Poly(N^5-hydroxyalkyl glutamines): 2. The effect of conformation and solvent on the kinetics of poly(γ-alkyl glutamate) aminolysis*

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(Received 1 February 1988; revised 12 May 1988; accepted 18 May 1988)

Aminolysis of side-chain esters of poly(γ-benzyl-L-glutamate) (PBG), poly(γ-methyl-L-glutamate) (PMG) and copolymers containing various ratios of L and D enantiomers of γ-benzyl-glutamate was studied. The effects of temperature, solvent and conformation on the kinetics of aminolysis with 2-aminoethanol (AE) and 1-amino-2-propanol (AP) were evaluated. No significant difference in kinetic patterns was found between PBG and PMG or between AE and AP. For all polymers the rate of the reaction increased with increasing temperature. Aminolysis of all polyglutamates, if carried out directly in the amino alcohol, i.e. in the absence of solvent, followed pseudo-first order kinetics. In the presence of a helicogenic solvent (DMF, DMA), the reaction depended on the conformation of the polymer. The polymers in a helical conformation, i.e., homopolymers either of L or D enantiomers, had complex kinetic patterns, with the rate constant being conversion-dependent. The copolymers with an equal content of L and D enantiomers (random-coil copolymers) had a regular pseudo-first order kinetics. Data suggest that the aminolysis of the side-chain ester groups is much faster in random-coil parts of the polymer chain in contrast to the helical segments. The role of the helicogenic solvent rests in the stabilization of the helical conformation.

(Keywords: poly(γ-alkyl glutamate); poly(N^5-hydroxyalkyl glutamine); conformation; helix; random coil; aminolysis)

INTRODUCTION

Poly(N^5-hydroxyalkyl glutamines) are neutral, water-soluble polymers, which are considered as prospective degradable biomedical materials[1,2]. Usually they are prepared by the aminolysis of poly(γ-alkyl glutamates) with 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 resulting polymers in water[3]. Attention has been paid also to the polymerization degree of the resulting poly(N^5-hydroxyalkyl glutamines), which usually decreases during the aminolysis in comparison with the polymerization degree of initial polyglutamates. Optimal conditions for aminolysis, under which the degree of polymerization was preserved up to 70% of the original value, have been suggested[4]. The effect of polymer conformation on the reactivity of ester side chains towards the amine has not yet been studied although polyglutamates are known to exist either in a random coil or in a highly ordered helical conformation, depending on properties of the solvent and on the polymer constitution. Generally, the reactivity of side chains can be modified by polymer conformation because of its effects on the solvation of side groups, steric hindrances, distance of vicinal groups, etc. It has been reported that the HBr debenzylation rate constant for poly(γ-benzyl-L-glutamate) in benzene depends on the degree of debenzylation[5]. Similarly, the apparent rate constant of debenzyloxycarboxylation of poly(*N*-carbobenzoxy-carbonyl-L-lysine) was conversion dependent in a helicogenic solvent, while being conversion independent in a random-coil solvent[6].

In the present study, the effects of solvent and polymer conformation on the kinetics of aminolysis of poly(γ-alkyl glutamates) have been studied. In order to separate these two effects, polymers with various fractions of a helical conformation were prepared by copolymerization of L and D enantiomers and the kinetics of their aminolysis was studied in comparable solvent systems.

EXPERIMENTAL

Chemicals

N,*N*-Dimethylformamide (DMF), *N*,*N*-dimethylacetamide (DMA), AE, AP and dioxane were purchased from Fluka AG and were purified before use by standard procedures. Dichloroacetic acid (DCA), chloroform and hexane (Fluka AG) of commercial quality analytical grade were used without further purification. γ-Aminobutyric acid (GABA) was recrystallized twice from water.

Monomers

γ-Benzyl-L-glutamate, γ-methyl-L-glutamate as well as their corresponding D enantiomers were prepared according to references 7 and 8, respectively. *N*-carboxyanhydrides of the respective γ-alkyl glutamates (NCA) were prepared by the reaction with phosgene according to Hirschmann *et al.*[9]. NCAs were crystallized three times from $CHCl_3$ and an ethyl acetate–hexane mixture. Their

* See reference 1 for part 1 of this series

0032-3861/88/112072-04$03.00
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Table 1 Aminolysis of poly(γ-alkyl glutamates). First order rate constants and molecular weight averages of the resulting poly(N^5-hydroxyalkyl glutamines). Molecular weights ($\bar{M}_w$) for poly(γ-benzyl glutamate) and poly(γ-methyl glutamate) were 210 000 and 200 000, respectively, D/L 0:1

Polymer	Amine/solvent	Temp. (°C)	Rate constant ($\times 10^{-6}$ s^{-1})	$\bar{M}_w$ ($\times 10^3$)	$\bar{M}_n$ ($\times 10^3$)
PMG	AE	60	78		
PMG	AP	60	84	33.0	18.3
PBG	AE	60	107	51.6	23.9
PBG	AP	60	75	41.8	16.7
PBG	AE	37	6	95.0	39.7
PBG	AP	37	5	111.8	48.4
PBG	AE/DMA(1:1)	60		48.0	17.0
PBG	AE/DMF(1:1)	60			
PBG	AP/DMA(1:1)	60		17.2	7.9
PBG	AE/DMA(1:1)	37		52.8	27.0
PBG	AP/DMA(1:1)	37		16.7	19.3
P(BDG$_{0.5}$-*co*-BLG$_{0.5}$)[a]	AE/DMA(1:1)	60	126	–	–
P(BDG$_{0.5}$-*co*-BLG$_{0.5}$)[a]	AE	60	77	–	–
PBDG	AE	60	105	–	–

[a] D/L 1:1

purity was verified by m.p., 94–96°C for NCA of γ-benzyl glutamate (93–94°C) and 97–98°C for NCA of γ-methyl glutamate (96–98°C)[11].

Polymers

Poly(γ-alkyl glutamates) were prepared by the polymerization of the respective NCAs in dioxane at a concentration of monomers 0.1 mol l^{-1}, initiated by triethylamine (monomer: initiator ratio = 200:1). Mole ratios of NCAs of γ-benzyl-L-glutamate and γ-benzyl-D-glutamate in the copolymerization mixture were 10:0, 8:2, 6:4, 5:5, 4:6, 2:8 and 0:10 L and D enantiomers, respectively. All polymerizations were carried out for 6 days at room temperature with the exclusion of moisture. Polymers were precipitated in water, washed with ethanol and dried. The content of the L enantiomer in the copolymer was determined from optical rotary dispersion (o.r.d.) measurement in DCA using the equation[12]:

$$\text{L } \% = 100\,\frac{m' - m'_D}{m'_L - m'_D}$$

Kinetic measurements

Aminolysis of poly(γ-alkyl glutamates) was carried out using 2-aminoethanol (AE) and 1-amino-2-propanol (AP) at 37°C and 60°C. In a typical experiment 40 mg of the polymer was reacted with 5.0 ml of the respective amine (mole ratio of the amine to the alkylester was 500:1). The polymer was either suspended directly in the amine or it was first dissolved in DMA (or DMF) and then 0.5 ml of the amine was added to this solution to achieve the same molar ratio of the amine to the ester. The volume of the solvent was chosen in such a way that its ratios to the volume of amine were 1:1, 3:4, 1:2 and 1:4. After appropriate time intervals the reaction was stopped by neutralization with the ethanol–acetic acid (1:1) mixture and the polymer was dialysed against water. The polymer was recovered from the dialysed sample by freeze-drying.

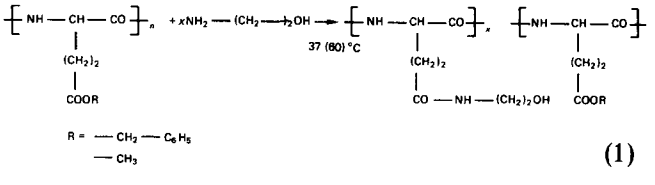
The conversion of aminolysis was determined in two ways: (1) residual benzyl esters were saponified with 2 M NaOH (1 h) and the resulting benzyl alcohol was determined spectrophotometrically upon its extraction into hexane[13]; (2) freeze-dried polymer was completely hydrolysed with concentrated HCl (120°C, 24 h). The hydrolysate was dried over NaOH and dissolved in a 0.05 mol l^{-1} phosphate buffer, pH 2.0. The content of amino alcohol and glutamic acid was determined using high performance liquid chromatography (h.p.l.c.) on a cation-exchange column with GABA as a standard.

Analyses

A Spectra Physics SP 8100 and SP 4200 HPLC system with a Waters RI detector was used for chromatographic measurements. The sulphone-exchange column was prepared by Dr P. Špaček (IMC Prague). The molecular weight of the poly(γ-alkyl glutamates) was determined from the viscometry in DCA using the equation $\eta = 2.78 \times 10^{-5} M^{0.87}$ (ref. 14). The molecular weight distribution of water-soluble poly(N^5-hydroxyalkyl glutamines) was determined by gel permeation chromatography (g.p.c.)[1], and $\bar{M}_w$ and $\bar{M}_n$ were calculated from g.p.c. data. O.r.d. in the range 300–600 nm was measured at 20°C using a Spectropolarimeter ORD UV 5.

RESULTS AND DISCUSSION

The aminolysis of poly(γ-alkyl glutamate) with hydroxyalkylamines follows the scheme in equation (1) for 2-amino alcohol.



(1)

Poly(N^5-hydroxyalkyl glutamine) is the only product. It was checked that no base-catalysed transesterification takes place. The reaction was carried out in a large excess of amino alcohol, so that pseudo first-order kinetics were anticipated. The rate constants were calculated according to the equation $kt = \ln a/x$, where a represents the initial concentration of benzyl ester groups and x holds for their concentration at a time t. To achieve sufficient accuracy throughout the whole conversion range, the conversion of the reaction was followed in two ways.

Data from h.p.l.c. analysis of the amount of amino alcohol already bound to the polymer ensured high accuracy even at very low conversion, while the determination of residual benzyl ester groups made it also possible

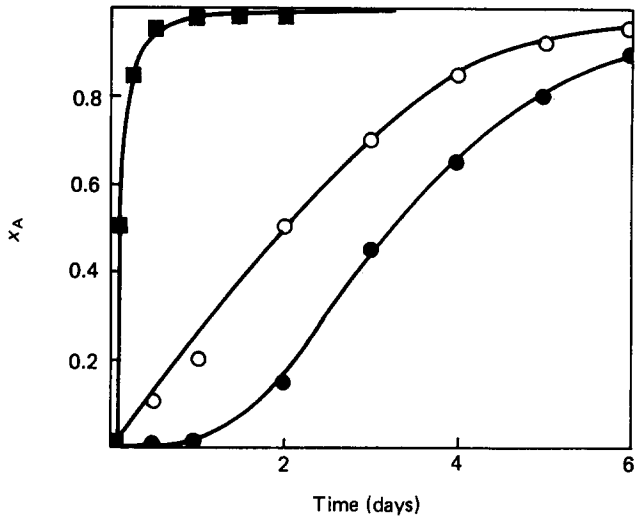


Figure 1 Aminolysis of PBG at 37 and 60°C. Dependence of degree of conversion x_A (mol % hydroxyethyl glutamine units) on time: ●, DMA:AE (1:1), 37°C; ○, DMA:AE (0:1), 37°C; ■, DMA:AE (1:1), 60°C

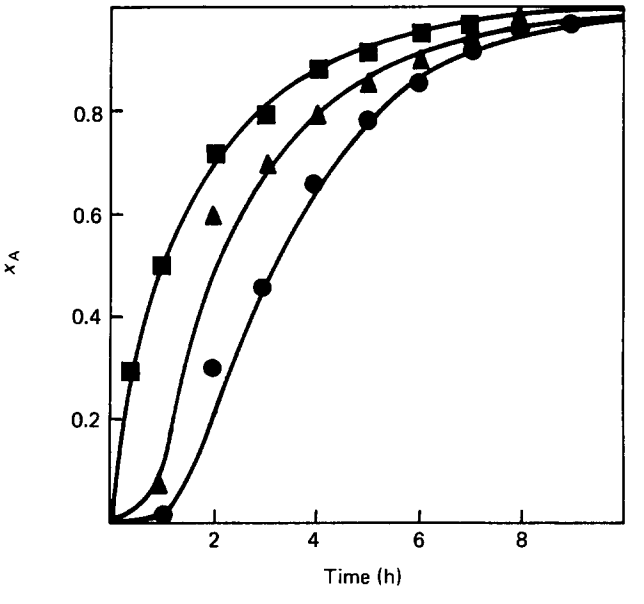


Figure 2 Aminolysis of PBG at 60°C for various ratios of DMA:AE. Dependence of degree of conversion x_A (mol % hydroxyethyl glutamine units) on time: ■, DMA:AE (0:1); ▲, DMA:AE (1:2); ●, DMA:AE (1:1)

to follow the reaction at high conversion and to check the degree of completion of aminolysis. In an overlapping range both determinations were in good agreement. The rate constants together with the molecular weight averages of the resulting poly(N^5-hydroxyalkyl glutamines) are presented in *Table 1*. The reaction was faster with increasing temperature. From the practical point of view, it is worth mentioning that aminolysis is virtually complete after 48 h or 7 days at 60 or 37°C, respectively (*Figure 1*). However, for a higher temperature, i.e., when the reaction is faster, the molecular weight of products is lower. No significant difference was found in the rate of aminolysis between PBG and PMG, or between 2-aminoethanol and 1-amino-2-propanol. On the other hand, a very pronounced effect on the course of the reaction was found in presence of helicogenic solvents (DMF, DMA). Furthermore, it was found that this 'solvent effect' is closely related to the conformation of the polymer.

Aminolysis of both homopolymers, as well as D, L-copolymers, followed pseudo first-order kinetics when it was carried out directly in the amino alcohol. In the presence of a helicogenic solvent (DMF, DMA), the onset of the reaction with amine was delayed and then the reaction rate gradually increased. The magnitude of this delay was higher for a higher solvent-to-amine ratio (*Figure 2*). In copolymers it was also dependent on the ratio of the D and L enantiomers. The effect was largest for homopolymers, either of D or L conformation, while it diminished with increasing content of the minor comonomer (*Figure 3*). The content of helical conformation in copolymers follows an analogous pattern, being highest in homopolymers and diminishes in copolymers with the ratio of D and L enantiomers approaching 1. For instance, the copolymer D/L = 1/1 which forms a meso form conformation in both DMF as well as DMA (*Table 2*) obeys regular pseudo-first order kinetics irrespective of the presence of a solvent (*Figure 4*).

These findings imply an obvious conclusion that the

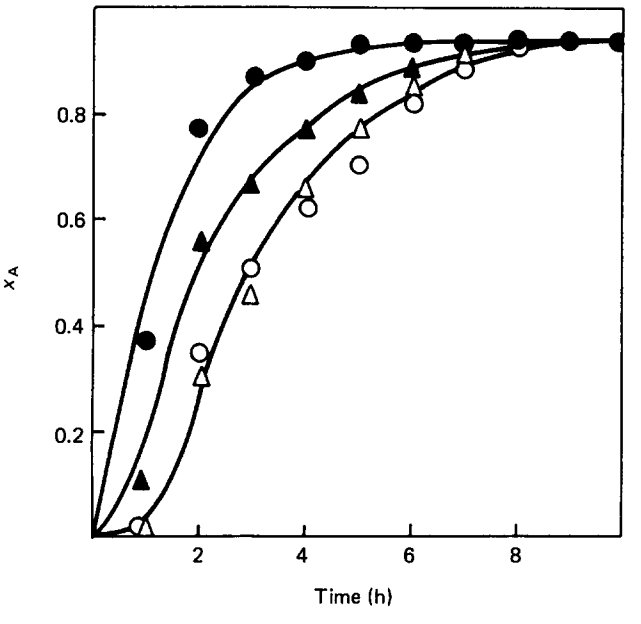


Figure 3 Aminolysis of benzyl glutamate copolymers with various ratios of D and L enantiomers, DMA:AE (1:1), 60°C. Dependence of degree of conversion x_A (mol % hydroxyethyl glutamine units) on time: ●, D:L (1:1); ▲, D:L (8:2); △, D:L (0:1); ○, D:L (1:0)

Table 2 The conformation (% of helix) of polyglutamates and polyglutamines as determined from o.r.d. measurements

Polymer	Solvent	Moffit parameter b_0[a]	% of helix[b]
PBG	DMF	−841.0	100
PBG	DMA	−772.0	100
PBG	DMF/AE[c]	−524.8	83
PHEG	AE	− 89.2	14
PHPG	DMA	−634.0	100
PHEG	DMF	−639.0	100

[a] Moffit parameter b_0 was calculated according to the equation[15]:

$$(\lambda^2-\lambda_0^2)m'=a_0\lambda_0^2+b_0/\lambda_0^4(\lambda^2-\lambda_0^2)$$

[b] % helix was calculated according to the relation[15]:

$$\%\ \text{helix}=(-630/b_0)\times 100$$

[c] Ratio of DMF to AE 10:1

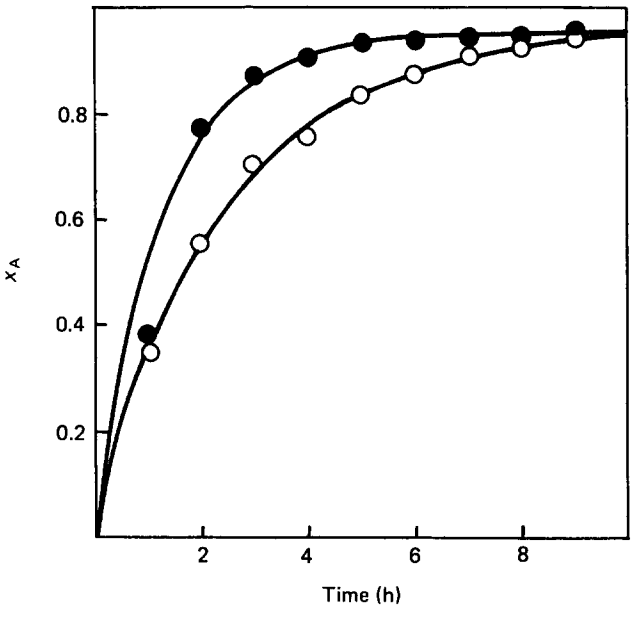


Figure 4 Aminolysis of poly($BLG_{0.5}$-*co*-$BDG_{0.5}$) at 60°C. Dependence of degree of conversion x_A (mol % hydroxyethyl glutamine units) on time: ●, DMA:AE (1:1); ○, DMA:AE (0:1)

aminolysis of side-chain ester groups is much faster in parts of the polymer chain which are not in the helical conformation and that the conformational transition helix-coil should occur before the reaction can proceed. However, this view does not fully explain the role of the solvent. Note that all homopolymers are at the beginning of the reaction in a helical form because they were prepared and isolated in dioxane—a strongly helicogenic solvent. Nevertheless, when aminolysis is carried out directly in amino alcohol, the conformation has no effect on the reaction rate. Note that both homopolymers as well as the D,L(1:1)-copolymer react at a comparable rate (see *Table 1*). On the other hand, when a helicogenic solvent (DMF, DMA) is present, it can be supposed, that it forms a solvation shell surrounding the helical parts of the polymer chain and hindering the access of reagent (amino alcohol) to the side-chain ester carbonyls. Due to this hindrance, the reaction of amino alcohol with helical polymers is extremely slow, in comparison with the same reaction on the random coil copolymers which proceeds with unchanged rate because of the absence of a highly organized solvation shell. Due to the solvation the helix is stabilized, and only following the reaction of some of the side-chain ester groups is the solvation shell perturbed, so the helix can uncoil and the reaction rate gradually increases.

CONCLUSIONS

The results of our kinetic studies show that the aminolysis of side-chain esters of poly(γ-alkyl glutamate) can be greatly affected by the conformation of the polymer chain. In the presence of a helicogenic solvent both effects, i.e., that of helical conformation and of its stabilization by the solvent solvation, are important. Data suggest that the aminolysis of side-chain ester groups is much faster in random-coil parts of the polymer chain, while it is very slow in helical segments. The role of helicogenic solvent (DMF, DMA) consists in a stabilization of the helical conformation.

ACKNOWLEDGEMENT

We thank Dr J. Kubín and Dr P. Špaček for many stimulating discussions during the h.p.l.c. work.

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