SYNTHESIS OF N^2-[N^5-(2-HYDROXYETHYL)-L-GLUTAMINYL]-N^5-(2-HYDROXYETHYL)-L-GLUTAMINE

Rudolf KOTVA, Jindrich PYTELA and Frantisek RYPACEK

Institute of Macromolecular Chemistry,
Academy of Sciences of the Czech Republic, 162 06 Prague 6, The Czech Republic

Received April 5, 1994
Accepted May 3, 1994

The synthetic, water-soluble polypeptide, poly[N^5-(2-hydroxyethyl)-L-glutamine] (PHEG), was proposed as a suitable polymer for biomedical applications[1,2]. Its biodegradability was demonstrated both in vitro and in vivo[3,4]. Ultimate degradation fragments resulting from the degradation of PHEG by endopeptidases, e.g. papain, are oligomers (down to tetramers) of N^5-(2-hydroxyethyl)-L-glutamine (HEG), which can be further degraded to dimeric and monomeric HEG by exopeptidases[5]. For in vivo studies related to the toxicology and metabolic disposition of these low-molecular-weight fragments of PHEG degradation as well as for an accurate calibration of analytical methods used, such as size exclusion chromatography (SEC), the standards, monomeric and dimeric HEG, are of crucial importance.

This communication reports on the synthesis of dimer of HEG, N^2-[N^5-(2-hydroxyethyl)-L-glutaminyl]-N^5-(2-hydroxyethyl)-L-glutamine (Scheme 1). Its applicability as a calibration standard is demonstrated in Fig. 1 showing its elution curve together with the curves of PHEG and HEG monomer.

EXPERIMENTAL

All chemicals were purchased from Fluka or Aldrich and were used without further purification. Solvents were dried by standing over molecular sieve 4 Å, if necessary. Melting points are given without correction.

Mass spectroscopy measurements were performed on a ZAB-EQ spectrometer (V.G. Analytical, Manchester, U.K.). ^{1}H NMR spectra were run on a Bruker ACF-300 spectrometer at 300.1 MHz. Chemical shifts are given in δ (ppm) from the respective internal standard. SEC analysis was carried out on a HEMA-BIO 300 (8 × 250 mm) column (Tessek Ltd., Prague, The Czech Republic) using a Waters GPC–HPLC system with Baseline 810 Datastation. As a mobile phase 0.15 M NaCl buffered with 0.005 M phosphate (pH 7.8) was used at a flow rate 1.0 ml/min. The samples (10 μl) of HEG, *IV* and PHEG were applied dissolved in the mobile phase. Their elution curves were monitored by the absorbance at 220 nm using a Model 484 UV-VIS detector. The peak of water (corresponding to the total volume of the column) was obtained from the refraction index detection using a Model 410 RI detector.

N^5-(2-Hydroxyethyl)-L-glutamine (HEG) was synthesized by the reaction of L-glutamic acid with ethanolamine adopting the procedure described in ref.[6].

SCHEME 1

Poly[N^5-(2-hydroxyethyl)-L-glutamine] (PHEG) (M.w. 16 000) was synthesized by the method described previously[2] involving polymerization of poly(γ-benzyl L-glutamate) by the NCA method and total aminolysis of polymer benzyl esters with ethanolamine.

γ-Benzyl L-glutamate[7] and γ-benzyl benzyloxycarbonyl-L-glutamate[8] were prepared as previously described.

γ-Benzyl α-(4-nitrophenyl)benzyloxycarbonyl-L-glutamate (*I*) was prepared by the reaction of γ-benzyl benzyloxycarbonyl-L-glutamate with 4-nitrophenol in the presence of *N,N′*-dicyclohexylcarbodiimide[9].

Bis(γ-benzyl) (Benzyloxycarbonyl-α-L-glutamyl)-L-glutamate (*II*)

Glutamate *I* (5.42 g, 11.0 mmol) in tetrahydrofuran (30 ml) was added to the suspension of γ-benzyl L-glutamate (3.13 g, 13.2 mmol) and $NaHCO_3$ (1.11 g, 13.2 mmol) in water (30 ml) and the reaction mixture was stirred overnight at room temperature. The resulting solution was evaporated and the residue was mixed with a 10% solution of citric acid (pH 3 – 4). A white precipitate was filtered off, washed with water and dried yielding 6.08 g (93.6%) of the crude product, which was further purified by repeated crystallization from an acetone–hexane mixture, m.p. 58 – 60 °C. For $C_{32}H_{34}N_2O_9$ (590.6) calculated: 65.07% C, 5.80% H, 4.74% N; found: 64.72% C, 5.78% H, 4.65% N. ^{1}H NMR spectrum ($CDCl_3$, hexamethyldisiloxane): 1.85 m, 1 H; 1.95 m, 1 H; 2.04 m, 1 H; 2.14 m, 1 H and 2.38 m, 4 H (b,b′,c,c′); 4.27 q, 1 H and 4.48 q, 1 H (a,a′); 4.98 m, 6 H (d); 7.23 m, 15 H (e).

N^2-[N^2-Benzyloxycarbonyl-N^5-(2-hydroxyethyl)-L-glutaminyl]-N^5-(2-hydroxyethyl)-L-glutamine (*III*) 2-Hydroxyethylammonium Salt

A solution of *II* (1.549 g, 2.62 mmol) and 4-(dimethylamino)pyridine (0.064 g, 0.524 mmol) in DMF (5.5 ml) was mixed with ethanolamine (7 ml) under stirring. The resulting solution was stirred for 24 h at room temperature and then poured into 700 ml of ether. After standing for one week the supernatant was poured off and the remaining semisolid product was crystallized from ethanol–ether mixture. The product was obtained as white crystals in the yield 1.228 g (84%). It was further purified by recrystallization from the same solvents. M.p. 145 – 150 °C. For $C_{24}H_{39}N_5O_{10}$ (557.6) calculated: 51.70% C, 7.05% H, 12.56% N; found: 51.40% C, 6.72% H, 12.21% N.

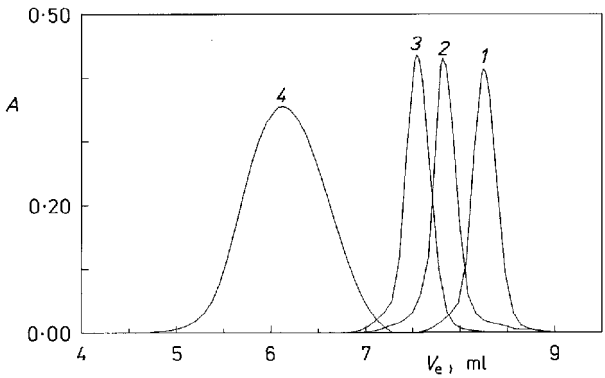

FIG. 1
SEC analysis (V_e elution volume, *A* absorbance). *1* Water; *2* N^5-(2-hydroxyethyl)-L-glutamine; *3* N^2-[N^5-(2-hydroxyethyl)-L-glutaminyl]-N^5-(2-hydroxyethyl)-L-glutamine (*IV*); *4* PHEG (M.w. 16 000)

N^2-[N^5-(2-Hydroxyethyl)-L-glutaminyl]-N^5-(2-hydroxyethyl)-L-glutamine (*IV*)

2-Hydroxyethylammonium salt of *III* (0.836 g, 1.5 mmol) in 80% acetic acid (105 ml) was hydrogenated in the presence of 5% Pd/C (0.5 g) for 18 h at room temperature. The catalyst was removed by filtration, the filtrate was evaporated at low pressure and the residue was crystallized from aqueous ethanol. Yield 0.421 g (78%), m.p. 216 – 220 °C (decomp.). For $C_{14}H_{26}N_4O_7$ (362.4) calculated: 46.40% C, 7.23% H, 15.46% N; found: 46.23% C, 7.14% H, 14.96% N. Mass spectrum, *m/z* : 363.3 (M + 1), 349.3, 327.3, 319.3, 301.3, 283.3, 263.2, 247.2, 217.2, 207.2, 175.2, 158.2, 142.2, 128.1, 115.1. ^{1}H NMR spectrum (D_2O, sodium 3-trimethylsilylpropanesulfonate): 1.98 m, 1 H; 2.12 m, 1 H and 2.20 m, 2 H (b,b′); 2.33 t, 2 H and 2.48 t, 2 H (c,c′); 3.32 m, 4 H (e,e′); 3.64 m, 4 H (d,d′); 4.07 t, 1 H and 4.22 t, 1 H (a,a′).

REFERENCES

1. Anderson J. M., Gibbons D. F., Martin R. L., Hiltner A., Woods R.: J. Biomed. Mater. Res. Symp. *5*, 197 (1974).
2. Rypacek F., Saudek V., Pytela J., Skarda V., Drobnik J.: Makromol. Chem., Suppl. *9*, 129 (1985).
3. Pytela J., Saudek V., Drobnik J., Rypacek F.: J. Controlled Release *10*, 17 (1989).
4. Dickinson H. R., Hiltner A., Gibbons D. F., Anderson J. M.: J. Biomed. Mater. Res. *15*, 577 (1981).
5. Rypacek F., Pytela J.: Unpublished results.
6. Lichtenstein N., Grossowicz N.: J. Biol. Chem. *171*, 387 (1947).
7. Guttmann S., Boissonas R. A.: Helv. Chim. Acta *41*, 1852 (1958).
8. Hanby W. E., Waley L. G., Watson J.: J. Chem. Soc. *1950*, 3239.
9. Losse G., Jeschkeit H., Langenbeck W.: Chem. Ber. *96*, 204 (1963).

Translated by the author (J. P.).