 respectively.

Results and discussion

Fradet and Marechal (16) had previously reported poor yields for the addition of PEG to acrylate esters. This result is in accord with a much earlier observation (27) that the ease of addition of acrylate esters to primary alcohols decreases as the molecular weight of the alcohol increases. Therefore, addition of mPEG to acrylate esters was ruled out as a viable synthesis route to methoxypoly(oxyethylene)propionic acid, **II**.

While mPEG can not be added efficiently to acrylate esters, we found that mPEG can be added to acrylonitrile easily and in high yields. The ^{1}HNMR of the product indicated a degree of substitution of 97-100% with the remaining being unreacted mPEG. Further, under the experimental conditions adopted, only one molecule of acrylonitrile was added to the end of each mPEG chain, as evidenced from GPC and ^{1}HNMR analyses of methoxypoly(oxyethylene)propionitrile, **I**. Recent reports, however, describe the synthesis of PEG-b-polyacrylonitrile from PEG and acrylonitrile in the presence of ceric ion in aqueous medium (28) and from block copolymerization of ethylene oxide and acrylonitrile using a bifunctional initiator with the characteristics of anionic and charge transfer polymerization (29).

It is well known that nitriles can be converted to acids under acidic or basic conditions. However, the base hydrolysis of **I** at reflux temperature yielded mainly mPEG, rather than the corresponding acid. In view of this results, hydrolysis of **I** under alkaline conditions, at ambient temperature was attempted. It was found that the hydrolysis reaction of **I** yielded approximately an equal mixture of mPEG and **II** (Scheme 2):



Scheme 2. Hydrolysis Reactions of methoxypoly(oxyethylene)propionitrile (**I**)

Similarly, direct acidic hydrolysis of **I** resulted in **II** as well as products resulting from chain cleavage of **I** or **II** during the reaction (Scheme 2). Details are in the experimental section.

The instability of this nitrile is probably attributable to the acidity of the α hydrogens, which, under alkali and heat, leads to deprotonation and formation of a resonance stabilized carbanion followed by formation of the corresponding alkoxide and acrylonitrile (or hydrolysis products of acrylonitrile or its polymer). This result is in accord with the view that "β-alkoxypropionitriles derived from primary alcohols with more than seven carbon atoms are unstable in the presence of alkali and heat" (30).

However, according to MacGregor and Paugh (31), β-ethoxypropionitrile can be converted to the corresponding acid by treatment with base (or acid) and heat.

Because the direct base and acid hydrolyses of methoxypoly(oxyethylene)propionitrile, **I** did not yield pure methoxypoly(oxyethylene)propionic acid, **II**, transformation of **I** to the corresponding amide was then considered. It was found that methoxypoly(oxyethylene)propionamide, **III**, could easily be prepared from **I** if the highly nucleophilic hydroperoxide ion (i.e., by using H_2O_2 and HO^-) or concentrated hydrochloric acid is used (32). Subsequent hydrolysis of **III** then resulted in **II** (Scheme 2). The efficiency of each of the above synthetic steps were monitored by ^{1}HNMR characterization of the products. It was found that these synthetic steps were very efficient, yielding almost complete functionalization. In the present work the degree of substitution of **II** was the same as the degree of substitution of the starting material, **I** (97-100%); the remaining being unreacted mPEG. The unreacted mPEG was also identified by thin layer chromatography as described in the experimental section. GPC analyses of **III** and **II** indicated that no degradation of mPEG backbone took place during reaction with hydroperoxide ion (or concentrated HCI) with **I** and during the conversion of **III** to **II**.

Our synthesis of methoxypoly(oxyethylene)propionic acid, **II**, while multistep, is very efficient and affords high purity product. A shorter method to synthesize **II** involves reaction of mPEG with a very large excess of a salt of a halogenated propionic acid in the presence of an alkali metal hydroxide followed by acidification (15). However, the product contains small amounts of unreacted starting materials or by-products. Another short method to prepare **II** is that of Geckeler and Bayer who reacted PEG-alkoxide and ethyl 3-bromopropionate in dry tetrahydrofuran (THF) and obtained **II**, after base

hydrolysis of the resulting ester (23). However, when we carried out this same reaction in dry THF the product was mainly mPEG, i.e. the elimination product. Apparently, the acidity of the α hydrogens leads the reaction toward the elimination pathway rather than the desired substitution. The question as to how the above authors obtained the substitution product remains open. On the other hand, we note that the results of the above transformation are consistent with that of mPEG-alkoxide with 3-chloropropionaldehyde diethyl acetal in benzene which gave primarily elimination products (33).

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