Improved method for the preparation of poly[N^5-(2-hydroxyethyl)-L-glutamine] by aminolysis of poly(γ-benzyl-L-glutamate)

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This paper describes the synthesis of poly[N^5-(2-hydroxyethyl)-L-glutamine] (PHEG) by the aminolysis of poly(γ-benzyl-L-glutamate) (PBG) with 2-aminoethanol. The effects of the temperature and addition of the bifunctional catalyst, 2-hydroxypyridine (2-HP), on the kinetics of aminolysis were studied. The results suggest that the aminolysis reaction takes place much faster with increasing temperature and an increasing amount of added catalyst. Moreover, chain scission, due to aminolysis of the amide bonds of the polymer backbone, can be minimized by the addition of the appropriate amount of 2-hydroxypyridine. These data demonstrate the feasibility of the described method to prepare PHEG derivatives with well controlled molecular weights.

(Keywords: poly(hydroxyalkylglutamine)s; aminolysis; chain scission)

INTRODUCTION

Derivatives of poly(α-amino acids) have been suggested as potential biomedical polymers, plasma expanders or drug delivery systems[1–3]. Polypeptides which are derived from glutamic acid are considered as having the best prospects because of the convenient way in which their side chains can be modified. Poly(N^5-hydroxyalkylglutamine)s are neutral, water soluble, biocompatible polymers, which can be prepared with preserving their L-conformation[3]. Of the several carriers that have been suggested, those based on N^5-hydroxyethyl-L-glutamine (HEG) appear to have great potential. They are biodegradable and when used as plasma expanders have a low toxicity in humans[4–6].

Poly(N^5-hydroxyalkylglutamine)s are usually prepared by the aminolysis of poly(γ-alkylglutamate)s using amino alcohols. So far, this reaction has been mainly studied with respect to the choice of the amino alcohol and its effect on the solubility of the resulting polymer in water[7]. Attention has also been paid to the degree of polymerization of the resulting poly(N^5-hydroxyalkylglutamine)s, which usually decreases during the aminolysis, in comparison with the chain length of the initial polyglutamates. Optimal conditions for aminolysis, under which a degree of polymerization of up to 70% of the original value was preserved, have been suggested[8]. Moreover, both the influence of the solvent and the reaction temperature on the rate of aminolysis have been investigated[9]. However, extensive chain scission, due to aminolysis of the poly-α-L-amino acid amide linkages, has always been observed. This limits the preparation of well defined PHEG derivatives. Feijen and coworkers reported the use of the bifunctional catalyst, 2-hydroxypyridine (2-HP), in the aminolysis of poly(γ-benzylglutamate) (PBG) with 3-aminopropanol: application of 2-HP proved successful, both with respect to yield and molecular weight[10]. However, aminolysis occurred at 60°C and main-chain cleavage was still significant.

The present study reports the application of 2-HP as a bifunctional catalyst in the aminolysis of PBG with 2-aminoethanol. The effect of the amount of added catalyst on the rate of aminolysis and the molecular weight of the prepared PHEG was investigated, both at ambient temperature and at 40°C. The aim of this work was to optimize the reaction conditions for aminolysis, so that main-chain cleavage could be minimized and well defined PHEGs could be prepared.

EXPERIMENTAL

All chemicals were purchased from Janssen Chimica (Beerse, Belgium). ^{1}H n.m.r. spectra were recorded on a Brücker WH 360 spectrometer.

Preparation of monomer

γ-Benzyl-L-glutamate was prepared by the method of Guttmann and Boissonnas[11]. *N*-carboxyanhydride (NCA) was prepared by the reaction of ester with diphosgene in ethylacetate, and was recrystallized three times from ethylacetate.

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Preparation of poly(benzyl-L-glutamate)

Poly(γ-benzyl-L-glutamate) (PBG) was prepared by polymerization of the respective NCA in ethylacetate/dichloromethane (1/2 v/v), initiated by tri-n-butylamine (monomer/initiator ratio = 200/1) at 60°C[12].

Five grams of monomer are dissolved in 5 ml dry ethylacetate by warming on a water bath. To this solution are rapidly added 10 ml of CH_2Cl_2 and 4 ml of initiator solution (2.5 vol% in CH_2Cl_2) and the mixture is heated under reflux for 30 min, after which the mixture is kept at ambient temperature for a further 12 h. The polymer is precipitated in 500 ml methanol, and the resulting PBG is filtered and then vacuum dried. The molecular weight of the polymer is determined from viscometry measurements in dichloroacetic acid (DCA) by using the following relationship[13,14]: $\eta = 2.78 \times 10^{-5} \times M^{0.87}$.

Kinetic measurements

The aminolysis of PBG was carried out using 2-aminoethanol in the presence of different ratios of catalyst/ester groups, i.e. 0/1, 0.2/1, 1/1 and 5/1. In a typical experiment, 150 mg (0.68 mmol) of PBG and 13 mg (0.136 mmol) of 2-HP were dissolved in 2 ml of DMF, and 2-aminoethanol (850 μl, 13.6 mmol) was added dropwise. After appropriate time intervals, 300 μl aliquots were withdrawn from the reaction mixture and precipitated in a mixture of ether/ethanol (5/1 vol/vol). The polymer was filtered, dried and analysed by ^{1}H n.m.r. spectroscopy in CF_3COOD. By comparing the integrated signals for the aromatic and benzylic protons of the ester (7.2 and 5.15 ppm, respectively), the α-CH of the polymer backbone (4.75 ppm) and the methylene protons of the hydroxyethyl side-chain groups (4.55 and 3.75 ppm), the degree of conversion can be calculated.

After completion of the aminolysis reaction, the molecular weight of the prepared PHEG was determined by a high pressure/size exclusion chromatography (h.p./s.e.c.) method, using the following conditions: columns, Tessek Hema Bio 1000, 300, 100 and 40; eluent, citrate buffer pH = 6, 0.1 M; detection, RI; calibration, dextran standards, 123 600, 66 700, 43 500, 21 400 and 4440.

RESULTS AND DISCUSSION

The aminolysis of poly(γ-benzyl-L-glutamate) with 2-aminoethanol, yielding poly[N^5-(2-hydroxyethyl)-L-glutamine], is represented in *Figure 1*.

The aminolysis reaction of side-chain esters can be accelerated by increasing the reaction temperature. However, at higher reaction temperatures, the molecular weight of the prepared PHEG is always substantially lower, as a result of increased chain degradation. In order to prepare PHEG derivatives with well controlled molecular weights it is of importance to minimize the chain scission that occurs during aminolysis.

The formation of amides by the aminolysis of esters is considerably accelerated by bifunctional compounds which possess both a basic and an acidic group, mutually situated in such a manner that a cyclic transition state, allowing a concerted displacement, may be postulated[15]. Among various bifunctional compounds, 2-hydroxypyridine (2-HP) is the preferred catalyst, and has proved extremely useful in the amination of 'low energy' esters (e.g. methyl esters) at room temperature[16–18].

For preparing poly[N^5-(2-hydroxyethyl)-L-glutamine] (PHEG) by the aminolysis of poly(γ-benzyl-L-glutamate) (PBG) with minimal main-chain cleavage, the influence of the amount of added catalyst on the rate of aminolysis was investigated at ambient temperature and at 40°C. The rate of aminolysis was also investigated in the presence of different ratios of catalyst to ester groups. The degree of conversion was calculated, after isolation of the intermediate copolymer, by ^{1}H n.m.r. spectroscopic analysis. *Figures 2* and *3* show the dependence of the conversion rate on the various catalyst/ester ratios, measured at room temperature and at 40°C, respectively. The times required for 50% conversion, for different proportions of catalyst, are summarized in *Table 1*.

These data indicate that the reaction proceeds much faster with increasing temperature. The conversion of the benzylester groups into hydroxyethyl groups is clearly accelerated by addition of the catalyst. Aminolysis at ambient temperature (without additional catalyst) required 4 days to achieve 50% conversion. However, in the presence of a fivefold excess of 2-HP, a 50% conversion is reached within 8 h. At 40°C, the effect of the catalyst is even more pronounced, with no further benzylester groups being detectable after 24 h of reaction.

Furthermore, the reduction of chain scission, by aminolysis of the main-chain amide bonds in PBG, in the presence of different proportions of catalyst, was studied. The molecular weight of the initial PBG was determined by viscometry. For the analysis of the

Table 1 Reaction time required for a 50% conversion of the side-chain ester into hydroxyethyl groups in the aminolysis of PBG

Ratio 2-HP/ester	R.t.	40°C
0/1	96	10
0.2/1	96	10
1/1	16	8
5/1	8	4

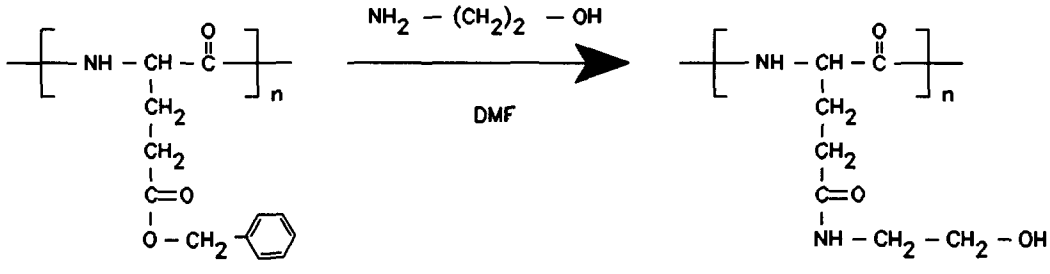

Figure 1 Reaction scheme for the aminolysis of PBG

molecular weight of the prepared PHEG a h.p./s.e.c. method was applied. The calculated molecular weights of PBG and PHEG for the reactions carried out at ambient temperature, and at 40°C, are presented in *Tables 2* and *3*, respectively.

These data indicate that chain scission increases with increasing reaction temperature: the molecular weight drops from 180 000 to 100 400 for the reaction at room temperature, and from 160 000 to 31 250 for the reaction at 40°C. It should be noted that the molecular weight data for the PBG are derived from viscosity measurements, whereas those of the PHEG are determined by g.p.c. analysis. The molecular weight data are therefore only approximative. The addition of a bifunctional catalyst minimizes chain degradation at room temperature: when

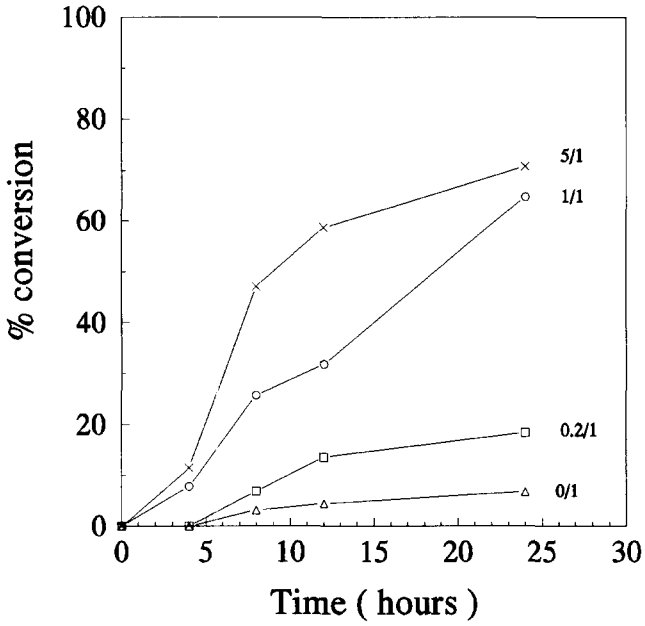

Figure 2 Dependence of the conversion on time, measured at room temperature, for different ratios of 2-HP/PBG: △, 0/1; □, 0.2/1; ○, 1/1; ×, 5/1

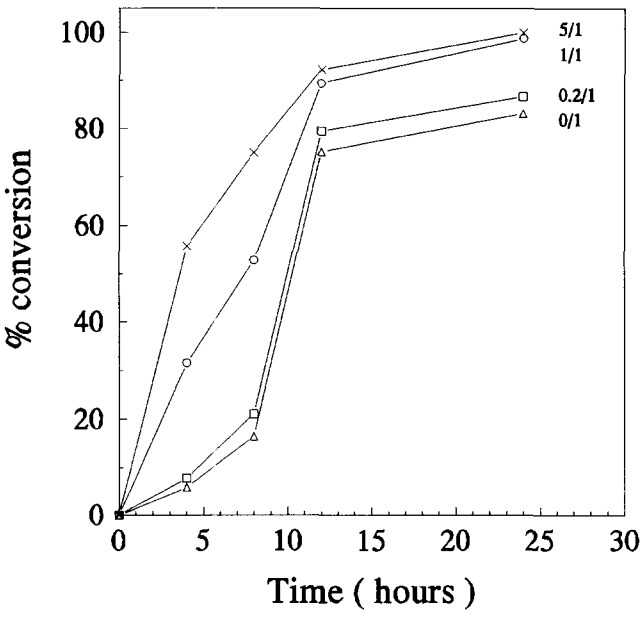

Figure 3 Dependence of the conversion on time, measured at 40°C, for different ratios of 2-HP/PBG: △, 0/1; □, 0.2/1; ○, 1/1; ×, 5/1

Table 2 Effect of the ratio of catalyst (2-HP) to ester (PBG) on the molecular weight of the PHEG prepared at room temperature. The molecular weight of the initial PBG is given for comparison

Ratio 2-HP/ester	M_v(PBG)	M_w(PHEG)	M_n(PHEG)	M_n/M_v
0/1	180 000	100 400	42 750	0.238
0.2/1	180 000	134 500	58 200	0.323
1/1	180 000	139 900	73 000	0.406
5/1	160 000	119 700	62 600	0.391

Table 3 Effect of the ratio of catalyst (2-HP) to ester (PBG) on the molecular weight of the PHEG prepared at 40°C. The molecular weight of the initial PBG is given for comparison

Ratio 2-HP/ester	M_v(PBG)	M_w(PHEG)	M_n(PHEG)	M_n/M_v
0/1	160 000	31 250	6 175	0.039
0.2/1	160 000	57 100	19 900	0.124
1/1	220 000	68 700	29 100	0.132
5/1	220 000	154 300	76 400	0.347

the M_n values of the prepared PHEG derivatives are compared with the molecular weight of the original PBG, a clear decrease of chain scission with an increasing proportion of catalyst is observed.

The effect of the amount of added catalyst on the M_n of the resulting polymer is even more pronounced at higher temperatures: the ratio of M_n/M_v increases rapidly with increasing amounts of 2-HP.

These results demonstrate that the aminolysis of PBG with 2-aminoethanol can be accelerated by addition of the appropriate amount of bifunctional catalyst, both at ambient temperature and at 40°C. Moreover, application of this catalyst can minimize degradation of the polymer backbone, even at higher temperatures. This method therefore allows the preparation of PHEG derivatives with controlled molecular weights by varying the molecular weight of the corresponding PBG polymers.

CONCLUSIONS

These results clearly show that aminolysis of the side-chain ester of PBG can be greatly affected by the presence of the bifunctional catalyst, 2-hydroxypyridine. The reaction time and chain scission can be reduced, both at room temperature and at 40°C. This indicates that the described method may be useful for the preparation of PHEG derivatives with well defined molecular weights, which can be used for biomedical purposes.

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