

Debenzylation Reaction of Poly(γ -benzyl-L-glutamate) in Helicogenic Solvent

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ABSTRACT: In order to investigate the reactivity of the side chains of a polypeptide in a helicogenic solvent, poly(γ -benzyl-L-glutamate) (PBLG) was debenzylated in benzene with anhydrous hydrogen bromide, and the results were compared with those of the debenzylation reaction of benzyl *n*-butyrate as a model compound. The debenzylation reaction constant of PBLG assuming second-order reaction kinetics was a function of the degree of debenzylation and by extrapolation the initial rate constant was obtained. The debenzylation of benzyl *n*-butyrate obeyed second-order reaction kinetics. The initial rate constant for PBLG was much smaller than for the model compound and the entropy of activation much greater. The rate of debenzylation of PBLG accelerated with increasing debenzylation on account of the steric effect of the nearest-neighboring side chains distributed in the α -helical backbone chain.

KEY WORDS Poly(γ -benzyl-L-glutamate) / Benzyl *n*-Butyrate / α -Helix Conformation / Debenzylation / Rate Constant / Activation Energy /

The reactivity of functional groups attached to a polymer backbone is different from that of small molecules in that the reactivity of a given functional group is influenced by its juxtaposition to neighboring groups. Steric hindrance, adsorption of some specific component, hydrophilic and hydrophobic interactions, etc., are examples of steric effects ascribable to neighboring groups.¹ The rate of reaction may be either enhanced or reduced depending on the type of steric effect.

The distance between functional side chains of a polymer is an important factor affecting their reactivity, and varies with the configuration and conformation of the polymer chain. Sakurada, Sakaguchi, and coworkers have investigated saponification of poly(vinyl acetate)² (PVAc) and poly(methyl acrylate)^{3,4} (PMA) in a mixture of acetone—water using NaOH as a catalyst, in particular, reference to the stereoregularity of the polymers. In the case of PVAc, isotactic PVAc showed a lower initial rate and a larger autocatalytic effect than syndiotactic

PVAc, while atactic PVAc behaved in nearly the same way as syndiotactic PVAc. On the other hand, syndiotactic PMA showed a much higher initial rate of saponification (about 20 times) than the PMA rich in isotactic configuration. Such a marked difference in saponification behaviors of both types of PMA was attributed to steric effects such as the adsorption of alkali on the saponified side chain and steric hindrance due to side chains adjacent to the saponified one.

In the saponification of poly(methyl methacrylate) (PMMA) where the internal rotations are rather more restricted than PMA, Glavis⁵ reported that saponification of isotactic PMMA showed a larger autocatalytic effect than syndiotactic PMMA. Semen, *et al.*,⁶ investigated the chemical composition of partially saponified PMMA by means of infrared spectra, and found that the carboxylic acid residues in the isotactic polymer has a strong tendency to form block sequences, whereas those in the syndiotactic polymer showed a random distribution, as demonstrated by Klesper.⁷ These facts were

explained in terms of steric effects due to the stereoregularity.

As mentioned above, the reactivity of linear homopolymers which exist in random coil conformation in the reaction medium is influenced by the stereoregularity of the chain. However, for those polymers that assume ordered chain conformations in the reaction medium, *e.g.*, synthetic polypeptides and proteins, the reactivity of the molecules may vary markedly according to their chain conformations. In our previous work, Hayashi, *et al.*,⁸ examined the effect of polypeptide chain conformation on the rate constant of decarbobenzyloxylation of poly(ϵ -*N*-carbobenzyloxy-L-lysine) (PCBL). The apparent second-order rate constant of the reaction of PCBL in the helicogenic solvent system gradually decreased as the degree of conversion increased, whereas, in the coil solvent system the second-order rate constant was independent of the degree of conversion.

In the present study, we investigated the debenzoylation reaction of poly(γ -benzyl-L-glutamate) (PBLG) with anhydrous hydrogen bromide (HBr), which is thought to be the most satisfactory reagent for the debenzoylation of poly(α -amino acid) esters,⁹ in order to evaluate quantitatively the reactivity of the polypeptide ester groups in a helicogenic solvent, benzene, and to elucidate the effect of α -helical conformation on the reaction. For comparison the debenzoylation of benzyl *n*-butyrate, a model compound for PBLG, was also investigated.

EXPERIMENTAL

Materials

Poly(γ -benzyl-L-glutamate) samples were synthesized by the NCA method. The polymerization was carried out at room temperature in a 1:1(v/v) mixture of dioxane and methylene dichloride in the presence of triethylamine as an initiator. The numerical value of the molar ratio $[M]/[I]$ of the NCA monomer (M) to the initiator (I) was 250 in a 3.0-% solution of the NCA.

In Table I, the limiting viscosity number $[\eta]$ of PBLG in dichloroacetic acid (DCA) at 25°C is given together with the b_0 value of the Moffitt equation. The limiting viscosity number was measured with a Ubbelohde-type viscometer.

Table I. Characterization of PBLG

Sample	$[\eta]$, dl/g	b_0
PBLG-I	3.75	-630
PBLG-II	3.26	-620
PBLG-III	4.17	-610

The optical rotatory dispersion (ORD) spectra were measured, over the wavelength range 310–510 nm, with a Yanagimoto OR-100-type spectropolarimeter using a tungsten lamp as the light source. The concentration of sample in benzene was 0.4 g/dl throughout the ORD measurements. The temperatures of ORD measurements were 30, 35, and 40°C, respectively, for PBLG-I, -II, and -III.

The reagent for the debenzoylation was prepared as follows; anhydrous hydrogen bromide gas was produced by dropping the desired volume of bromine in tetralin, and was then absorbed in benzene.

Debenzoylation of PBLG and Benzyl *n*-Butyrate

Debenzoylation of PBLG and of benzyl *n*-butyrate was carried out in benzene with anhydrous HBr under the experimental conditions listed in Table II. The reaction mixtures were stirred during the reaction, and the temperature was maintained within $\pm 0.3^\circ\text{C}$ by means of a thermostat.

In the case of debenzoylation of PBLG, the reaction mixtures were homogeneous provided the degree of debenzoylation was less than *ca.* 0.25. Therefore, the debenzoylation reaction was only studied up to this point. The reaction was stopped at appropriate time intervals and the reaction mixture was poured into a mixture of acetone and methanol. The precipitated partially saponified poly(γ -benzyl-L-glutamate) (PABLG) was washed thoroughly with ether and acetone, and dried for two days. Elementary analyses of carbon and hydrogen in PABLG were adopted for quantitative determination of the degree of debenzoylation.

On the other hand, in the case of the debenzoylation of benzyl *n*-butyrate, the reaction mixture remained homogeneous throughout the reaction. The reaction mixtures at appropriate time intervals were poured into water, and *n*-butyric acid and the remaining HBr were

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Table II. Experimental conditions for debenzilation

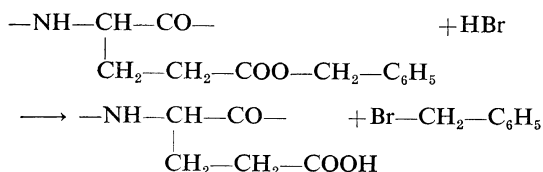
Code No.	Temp, °C	Sample	Solvent	<i>a</i> , mol/l	<i>b</i> , mol/l	<i>b/a</i>
1	30	PBLG-I	Benzene	0.0207	0.0303	1.47
1'	"	"	"	0.0103	0.0564	5.48
1''	"	"	"	0.0163	0.1176	7.22
1'''	"	"	"	0.0209	0.0161	0.77
2	35	PBLG-II	"	0.0191	0.0237	1.24
2'	"	"	"	0.0148	0.0218	1.48
3	40	PBLG-III	"	0.0167	0.0301	1.80
4	30	PBLG-II	7 : 1 Bez—DCA	0.0200	0.0307	1.53
5	30	Benzyl <i>n</i> -butyrate	Benzene	0.0229	0.0362	1.58
6	35	"	"	0.0232	0.0357	1.54
7	40	"	"	0.0229	0.0363	1.59

thoroughly transferred from benzene to water phase by shaking the vessel slowly. The aqueous solution thus obtained was titrated conductometrically with 0.1-*N* NH₄OH. The degree of debenzilation was quantitatively determined from the concentrations of HBr and of *n*-butyric acid independently. These two results satisfactorily agreed with each other.

The initial concentration of HBr was also measured conductometrically in the same manner.

RESULTS AND DISCUSSION

The debenzilation of a residue of PBLG with HBr is expressed by bimolecular reaction.



Thus, it may be adequate to assume second-order reaction kinetics for the debenzilation of PBLG

$$dx/dt = k_{app}(a-x)(b-x) \quad (1)$$

where *x* is the molar concentration of the reacted polypeptide after time *t*, and *a* is the initial concentration of the polypeptide, both *x* and *a* being denoted in terms of the base-moles of the ester group, *b* is the initial molar concentration of HBr, and *k*_{app} is the apparent rate constant. If *k*_{app} is assumed to be constant, integration of eq 1 leads to eq 2.

$$k_{app}t = \frac{1}{b-a} \ln \frac{a(b-x)}{b(a-x)} \quad (2)$$

Thus, the rate constant at time *t* in the initial reaction stage is calculated with little error if the right side of eq 2 is divided by reaction time *t*. However, if *k*_{app} is not constant, even at the initial reaction stage, then it is necessary to obtain the differential value *dx/dt* of eq 1 from the experimental curve of conversion against *t*.

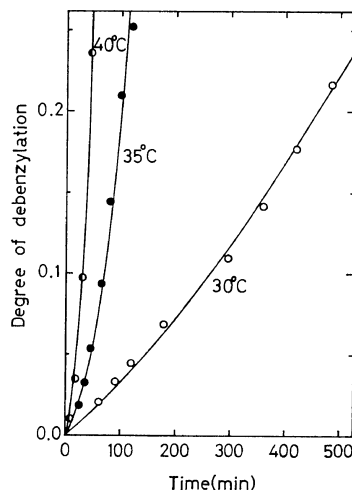


Figure 1. Degrees of debenzilation of PBLG plotted against reaction time at 30°C (○, No. 1), 35°C (●, No. 2), and 40°C (●, No. 3).

In Figure 1, the degrees of debenzilation of PBLG at 30, 35, and 40°C are plotted against the reaction time *t*. The second-order plots based on eq 2 are given in Figure 2. As is

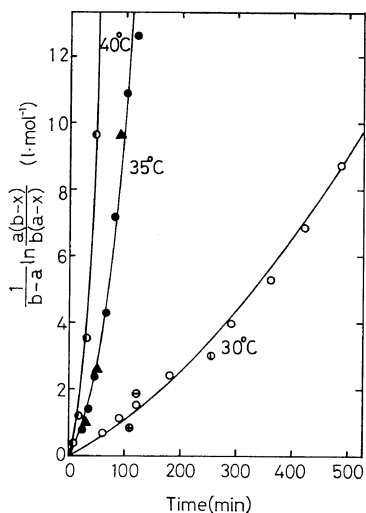


Figure 2. Second-order plots for debenzilation of PBLG at 30°C (○, No. 1; ⊕, No. 1'; ⊖, No. 1''; ⊙, No. 1'''), 35°C (●, No. 2; ▲, No. 2'), and 40°C (◐, No. 3).

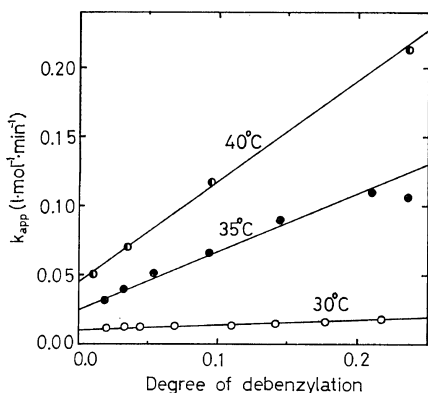


Figure 3. Plots of apparent rate constant vs. degree of debenzilation of PBLG at 30°C (○, No. 1), 35°C (●, No. 2), and 40°C (◐, No. 3).

obvious from Figure 2, the plot of No. 2' falls precisely on the experimental curve of No. 2, and further the experimental points of No. 1', 1'', and 1''' which differ greatly from No. 1 in numerical values of b/a , are situated on the curve of No. 1 within experimental error. Such results should prove that the rate equation for debenzilation of PBLG is sufficiently analyzed by the second-order reaction scheme.

The apparent rate constant k_{app} is plotted against the degree of debenzilation in Figure 3.

It is apparent that, k_{app} is not independent of the degree of debenzilation. In discussing the reactivity of polymer molecules, it is important to consider the initial rate constant k_0 estimated by extrapolating the apparent rate constant k_{app} to zero degree of debenzilation. Figure 3 indicates that k_{app} increases almost linearly with the increase of conversion up to a molar fraction of *ca.* 0.2, and then deviates slightly downwards at higher conversion. Such a linear relation between k_{app} and the degree of debenzilation x/a is the same as that for deacetylation of poly(vinyl acetate) proposed by Sakurada and Osugi.¹⁰ Thus k_{app} is denoted by the equation

$$k_{app} = k_0 \left(1 + m \frac{x}{a} \right) \quad (3)$$

where the empirical parameter m is a kinetic constant and is considered to be a measure of autoaccelerated reaction.¹⁰

In Figure 4, the degree of debenzilation of benzyl *n*-butyrate, the model compound, is plotted against the reaction time t . Figure 5 shows the second-order plots based on eq 2. It is apparent that eq 2 holds perfectly for the model compound. In other words, k_{app} in eq 2 is constant and is independent of the degree of debenzilation. Thus we designate the rate constant of the model compound by k .

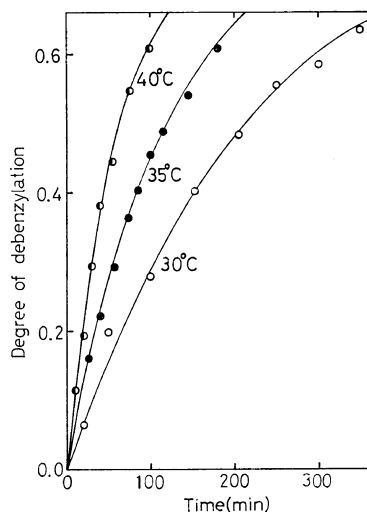


Figure 4. Degrees of debenzilation of benzyl *n*-butyrate plotted against reaction time at 30°C (○, No. 5), 35°C (●, No. 6), and 40°C (◐, No. 7).

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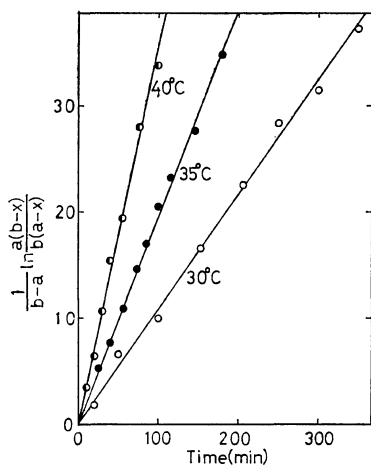


Figure 5. Second-order plots for debenzilation of benzyl *n*-butyrate at 30°C, 35°C, and 40°C. Symbols are the same as those in Figure 4.

Table III. Initial rate constant k_0 and kinetic parameter m for debenzilation of PBLG, and rate constant k for debenzilation of benzyl *n*-butyrate

Temp, °C	Benzyl <i>n</i> -butyrate k , l/mol min	PBLG	
		k_0 , l/mol min	m
30	0.108	0.0118	5.3
35	0.197	0.0252	16.1
40	0.357	0.0453	15.8

In Table III, the initial rate constant k_0 and the kinetic parameter m for PBLG at various temperatures are given together with the rate constant k for benzyl *n*-butyrate.

The products from the debenzilation reaction were characterized by measuring the limiting viscosity number $[\eta]$ and the Moffitt parameter b_0 . The results for PBLG-I system are given in Table IV, in which $[\eta]$ and b_0 are measured at 30°C in DCA and in benzene, respectively. These data suggest that 1) little scission of the backbone chain takes place; the small decrease in $[\eta]$ observed with increasing degree of debenzilation may be attributed to a decrease in the interaction between the polymer and DCA as the degree of benzylation of the polymer decreases. 2) The reaction products, PABLG copolymers, keep 100-% right-handed α -helix

Table IV. Characterization of partially debenzilated products of PBLG-I

Degree of debenzilation	$[\eta]$, dl/g	b_0
0	3.41	-630
0.069	3.25	-560
0.110	3.21	-630
0.177	3.15	—
0.217	3.12	—

conformation during the reaction since b_0 , the helix parameter, is almost constant. Such results are in accord with our previous finding¹¹ that the helix stability of PABLG copolymer increases as the degree of debenzilation increases.

The side chains of polypeptides in the α -helix conformation are oriented regularly around the backbone chain, such that the distance between the nearest-neighboring side chains is constant along the backbone chain. Thus the side chain of i -th residue is enclosed by four side chains of the $(i-3)$ th, $(i-1)$ th, $(i+1)$ th, and $(i+3)$ th residues. Taking such circumstance into consideration, self acceleration in debenzilation reaction may be explained as follows. The side chains of PBLG are solvated with benzene molecules as a result of the hydrophobic interaction between benzyl group of the side chain and benzene.¹² Once an ester side chain is converted into carboxylic acid side chain, benzene molecules will move to ester groups because of the hydrophobic effect. In other words, the concentration of HBr will increase around the acidic residues making it easier for ester groups in the neighborhood of the carboxylic acid side chain to be attacked by HBr. Thus the debenzilation of PBLG is autoaccelerated as the content of carboxylic acid residues increases.

In this manner, the helical conformation of PBLG accelerates the debenzilation reaction. However, the rate of increase of the apparent rate constant is reduced at higher degrees of debenzilation as is seen from curves No. 2 and No. 3 in Figure 3. To study this effect further PBLG-II was debenzilated in a 7:1(v/v) benzene-DCA mixture, in which the polymer had a value of $b_0 = -520$ at zero conversion. The experimental results are shown in Figures 6 and 7, and are compared with the results of

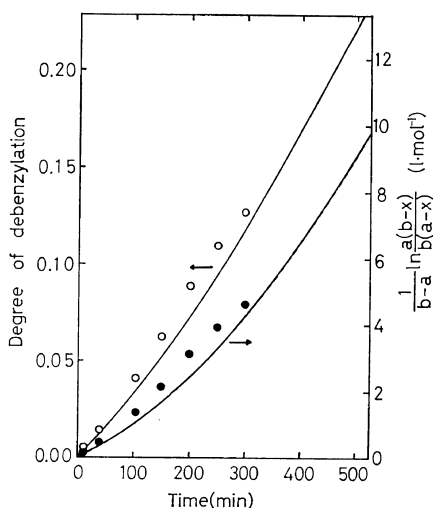


Figure 6. Debzoylation of PBLG-II in a 7:1 (v/v) mixture of benzene and DCA at 30°C. Solid lines are the results of debzoylation in benzene at 30°C as given in Figures 1 and 2.

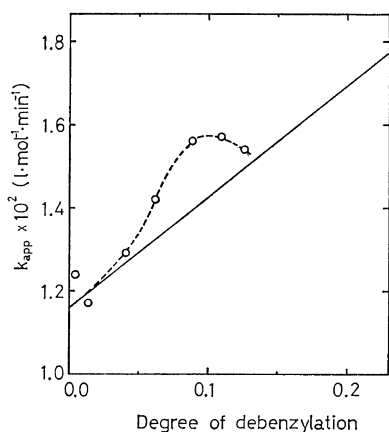


Figure 7. Apparent rate constant for debzoylation of PBLG-II in the 7:1 mixture of benzene and DCA plotted against degree of debenzoylation. Solid line denotes the result of debzoylation in benzene.

debzoylation in benzene (see solid curves in Figures 6 and 7). In the benzene—DCA mixture, PBLG exists 80% in helix and 20% in random coil conformations. The random coil portion seems to be responsible for accelerating the reaction during the initial stage of debzoylation, while the helix portion as the major component gradually suppresses the rate of in-

crease of the apparent rate constant as the reaction proceeds. As can be seen in Figure 7, the plot of k_{app} against x/a for the benzene—DCA mixture system is convex upwards. The apparent rate constant of PBLG cannot exceed the debzoylation rate constant for benzyl *n*-butyrate, because of the suppressing effect. Comparison of k_0 with k in Table III gives some insight into the role of α -helix conformation in the reaction of ester residues. The reason why benzyl *n*-butyrate was used as a model compound for γ -benzyl-L-glutamic acid (BLG) is that in the case of the monomer unit the reactivity of the carbonyl group separated by two methylene groups from the α -carbon of the backbone may be slightly dependent upon the bulkiness of amino and carbonyl groups bonded to α -carbon. Thus in the hydrolysis of poly(allyl acetate), in which one oxymethylene group separates a carbonyl group from the backbone chain, the backbone chain had scarcely any influence during the initial reaction stage.¹³

The Arrhenius plots of the initial rate constant k_0 for PBLG and of the rate constant k for benzyl *n*-butyrate are shown in Figure 8, from which the activation energy E_a and the frequency factor A were calculated using the least-square method by means of

$$k_0(\text{or } k) = A \exp(-E_a/RT) \quad (3)$$

On the basis of E_a and A values, such thermodynamic quantities as the enthalpy of activation, ΔH^\ddagger , the entropy of activation, ΔS^\ddagger , and the

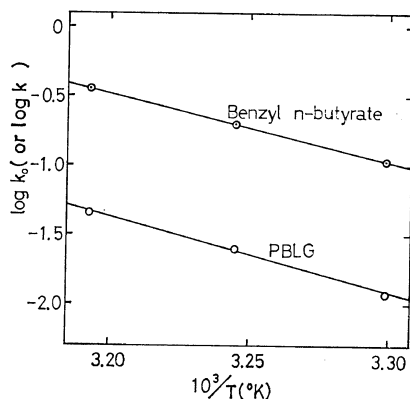


Figure 8. Arrhenius plots for k_0 of PBLG and for k of benzyl *n*-butylate.

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Table V. Activation energy E_a , frequency factor A , and thermodynamic quantities, ΔH^\ddagger , ΔS^\ddagger , and ΔG^\ddagger , at 25°C^a

Sample	E_a , kcal/mol	A , l/mol min	ΔH^\ddagger , kcal/mol	ΔS^\ddagger , eu	ΔG^\ddagger , kcal/mol
PBLG	25.1	1.99×10^{16}	24.5	-26.1	32.3
Benzyl <i>n</i> -butyrate	22.5	1.71×10^{15}	21.9	-31.0	31.1
Syndiotactic PMA ^a	12.0	8.42×10^8	11.4	-59.8	28.0
Methyl isobutyrate ^b	11.0	5.24×10^8	10.4	-60.8	27.3

^a and ^b Calculated from the data in ref 3 and 15, respectively.

free energy of activation, ΔG^\ddagger , were evaluated from the following equations¹⁴

$$\Delta H^\ddagger = E_a - RT \quad (4)$$

$$A/60 = e(kT/h) \exp(\Delta S^\ddagger/R) \quad (5)$$

$$\Delta G^\ddagger = \Delta H^\ddagger - T\Delta S^\ddagger \quad (6)$$

where k is the Boltzmann constant and h the Plank constant. The standard state refers to a unit concentration of one mole per liter.

The numerical values of the activation energy, the frequency factor, and three thermodynamic quantities of activation are summarized in Table V. For comparison the results for hydrolysis of poly(methyl acrylate)³ and of methyl isobutyrate¹⁵ have been included. Judging from the ΔG^\ddagger values of 25°C in Table V, the relative reactivities of PBLG and benzyl *n*-butyrate are almost the same, while actual activation is energetically a little more unfavorable with PBLG than with the model compound. The values of ΔH^\ddagger and ΔS^\ddagger of PBLG are different from those of benzyl *n*-butyrate. With respect to poly(methyl acrylate), however, the thermodynamic quantities for the activated complex of syndiotactic PMA are very similar to those of methyl isobutyrate as model compound. Such results on PMA may be attributed to the fact that in syndiotactic PMA which exists in random coil conformation, there is little steric hindrance between the nearest side chains because of the large distance between them. It may be reasonable to conclude that polymer in random coil conformation has nearly the same thermodynamic values of activation as low molecular weight compound does.

In the debenzylolation reaction of PBLG in α -helix conformation there are remarkable differences: that is, (1) the ΔH^\ddagger value of PBLG is considerably larger than that of benzyl *n*-

butyrate, and (2) the ΔS^\ddagger value of PBLG is also appreciably larger than that of benzyl *n*-butyrate. First, the difference in the ΔH^\ddagger values between PBLG and benzyl *n*-butyrate is probably attributed to differences in solvation with benzene. Second, the large difference in ΔS^\ddagger and the frequency factor can be attributed to the role of the helical conformation of the polypeptide. The ester side chains of PBLG are fixed rigidly onto the backbone chain, and their degrees of translational or rotational freedom are severely restricted,¹⁶ whereas the degrees of translational freedom of benzyl *n*-butyrate are not restricted except by solvation. Hence, the ΔS^\ddagger value of activated complex of benzyl *n*-butyrate is lower than that of PBLG. Of course, strictly speaking the comparison should be with PBLG 100% in coiled conformation, but taking into consideration the fact that the ΔS^\ddagger value of randomly coiled flexible polymer is almost equal to that of low molecular compound, the difference of *ca.* 5 eu may be ascribed to α -helix conformation of polypeptide. Thus we conclude that the reactivity of side chains of PBLG is inevitably influenced during the reaction by the secondary structure, *i.e.*, α -helix conformation, of the polypeptide.

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