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Preparation of (vinylbenzylimino)oligo(DL-phenylalanine NCA) macromer with narrow molecular weight distribution

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SUMMARY

Polymerization conditions of DL-phenylalanine *N*-carboxyanhydride (NCA) by *m,p*-vinylbenzylamine were examined for the preparation of (vinylbenzylimino)oligo(DL-phenylanaline NCA) macromer (VB-OPhe) with narrow molecular weight distribution. According to the results obtained, i.e., under the condition of short polymerization time (ca. 2 h) and in relatively high concentrations of the NCA in THF, the polymerizations of the NCA were carried out to give VB-OPhe macromers, which were found to have very narrow molecular weight distributions. Furthermore, their functionalities were proved to be approximately unity by the polymerization method.

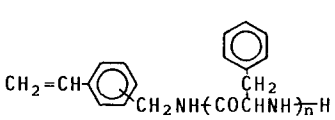
INTRODUCTION

Synthetic oligopeptides and polypeptides, which are structurally similar to proteins, have been received much interest as physiologically active substances, biomedical materials, and highly functional materials such as functional membranes and fibers for improvement of dyeability. For the synthesis of peptide chains, the polymerization of α-amino acid NCA is most extensively used as reviewed by Imanishi (1).

Usually, polypeptides as well as oligopeptides by the NCA method, however, does not have an enough mechanical strenght to use as materials. In order to overcome this disadvantage and/or to make a microphase separated structure in the solid state, a variety of attempts have been made on the synthesis of block and graft copolymers bearing peptide chains and polyvinyl segments (2). These attempts involved the nucleophilic-addition polymerizations of α-amino acid NCAs by polymeric initiators. We also have prepared graft copolymers having peptide chains as branches by the polymerization of α-amino acid NCAs by using vinylbenzylamine copolymers as initiators (3).

As an alternative method for the preparation of graft copolymer of α-amino acid NCA, there has been a so-called macromer method, in which Maeda and Inoue (4) successfully performed the synthesis and copolymerization of poly (γ-benzul-L-glutamate NCA) macromer.

In the present work, we carried out the polymerization of DL-phenylalanine NCA by *m,p*-vinylbenzylamine to obtain (vinylbenzylimino)oligo(DL-phenylalanine) macromer (VB-OPhe),



VB-OPhe

as an extension work of the grafting polymerization of NCAs by the vinylbenzylamine copolymers (3). Also, in the preparation of the macromer, much attention has been paid on narrow molecular weight distribution (MWD), which would be very important, e.g., for the accurate elucidation of relationship between physical properties and structure of polymer having peptide chains as pendants.

EXPERIMENTAL

Materials

Trichloromethyl chloroformate (TCF) was used as received from Hodogaya Chemical Co., LTD, Tokyo. *m,p*-Vinylbenzylamine (VBA: *m*-, 60%; *p*-, 40%) was synthesized by N-vinylbenzylation of phthalimide followed by treatment of the *N*-vinylbenzylphthalimide with hydrazine (Gabriel Synthesis (5)). It was stirred with CaH_2 for 24 h and distilled on a vacuum line just before its use. DL-Phenylalanine NCA was prepared by a TCF method (6-8). The light brown NCA obtained was twice recrystallized from ethyl acetate-hexane to give its colorless crystals, and the final recrystalization was carried out prior to use. Tetrahydrofuran (THF) was purified by the usual method employed in anionic polymerization. Acetonitrile (AN) and *N,N*-dimethylformamide (DMF) were dried over CaH_2 and 4A molecular sieves, respectively according to the literature (9) and then rectified.

Polymerization procedures

Polymerization of the NCA was carried out at 30 or 40 ± 0.1°C under dry nitrogen in a Erlenmeyer flask equipped with a three-way stopcock. After the prescribed polymerization time, the reaction mixture was poured into a large excess of diethyl ether. The precipitate was filtered, washed with dietlyl ether, and dried in vacuo at room tempereture for 2 days.

Measurements

Gel permeation chromatography (GPC) was performed at a column-oven temperature of 38°C on a Toyo Soda HLC-802UR. THF was used as the eluent, and the flow rate was 1.0 mL/min. Two systems of $G2000H_8$-$G3000H_8$ columns (61+61cm, Toyo Soda) and two GMH_6 columns (61cmx2, Toyo Soda) were used for the characterization of VB-OPhe macromer and polymerized macromer, respectively. The column systems were calibrated with polystyrene standards, and thus the molecular weights and MWD (M_w/M_n) values determined with GPC are apparent values. UV spectrum was recorded on a Hitachi Model 200-20 spectrophotometer using THF as solvent. IR measurement was carried out for ca. 1% dispersion in a KBr disk with an infrared spectrophotometer (Jasco A-102, Japan).

RESULTS AND DISCUSSION

Preparation of VB-OPhe

The polymerization of DL-phenylalanine NCA by VBA was carried out under various conditions in order to find out polymerization conditions which provid a narrow MWD. Figure 1 shows the effects of the concentration of NCA and the molar ratio of the NCA to VBA on the MWD of the macromer. The comparison between Curves a and b in Figure 1 suggests that a high concentration of the NCA is favorable for narrow MWD. Also, Curves b and c indicates that polymer having higher molecular weight than expected one forms to a considerable extent at a relatively high [NCA]/[VBA] ratio

(e.g., [NCA]/[VBA] = 19). Figure 2 demonstrates the influence of polymerization time and the effect of solvent on the MWD of VB-OPhe. As a whole, GPC curves of VB-OPhe macromers became broad as the polymerization time was prolonged. This tendency was significant in the case of the polymerization in acetonitrile, whereas Oya et al. (10) reported that some NCAs were successfully polymerized by *n*-butylamine in acetonitrile rather than in THF. Considering the facts that the broadening of GPC curves appears in the high molecular weight side of the curve and also is remarkable in the late stage of the polymerization, we speculate that the broadening may result from a certain chain extension reaction.

Anyway, it is concluded that the VB-OPhe macromer with narrow MWD can be prepared by the polymerization of the NCA under the following conditions: high concentration of the NCA; solvent, THF; polymerization time, less than 3 h.

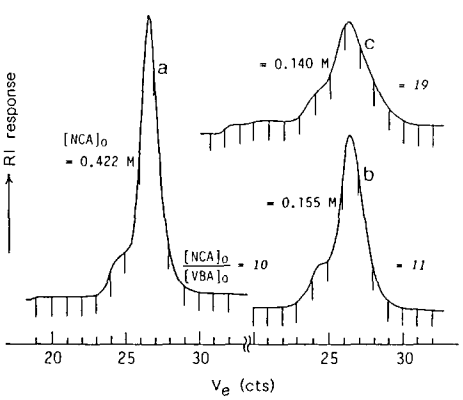


Figure 1. Influence of the NCA concentration and [NCA]/[VBA] ratio on the MWD of VB-OPhe. Polymerization time, 24 h; solvent, THF.

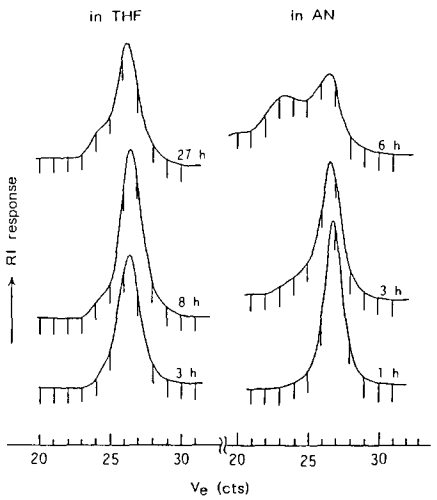


Figure 2. Effects of solvent and reaction time on the MWD of VB-OPhe. Polymn in THF; $[NCA]_0$ = 0.297 M; [NCA]/[VBA] = 3.25. Polymn in AN; $[NCA]_0$ = 0.300 M; [NCA]/[VBA] = 3.08.

Table I

Preparation of Monodisperse VB-OPhe Macromer[a)]

Expt	[NCA] (mol/L)	[VBA] x 10^2 (mol/L)	[NCA]/[VBA]	Time (h)	VB-OPhe macromer yield (%)	M_n x 10^{-3} calcd	M_n x 10^{-3} GPC	M_n x 10^{-3} UV	M_w/M_n
M-1	0.648	12.4	5.23	2.0	90	0.83	0.92	0.84	1.0_9
M-2	0.685	6.94	9.87	2.5	92	1.47	1.3	1.5	1.1_2
M-3	0.683	6.38	10.7	2.1	95	1.63	1.8	1.9	1.1_4

[a)] Polymerization temperature, 30°C; solvent, THF.

On the basis of these results, the polymerization of the NCA with VBA was carried out and the results were summarized in Table 1. The yield of VB-OPhe was above 90% even in short polymerization time of 2.0-2.5 h. The molecular weight of VB-OPhe determined by GPC and UV methods was in good agreement with calculated one from the the [NCA]/[VBA] ratio and the conversion of the NCA. Although the MWD of the macromers were obtained by the calculation with the calibration curve which was constructed from polystyrene standards, the values of MWD is considered to be virtually reliable since the molecular weight of the macromer determined by GPC is consistent with the values by the other methods. Figure 3 apparently supports the narrow MWD; namely, the GPC curve of VB-OPhe were sharp and symmetrical. This fact suggests that the polymerization of the NCA proceeds exclusively *via* nucleophilic-addition reaction to C^5 of the NCA without side reaction at least within polymerization time of 2 h.

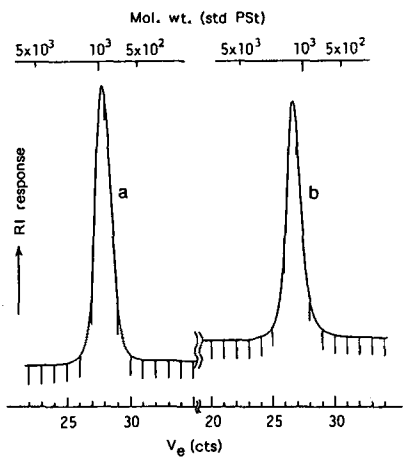


Figure 3. GPC curves of VB-OPhe obtained in Expts M-1 (Curve a) and M-2 (Curve b).

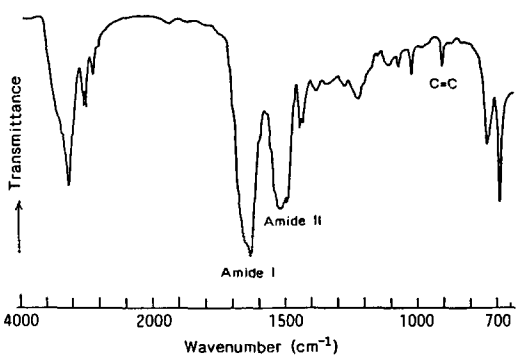


Figure 4. IR spectrum of VB-OPhe obtained in Expt M-2.

Table 2

Radical Polymerization of VB-OPhe Macromer[a)]

Expt	VB-OPhe	(mg)	Comonomer	(mg)	AIBN (mg)	Solvent	(mg)	Time (h)	Conv. of VB-OPhe[c)] (%)
HP-1	M-1[b)]	106	—	—	2.60	Bz	238	18	84
HP-2	M-1	99	—	—	2.59	THF	210	18	92
HP-3	M-2	105	—	—	2.46	THF	392	18	65
HP-4	M-2	99	—	—	2.30	THF	498	15	60
CP-1	M-1	52.3	MMA	207	2.90	THF	335	18	98
CP-2	M-1	51.5	St	205	2.58	THF	297	22	90
CP-3	M-2	85.5	St	280	4.70	THF	311	22	79

[a)]Polymerization temperature, 60°C. [b)]The same as in Table 1.
[c)]Determined by GPC.

Characterization and Radical Polymerization of the Macromer

IR spectrum of VB-OPhe (Expt M-1) had the characteristic bands of both vinyl groups (910 cm^{-1}) and peptide chains (Amide I band, around 1650 cm^{-1}; Amide II band, around 1530 cm^{-1}) as shown in Figure 4, exhibiting the formation of the macromer.

In order to examine the purity (functionality) and polymerizability of VB-OPhe, the radical homo- and copolymerizations of the macromer were performed. Polymerization conditions and results are summarized in Table 2. The extent of conversion of VB-OPhe was very high in the homopolymerization in the case of a high concentration of VB-OPhe. Also, the copolymerization of VB-OPhe with methyl methacrylate proceeded quantitatively, while in the copolymerization with styrene the extent of conversion of VB-OPhe was somewhat low, suggesting the dependence on the polymerizability of the comonomers. These are illustrated by GPC curves in Figure 5. In conclusion, the functionality of VB-OPhe is approximately unity and the homopolymerizability as well as the copolymerizability of VB-OPhe is very high.

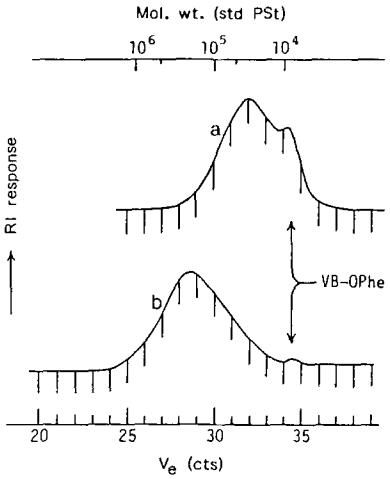


Figure 5. GPC curves of poly(VB-OPhe) (Curve a) and poly(VB-OPhe-*co*-MMA) (Curve b) obtained in Expts HP-2 and CP-1, respectively.

Acknowledgment

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