European Polymer Journal 108 (2018) 286–294

Contents lists available at ScienceDirect

European Polymer Journal

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

Mechanistic study on the hydrolytic degradation of polyphosphates

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A B S T R A C T

Ring-opening polymerization of cyclic phosphates offers a fast access to well-defined, water soluble and (bio)degradable polyphosphoesters (PPEs). In particular, poly (alkyl ethylene phosphate)s have been used as building blocks for nanocarriers or hydrogels. The molecular mechanism of their degradation is, however, not well understood. Herein, we study the hydrolytic degradation of two most frequently used PPEs, poly(methyl ethylene phosphate) (PMEP) and poly(ethyl ethylene phosphate) (PEEP). The degradation process is analyzed by NMR spectroscopy, which identifies and quantifies intermediates and degradation product(s). We prove that the major degradation pathway is backbiting, leading to one dominating hydrolysis product, ethyl or methyl ethylene phosphate (a diphosphate). Accelerated hydrolysis, performed in basic and acidic conditions, shows the high stability of PEEs under acidic conditions, while they readily degrade under basic conditions. The backbiting mechanism is further supported by the reduction of the degradation kinetics if the terminal OH-group is blocked by a urethane. Our findings help to develop degradable nanodevices with adjustable hydrolysis kinetics.

1. Introduction

Biodegradable polymers are interesting materials for applications ranging from materials science to biomedicine [1,2]. They are especially appealing for temporary therapeutic applications [3], e.g. for the design of surgical implants or drug delivery vehicles [4,5]. They remain inside of the body only temporarily but eventually are biodegraded. This degradation can happen either by hydrolytic or enzymatic degradation or a combination of both [6,7]. The majority of biodegradable polymers is based on synthetic polyesters or modified biopolymers that are susceptible to enzymatic and/or hydrolytic cleavage [8]. The pre-evaluation of enzymatic degradation *in vivo* is difficult, due to varying enzyme concentrations at different sites in the body and often *ex vivo* investigations with purified enzymes or accelerated hydrolysis conditions are conducted [9]. For a few years, polyphosphoesters (PPEs), e.g. polyesters based on phosphoric acid, have been considered as promising degradable materials for diverse biomedical applications [10,11]. Synthetic PPEs are inspired from natural PPEs, such as DNA and RNA or teichoic acids. The biodegradation of these biopolymers has been studied in detail [12–14]. In contrast, for synthetic PPEs, the potential biodegradability and biocompatibility are emphasized in most publications about PPEs, but only a few detailed studies of their degradation mechanism are available [15,16]. PPEs are, however, very interesting candidates for bioapplication due to their potential polyfunctionality and adjustable degradation kinetics [17]. For example, the enzymatic degradation of polyphosphates was reported to occur by phosphatases [18,19]. Also poly(alkyl ethylene phosphonate)s – with a stable P-C-bond as a pendant group- have been reported to undergo much faster hydrolysis than the respective polyphosphates [20]. First studies on the kinetics of PPE hydrolysis were presented by Penczek in the 1990s [21]. They evaluated the hydrolysis of poly(methyl ethylene phosphate) (PMEP) at pH-values ranging from 2 to 12 and suggested two different mechanisms for hydrolysis under basic and acidic conditions. Under basic conditions, they proposed a direct nucleophilic attack of the OH^- ion on the phosphorus atom leading to an intermediate five-binding phosphorus with a trigonal bipyramidal geometry in which the axial position is preferably shed. Due to comparable probabilities for the main and side chains to occupy this position, these chains should be cleaved with the probability proportional to the Boltzmann prefactor. Under acidic conditions, however, they proposed a nucleophilic attack of H_2O on the α-carbon, after protonation.

Herein, we systematically investigated the hydrolysis of two poly (alkyl ethylene phosphate)s (with alkyl = methyl or ethyl, i.e. PMEP and PEEP), the two most frequently used and water-soluble PPEs. They are prepared by the ring-opening polymerization of the respective 5-membered cyclic phosphate monomers and are accessible in high control over molecular weight, dispersity, and end-group chemistries [10,22]. We propose a different hydrolysis mechanism, mainly happening from the chain end by a backbiting reaction, leading to a single degradation product, namely alkyl (2-hydroxyethyl) phosphate. This mechanism is supported by the characterization of the degradation product and by a drastic slowdown of the degradation kinetics if the OH-chain end is blocked by a stable urethane linkage. This is the first report on the detailed back-biting degradation of poly(alkyl ethylene phosphate)s and will be helpful for the design of future degradable polymer architectures based on PPEs.

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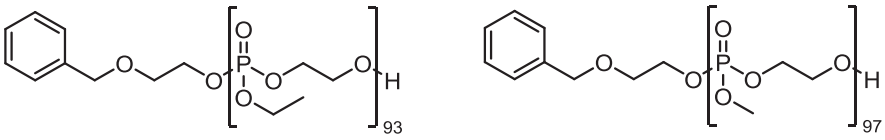
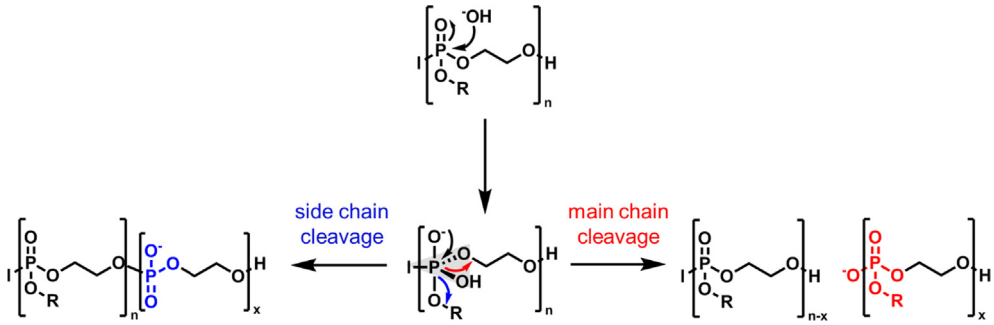
https://doi.org/10.1016/j.eurpolymj.2018.08.058
Received 16 July 2018; Received in revised form 28 August 2018; Accepted 31 August 2018
Available online 08 September 2018
0014-3057/



poly(ethyl ethylene phosphate)
(**PEEP**)

poly(methyl ethylene phosphate)
(**PMEP**)

Fig. 1. Chemical structures of the polyphosphates used in this study.



Scheme 1. Hydrolytic degradation of poly(alkyl ethylene phosphate)s (typically prepared by ring-opening polymerization of cyclic phosphoester) under basic conditions as reported in previous literature [21].

2. Experimental

2.1. General

All reagents were used without further purification unless otherwise stated. Solvents, dry solvents and deuterated solvents were purchased from Acros Organics, Sigma-Aldrich, Deutero GmbH or Fluka. 1,8-diazabicyclo[5.4.0]undec-7-ene was distilled prior to use, and stored over molecular sieve at 4 °C.

2.2. Methods

2.2.1. Size exclusion chromatography (SEC)

SEC measurements were conducted in DMF (containing 0.25 g L^{-1} of lithium bromide) at 60 °C on an Agilent 1100 Series as an integrated instrument, including a PSS GRAM columns (1000/1000/100 g), a UV detector (270 nm), and a RI detector at a flow rate of 1 mL min^{-1} at 60 °C. Calibration was carried out using PEO standards provided by Polymer Standards Service.

2.2.2. Nuclear magnetic resonance (NMR)

For nuclear magnetic resonance analysis ^{1}H, ^{13}C, and ^{31}P NMR spectra were recorded on a Bruker AVANCE III 300 or 500 MHz spectrometer. All polymer spectra were measured in either CDCl3 or CD_2Cl_2 and the spectra were calibrated against the solvent signal and analyzed using MestReNova 8 from Mestrelab Research S.L.

Degradation studies were recorded on a Bruker AVANCE III 500 MHz spectrometer. All degradation studies were conducted in H_2O containing 10% of D_2O.

To measure diffusion coefficient of ^{31}P-nuclei an INEPT based 2D measurement were used with non-selective polarisation transfer between protons and phosphorous nuclei using bipolar gradient pulses for diffusion.

In this work, the gradient strength was varied in 32 steps from 2% to 100%. The diffusion time was optimized at 50 ms and the gradient length to 1.4 ms. The chosen relaxation delay was set to 2 s.

2.2.3. DFT calculations

The structures were optimized at the B3LYP level of theory [23], in conjugation with the basis set of 6-311 + + G** [24]. The SMD solvation model was used to compute the solvation Gibbs free energies by employing water as the solvent [25]. All DFT calculations were carried out by means of Gaussian 09 package [26].

2.3. Synthetic procedures

2.3.1. Synthesis of poly(methyl ethylene phosphate) ($PMEP_{97}$)

All Schlenk-tubes were flame-dried prior to use. 2-(benzyloxy) ethanol was used as initiator and 1,8-diazabicyclo[5.4.0]undec-7-ene DBU (DBU)/1-(3,5-bis(trifluoromethyl)phenyl)-3-cyclohexylthiourea (TU) as catalyst/co-catalyst system. TU (121 mg, 328 µmol, 5 eq) was introduced into a flame-dried Schlenk-tube, dissolved in benzene, and dried by lyophilization. MEP (894 mg, 6.5 mmol, 100 eq) was added. 0.49 mL (9.9 mg, 64.8 µmol, 1 eq) of initiator stock solution (c = 20 mg mL^{-1} in dry DCM) was added and the monomer concentration was adjusted to 3 mol L^{-1}. The reaction mixture was cooled down to 0 °C and the polymerization was started by rapid addition of DBU (50 mg, 328 µmol, 5 eq). The polymerization was quenched after 1.4 h by addition of 2 mL of acetic acid in DCM (c = 20 mg mL^{-1}). The polymer was obtained as a colorless viscous liquid (819 mg, 92%) and purified by precipitation into diethyl ether and subsequent dialysis against water.

^{1}H NMR (300 MHz, Methylene Chloride-d_2) δ 7.33 (m, 5H), 4.54 (s, 2H), 4.51–4.02 (m, 378H), 3.78 (dd, J = 11.2, 5.9 Hz, 291H). ^{31}P NMR (121 MHz, Methylene Chloride-d_2) δ 1.04, −0.18, −1.42.

2.3.2. Synthesis of poly(methyl ethylene phosphate) ($PEEP_{93}$)

All Schlenk-tubes were flame-dried prior to use. 2-(benzyloxy) ethanol was used as initiator and DBU/TU as catalyst/co-catalyst system. TU (121 mg, 328 µmol, 5 eq) was introduced into a flame-dried Schlenk-tube, dissolved in benzene and dried by lyophilization. EEP (1026 mg, 6.7 mmol, 100 eq) was added to dissolve the TU co-catalyst. 0.51 mL (10.3 mg, 67.5 µmol, 1 eq) of initiator stock solution (c = 20 mg mL^{-1} in dry DCM) was added and the monomer concentration was adjusted to 3 mol L^{-1}. The reaction mixture was cooled down to 0 °C and the polymerization was started by rapid addition of DBU (50.0 mg, 328 µmol, 5 eq). The polymerization was quenched after 1.4 h by addition of 2 mL of acetic acid in DCM (c = 20 mg mL^{-1}). The polymer was obtained as a colorless viscous liquid (921 mg, 89%) and purified by precipitation into diethyl ether and subsequent dialysis against water.

^{1}H NMR (300 MHz, Methylene Chloride-d_2) δ 7.53–6.97 (m, 5H),