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Synthesis and characterization of thiomers of polyaspartamide type

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Abstract

Synthesis of poly[α,β-(*N*-2-hydroxyethyl-DL-aspartamide)]–thioglycolic acid (PHEA–TGA) conjugate as a new polyaspartamide thiomer is described. The parent polymer PHEA is chemically modified by introducing sulphydryl-bearing compound thioglycolic acid.

By varying the reaction conditions several batches of PHEA–TGA conjugates were prepared and analyzed. Tensile studies revealed that total work of adhesion of PHEA–TGA increased more than twice compared to the unmodified polymer. Microparticles prepared from the thiolated polymer preserved its bioadhesive properties.

Keywords: Thiomer; Polyaspartamide; Thioglycolic acid; Mucoadhesion; Microparticles

1. Introduction

Thiolated polymers (thiomers) represent a promising new generation of mucoadhesive polymers. They are supposed to interact with cysteine-rich subdomains of mucus glycoproteins thereby forming disulphide bonds between the mucoadhesive polymer and the mucus layer (Gum et al., 1992). Thiomers could provide prolonged residence time of drug delivery systems on various mucosal tissues compared to well established polymers, improved cohesive properties, show enzyme inhibitory capabilities and a permeation enhancing effect (Bernkop-Schnürch and Thaler, 2000; Bernkop-

Abbreviations: AS, atomic spectrometry; DMF, *N*,*N*′-dimethylformamide; DSC, differential scanning calorimetry; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid) (Ellman's reagent); DTT, dithiothreitol; EDAC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride; PHEA, poly[α,β-(*N*-2-hydroxyethyl-DL-aspartamide)]; SEM, scanning electron microscopy; SNF, simulated nasal fluid; TG, thermogravimetry; TGA, thioglycolic acid; TWA, total work of adhesion

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Schnürch et al., 2001a, 2003). These features render thiolated polymers as useful excipients for various drug delivery systems.

Numerous thiomers have been synthesized and evaluated based both on anionic (polycarbophil, carboxymethylcellulose, alginate) and cationic polymers (chitosan). In order to introduce thiol moieties, sulphydryl-bearing compounds, such as cysteine, cysteamine or thioglycolic acid (TGA) have been used (see, e.g., Bernkop-Schnürch and Steininger, 2000; Kast and Bernkop-Schnürch, 2001; Bernkop-Schnürch et al., 2001b).

In this paper we report preparation and characterization of poly[α,β-(*N*-2-hydroxyethyl-DL-aspartamide)]–thioglycolic acid conjugate (PHEA–TGA), a new type of thiolated polyaspartamide. PHEA is chosen for modification since it is a hydrosoluble, nontoxic and nonantigenic polymer useful in preparation of various polymer–drug conjugates (see, e.g., Antoni et al., 1979; Giammona et al., 1998; Martinac et al., 2002; Van der Merwe et al., 2002).

2. Materials and methods

2.1. Instruments and materials

IR spectra were recorded on a GX FT-IR spectrometer (Perkin Elmer, UK). ^{1}H and ^{13}C NMR spectra were taken on a Varian Gemmini spectrometer (Varian, USA). Differential scanning calorimetry (DSC) was carried out with a type DSC Pyris 1 instrument (Perkin Elmer, UK). Thermogravimetric analyzer TGA 7 (Perkin Elmer, USA) was used for thermogravimetry (TG). Atomic spectrometry (AS) was carried on inductively coupled plasma atomic emission spectrometer (Vista Pro, Varian, USA). The weight average molecular weight was determined by size exclusion chromatography (SEC) with UV detector (Series II, Hewlet Packard, USA). Microparticles were prepared using spray dryer (Büchi 190, Flawil, Switzerland). Their characterization was done by Olympus BH-2 microscope, equipped with a computer-controlled image analysis system (Optomax V, Cambridge, UK) and JSM-5800 scanning electron microscope (Joel, Japan). The centrifugation was performed at spin 3500 × *g* on Labofuge 400 (Heraeus, Germany) using filter device Centricon® Plus 20 (molecular weight cut-off 5000, Amicon Bioseparations, Millipore, USA). Dialysis was made with cellulose dialysis tubings with a molecular weight cut-off 8,000–12,000 (Sigma, USA). For thin layer chromatography, silica gel sheets Kieselgel 60 F_{254} (Merck, Germany) were used. Solvent system was dichloromethane/methanol 1:1. For spot detection iodine vapour was used. Gel filtration molecular weight standards were purchased from Bio Rad Laboratories CA (USA). Thioglycolic acid and Ellman's reagent were purchased from Sigma-Aldrich (Germany), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride and dithiothreitol from Sigma (USA), L-aspartic acid and ethanolamine from Kemika (Croatia). The amine was distilled prior to use. All solvents were of analytical grade purity and dry.

2.2. Synthesis

2.2.1. Synthesis of poly[α,β-(N-2-hydroxyethyl-DL-aspartamide)] (PHEA, **1**)

PHEA was synthesized following the procedure published previously (Neri et al., 1973; Zorc et al., 1993).

2.2.2. Synthesis of poly[α,β-(N-2-hydroxyethyl-DL-aspartamide)]–thioglycolic acid conjugates (PHEA–TGA, **2a–f**). General procedure

PHEA and the corresponding amount of TGA were dissolved in demineralized water in order to obtain 0.8% solution of PHEA (Table 1). The solution was cooled in ice bath and 1-ethyl-3-(3-dimetylaminopropyl)carbodiimide (EDAC) was added. The reaction mixture was stirred for 24 or 48 h at room temperature, protected from light. The analogous reaction without carbodiimide served as a control. The reaction mixture was dialyzed protected from light, lyophilized and stored at 4 °C until use. Yields: 68–96%.

2a: PHEA/TGA mass ratio 5:1; EDAC concentration 58 mM; reaction time 24 h; dialysis 3 days against 5 mM HCl in the presence of small amount of $Na_2S_2O_4$, 1 day against 1 mM HCl, room temperature. Yield: 96%.

2b: PHEA/TGA mass ratio 2:1; EDAC concentration 58 mM; reaction time 24 h; dialysis 3 days against 5 mM HCl in the presence of small amount of $Na_2S_2O_4$, 1 day against 1 mM HCl, room temperature. Yield: 92%.