The Estimation of the β-Sheet-Structure Stability of Protected Peptides in Organic Solvents[1)]

Mitsuaki NARITA,* Jin-Shik LEE, Shizue HAYASHI, and Mitsuo HITOMI
Department of Biotechnology, Faculty of Technology, Tokyo University of Agriculture and Technology, Nakamachi, Koganei 184

(Received June 1, 1992)

The β-sheet-structure stability in organic solvents was examined for protected tri- to heptapeptide fragments of *E. coli* ribosomal protein L7/L12. This was evaluated by monitoring the peptide IR absorption band around 1630 cm^{-1}, assigned to a β-sheet structure, in CH_2Cl_2 alone or CH_2Cl_2 containing various concentrations of DMSO. For each peptide with a chain length up to a heptapeptide, the $<SP_\beta>$ values, defined in a previous paper, of a protected peptide were in harmony with their β-sheet-structure stability almost regardless of their amino acid sequences. This fact indicates that the $<SP_\beta>$ value is useful for the estimation of the β-sheet-structure stability of protected peptides in organic solvents. It also supports the assumption that the β-sheet-structure stability of protected peptides is strongly dependent on their amino acid compositions and weakly dependent on their amino acid sequences. Furthermore, it was shown that the $<SP_\beta>$ value of a protected peptide can provide a basis for the choice of reaction solvent. The significance of the present study in the design of synthetic routes for peptides and proteins is briefly discussed.

In solid- and liquid-phase peptide syntheses, the intermolecular hydrogen-bonded β-sheet aggregation of protected peptides in organic solvents causes difficulty in deprotection and subsequent coupling reactions. Thus, for the design of synthetic routes for peptides and proteins, it is important to estimate the stability of the β-sheet structure of protected peptides in organic solvents. In previous papers,[2—4)] we demonstrated that the stability of the β-sheet structure in organic solvents is principally influenced by two factors, namely, the nature of the organic solvents and that of the protected peptides. As for the former, the electron-accepting and -donating abilities of organic solvents dominate the stability of the β-sheet structure in organic solvents because the electron donor-acceptor interaction between the solvent and the peptide bond is a determinant for β-sheet structure disruption.[5—8)] With respect to the latter, the stability of the β-sheet structure of protected peptides is dependent on the β-sheet-structure-stabilizing potentials of the amino acid residues composing protected peptides as well as their peptide chain lengths.[14—18)] For the design of synthetic routes for peptides and proteins, the estimation of the β-sheet-structure stability of protected peptides is so important that we proposed an estimation method for the β-sheet-structure stability of protected peptides using their $<SP_\beta>$ values, which are defined as the arithmetic average of the β-sheet-structure-stabilizing potentials, SP_β, of the amino acid residues composing the protected peptides.[9,10)]

Using 77 kinds of protected tri- to heptapeptide fragments of *E. coli* ribosomal protein L7/L12,[11—13)] we show here that their $<SP_\beta>$ values are useful for the estimation of their β-sheet-structure stability in organic solvents and that the potentials, SP_β, previously obtained for the 20 common amino acid residues can be used for the estimation of the β-sheet-structure stability of protected peptides. The amino acid sequence of *E. coli* ribosomal protein L7/L12 is shown by one-letter symbols in Fig. 1, in which its fragmentation into 20 kinds of peptides is also illustrated. The protected tri- to heptapeptides used in this study are summarized in Tables 1 and 2.

The *N*- and *C*-terminals of each peptide were protected by Boc and Pac groups, respectively. Side-chain functional groups in the peptides were also protected by suitable groups commonly used in peptide synthesis. Namely, those of Asp, Glu, Ser, Thr, and Tyr were blocked by a Bzl group, and those of Arg and Lys by Mts and Z groups, respectively. Met was converted to Met(O). The protecting groups in the peptides are not shown in Tables 1 and 2.

Experimental

Materials. The seventy-seven kinds of protected tri- to heptapeptides summarized in Tables 1 and 2 were prepared in CH_2Cl_2 alone or a mixture of CH_2Cl_2 and DMF by common stepwise elongation using DCC and HOBt as coupling reagents.[19)] The elongation was started with the Pac esters of each *C*-terminal amino acid residue for the 20 kinds of peptide fragments shown in Fig. 1. The details of the preparation will be reported elsewhere. The elemental and amino acid analyses were examined for the 20 kinds of each final fragment and were in good agreement with their calculated values. Protected tri- to heptapeptides were purified by repeated recrystallization and, as a result of this

SITKDQ IIEAVA AMSVMD VVELISA MEEKFG VSAAAA VAVAAG

PVEAAEE KTEFDVI LKAAG ANKVAVI KAVRG ATGLG LKEAKD

LVESAP AALKEG VSKDDAE ALKKA LEEAG AEVEVK

Fig. 1. The amino acid sequence of *E. coli* ribosomal protein L7/L12.

Table 1. The Stability of the β-Sheet Structure[a] of Tri- to Tetrapeptides in Mixed Solvents[b]

	SP_β	1	2	3	4
SAP	2.7	r	—	—	—
AKD	3.0	r	—	—	—
DAE	3.0	r	—	—	—
KDQ	3.0	r	—	—	—
VMD	3.3	r	—	—	—
AEE	3.7	r	—	—	—
KEG	3.7	r	—	—	—
KKA	3.7	r	—	—	—
EVK	4.0	r	—	—	—
KFG	4.0	r	—	—	—
DVI	4.0	r	—	—	—
EAG	4.3	r	—	—	—
GLG	4.3	r	—	—	—
ISA	4.0	r	—	—	—
AAG	5.0	r	—	—	—
AVA	5.3	β	β	β/r	r
AVI	5.3	r	—	—	—
VRG	5.7	r	—	—	—
DDAE	2.5	r	—	—	—
TKDQ	2.8	r	—	—	—
ESAP	2.8	r	—	—	—
SVMD	3.0	r	—	—	—
EAKD	3.0	r	—	—	—
LKKA	3.5	r	—	—	—
LKEG	3.5	r	—	—	—
TGLG	3.8	r	—	—	—
EKFG	3.8	r	—	—	—
LISA	3.8	r	—	—	—
EEAG	4.0	r	—	—	—
AAEE	4.0	r	—	—	—
FDVI	4.0	r	—	—	—
VEVK	4.5	r	—	—	—
KAAG	4.5	r	—	—	—
EAVA	4.8	β	β	β	r
AAAA	5.0	β/r	β(s)/r	r	—
VAAG	5.3	β	β/r	r	—
AVRG	5.5	β	r	—	—
VAVI	5.5	β	r	—	—

a) Structure: β, β-sheet structure; r, random and/or α-helix structure. b) Solvents: **1**, CH_2Cl_2 only; **2**, CH_2Cl_2 : DMSO = 9 : 1 (v/v); **3**, CH_2Cl_2 : DMSO = 4 : 1 (v/v); **4**, DMSO only.

Table 2. The Stability of the β-Sheet Structure[a] of Penta- to Heptapeptides in Mixed Solvents[b]

	SP_β	1	2	3	4
KDDAE	2.6	r	—	—	—
KEAKD	3.0	β	r	—	—
MSVMD	3.0	β/r	r	—	—
ITKDQ	3.2	β/r	r	—	—
VESAP	3.4	β/r	r	—	—
EEKFG	3.6	β(s)/r	r	—	—
ELISA	3.6	β/r(s)	r	—	—
ALKEG	3.8	β/r	r	—	—
LEEAG	3.8	β(s)/r	r	—	—
EAAEE	3.8	β/r	r	—	—
ALKKA	3.8	β	r	—	—
EFDVI	3.8	r	—	—	—
ATGLG	4.0	β/r	r	—	—
LKAAG	4.2	β/r(s)	r	—	—
EVEVK	4.2	β/r	r	—	—
SAAAA	4.4	β	β	β/r(s)	—
IEAVA	4.8	β	β	β	r
KAVRG	5.0	β/r(s)	β(s)/r	r	—
KVAVI	5.0	β/r(s)	β/r	β(s)/r	r
AVAAG	5.2	β	β/r	β/r	r
SKDDAE	2.5	r	—	—	—
SITKDQ	3.0	β/r	r	—	—
LKEAKD	3.0	β	r	—	—
LVESAP	3.3	β	r	—	—
AMSVMD	3.3	β	β/r	r	—
TEFDVI	3.5	β/r	r	—	—
MEEKFG	3.5	β	r	—	—
AALKEG	4.0	β	r	—	—
VELISA	4.0	β	β(s)/r	—	—
VEAAEE	4.2	β	r	—	—
AEVEVK	4.3	β	β/r(s)	r	—
VSAAAA	4.7	β	β/r(s)	β/r(s)	β(s)/r
IIEAVA	4.8	β	β	β/r	β(s)/r
VAVAAG	5.3	β	β/r	β/r	β(s)/r
NKVAVI	5.3	β	β	β	r
VSKDDAE	3.0	r	—	—	—
KTEFDVI	3.4	β(s)/r	r	—	—
PVEAAEE	3.7	β	β(s)/r	r	—
ANKVAVI	5.1	β	β/r(s)	β/r(s)	r

a) Structure: β, β-sheet structure; r, random and/or α-helix structure. b) Solvents: **1**, CH_2Cl_2 only; **2**, CH_2Cl_2 : DMSO = 9 : 1 (v/v); **3**, CH_2Cl_2 : DMSO = 4 : 1 (v/v); **4**, DMSO only.

purification process, gave a single peak on HPLC and were negative for the Kaiser test.

IR Absorption Measurements. The IR absorption spectra of protected tri- to heptapeptides in solution or in the suspended state were recorded at room temperature on a JEOL Model JIR-100 FT-IR spectrometer by employing 0.5 mm path length cells with sodium chloride windows. The peptides were dissolved or suspended in CH_2Cl_2 alone or CH_2Cl_2 containing various concentrations of DMSO. The concentration of each peptide was kept at 3×10^{-2}—6×10^{-2} M (1 M = 1 mol dm^{-3}). The IR absorption spectra of the tripeptides were measured at a high concentration (6×10^{-2} M) and those of the heptapeptides at a low concentration (3×10^{-2} M).

Results

The stability of the β-sheet structure was examined for protected tri- to heptapeptides and is summarized in Tables 1 and 2. It was evaluated by monitoring the peptide IR absorption band around 1630 cm^{-1}, assigned to a β-sheet structure, in CH_2Cl_2 alone or CH_2Cl_2 containing various concentrations of DMSO. The β-sheet structure of protected peptides was disrupted in CH_2Cl_2 by adding increasing amounts of DMSO, resulting in the successive increase in the intensity of the band around 1670 cm^{-1}. Typical IR absorption spectra in the amide I region of peptides are shown in Fig. 2. The bands around 1630 and 1670 cm^{-1} are assigned

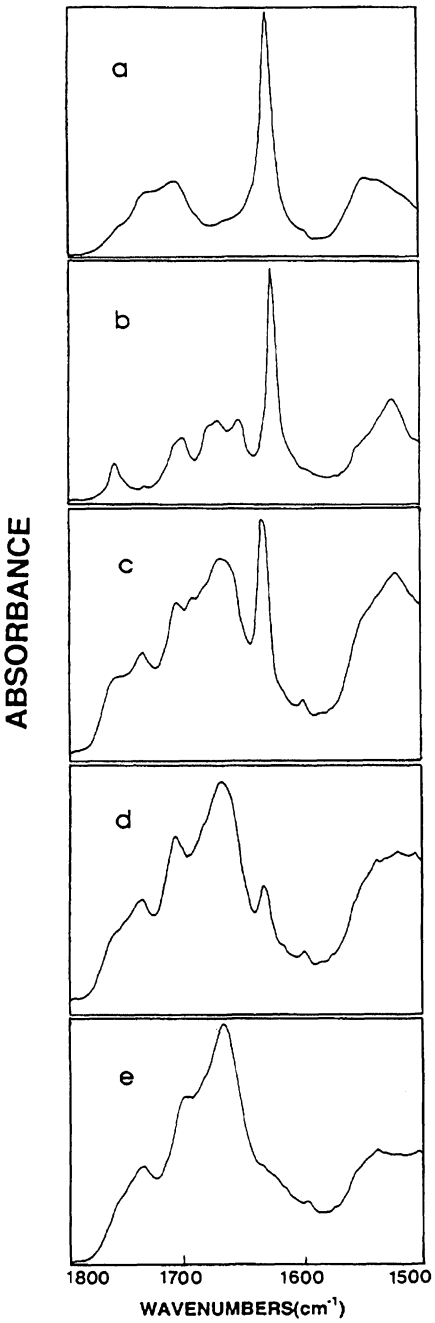

Fig. 2. The classification of IR absorption spectra of the peptides in Tables 1 and 2. a) LKEAKD in CH_2Cl_2; β. b) VSAAAA in CH_2Cl_2 : DMSO = 9 : 1 (v/v); β/r(s). c) IIEAVA in CH_2Cl_2 : DMSO = 4 : 1 (v/v); β/r. d) IIEAVA in DMSO; β(s)/r. e) LKEAKD in CH_2Cl_2 : DMSO = 9 : 1 (v/v); r.

to a β-sheet and mainly to an unordered structure, respectively. The intensity of the former was in harmony with that of the band around 3280 cm^{-1} in the amide A region, assigned to a β-sheet structure. In order to summarize the results of the IR absorption spectra in Tables 1 and 2, the IR absorption spectra of the peptides were classified into five groups. The first group (Fig. 2a) showed strong bands around 1630 cm^{-1} and were accompanied by no or weak shoulder bands around 1670 cm^{-1}. They are assigned to β in Tables 1 and 2. The second group (Fig. 2b) exhibited strong bands around 1630 cm^{-1} and medium shoulder bands around 1670 cm^{-1} (β/r(s) in Tables 1 and 2), the third (Fig. 2c), medium bands around both 1630 cm^{-1} and 1670 cm^{-1} (β/r in Tables 1 and 2), and the fourth (Fig. 2d), medium shoulder bands around 1630 cm^{-1} and strong bands around 1670 cm^{-1} (β(s)/r in Tables 1 and 2). The fifth group (Fig. 2e) showed strong bands around 1670 cm^{-1} and were accompanied with no or weak shoulder bands around 1630 cm^{-1} (r in Tables 1 and 2). In Tables 1 and 2, the $<SP_\beta>$ values of peptides are also included and the peptides are placed in the order of lower to higher $<SP_\beta>$ values. The results in Tables 1 and 2 clearly indicate that the $<SP_\beta>$ values of protected peptides with chain lengths up to a heptapeptide properly reflect the stability of their β-sheet structure in organic solvents.

Discussion

The purpose of this study was to investigate the relationship between the $<SP_\beta>$ value and the β-sheet-structure stability of a protected peptide with a chain length up to a heptapeptide. In this study, *E. coli* ribosomal protein L7/L12 was chosen by random sampling and the amino acid compositions and sequences of its fragments were not considered beforehand. As summarized in Tables 1 and 2, the stability of the β-sheet structure of protected peptides could be clearly evaluated using their $<SP_\beta>$ values. The protected tripeptides in Table 1 have $<SP_\beta>$ values of 2.7 to 5.7 continuously and the peptides except for Boc–Ala–Val–Ala–OPac have an unordered structure in CH_2Cl_2 alone, indicating that the β-sheet structure of the tripeptides is unstable in CH_2Cl_2 regardless of their $<SP_\beta>$ values. The β-sheet-like structure of Boc–Ala–Val–Ala–OPac, assigned by the bands at 1640, 3320, and 3290 cm^{-1}, was quite stable in CH_2Cl_2 alone and partially remained unchanged even in a mixture of CH_2Cl_2 and DMSO (volume ratio, 4/1) in spite of the high potential of DMSO for β-sheet-structure disruption. The protected tetrapeptides in Table 1 have $<SP_\beta>$ values of 2.5 to 5.5, and except for Boc–Glu(OBzl)–Ala–Val–Ala–OPac, the critical $<SP_\beta>$ value of tetrapeptides for the existence of a β-sheet structure is 5.0 in CH_2Cl_2 alone. In a mixture of CH_2Cl_2 and DMSO (volume ratio, 4/1), the tetrapeptides except for Boc–Glu(OBzl)–Ala–Val–Ala–OPac, exist in an unordered structure regardless of their $<SP_\beta>$ values. These unexpected β-sheet-structure stabilities as observed above for Boc–Ala–Val–Ala–OPac and Boc–Glu(OBzl) –Ala–Val–Ala–OPac may be due to an extraordinary contribution effect from the amino acid sequence of the AVA moiety. The protected pentapeptides in Table 2 also have $<SP_\beta>$ values of 2.6 to 5.2 continuously. In contrast with the tetrapeptides,

the critical $<SP_\beta>$ value of pentapeptides for the existence of a β-sheet structure is 3.0 in CH_2Cl_2 alone and 4.4 in a mixture of CH_2Cl_2 and DMSO (volume ratio, 9/1). Still, the pentapeptides have an unordered structure in DMSO alone regardless of their $<SP_\beta>$ values. The protected hexapeptides in Table 2 possess $<SP_\beta>$ values of 2.5 to 5.3 and the critical $<SP_\beta>$ value of hexapeptides is also 3.0 in CH_2Cl_2 alone and 4.0 in a mixture of CH_2Cl_2 and DMSO (volume ratio, 9/1). A different conformational behavior for hexapeptides is observed in DMSO alone. Namely, the β-sheet structure of hexapeptides having an $<SP_\beta>$ value above 4.7 is rather stable in DMSO alone, existing in mixture of β-sheet and unordered structures. Although only a few heptapeptides were examined, the critical $<SP_\beta>$ value of these heptapeptides is 3.7 in a mixture of CH_2Cl_2 and DMSO (volume ratio, 9/1).

The results obtained above are compiled in Table 3. They clearly exhibit that, for each peptide with chain lengths up to a heptapeptide, the $<SP_\beta>$ values of protected tripeptides are in harmony with their β-sheet-structure stability almost regardless of their amino acid sequences. As the $<SP_\beta>$ value increases, the β-sheet structure becomes more stable. This fact strongly supports the propriety of the estimation method for the β-sheet-structure stability of protected peptides in organic solvents and the supposition that the β-sheet-structure stability of protected peptides is strongly dependent on their amino acid compositions and weakly dependent on their amino acid sequences.[6—8] It also means that the SP_β potentials previously obtained[10] are useful for the estimation of the β-sheet-structure stability of protected peptides in organic solvents.

In order to design synthetic routes for peptides and proteins, the choice of reaction solvents is important because the β-sheet aggregation of protected peptides in reaction solvents prevents smooth deprotection and efficient coupling reactions. We show here that the $<SP_\beta>$ value of a protected peptide (shown in Table 3) provides a good basis for the choice of reaction solvents in liquid-phase peptide synthesis. The conformational behavior of protected peptides was examined at 3×10^{-2}—6×10^{-2} M in this study and the concentration is consistent with peptide synthesis. In practice, for coupling reactions between protected peptides having peptide chain lengths below a heptapeptide, a ratio of DMSO to CH_2Cl_2 should be chosen while taking into account the results in Table 3. On the other hand, with protected hexapeptides having $<SP_\beta>$ values above 4.7, it is difficult to disrupt β-sheet and unordered structures even by DMSO. The β-sheet-structure-disrupting potential of DMSO is nearly the same as DMF, NMP, and HMPA, while the potential of CH_2Cl_2 containing a small amount of TFE or HFIP is much larger than that of DMSO.[5] Thus, it is deduced that CH_2Cl_2 containing TFE as a reaction solvent is better than DMSO, DMF, NMP, or HMPA. Practically, we found that a mixture of CH_2Cl_2 and TFE was effective for coupling reactions between protected peptides which were insoluble in DMF, DMSO or HMPA.[8]

Table 3. The Critical $<SP_\beta>$ Values of Tri- to Heptapeptides for the Existence of a β-Sheet Structure in Mixed Solvents[a]

	1	2	3	4
Tripeptides	—	—	—	—
Tetrapeptides	5.0	5.0	—	—
Pentapeptides	3.0	4.4	5.0	—
Hexapeptides	3.0	4.0	4.7	—
Heptapeptides	3.4	3.7	—	—

a) Solvents: **1**, CH_2Cl_2 only; **2**, CH_2Cl_2 : DMSO=9 : 1 (v/v); **3**, CH_2Cl_2 : DMSO=4 : 1 (v/v); **4**, DMSO only.

In solid-phase peptide synthesis, resin matrix has a concentration efficacy for intermolecular hydrogen bonding which promotes the β-sheet aggregation of resin-bound protected peptides and thus prevents smooth deprotection and subsequent efficient coupling reactions.[6,20—23] The β-sheet aggregation in a resin matrix also can be disrupted in organic solvent mixtures having a high β-sheet-structure disruption potential.[6—8] Thus, the estimation of β-sheet-structure stability is essential for the successful achievement of solid-phase peptide synthesis. In this study, the estimation of β-sheet-structure stability was applied to protected peptides up to a heptapeptide. Our previous study[8] on conformational analysis of resin-bound human proinsulin *C*-peptide fragments indicates that the estimation can be independently applied to each peptide segment separated by tertiary peptide bonds such as X–Pro bonds, in which X stands for an arbitrary amino acid residue, namely, the β-sheet-structure stability of protected peptides can be treated separately for each peptide segment. The concept of peptide segment separation (PSS) proposed by us is expected to bring about an advantage for the estimation of the β-sheet-structure stability of protected peptides bound to or free from a resin matrix.[2,5,8]

The relationship between the $<SP_\beta>$ value and the β-sheet-structure stability of protected peptides further suggests that the solvating potential of organic solvents can be classified into some groups using the $<SP_\beta>$ value of protected peptides. In a following paper, we will report the classification of organic solvents by their solvating potential for protected peptides.

We wish to thank Professor Fukuji Higashi and Dr. Shokichi Ohuchi of our department for useful discussions.

References

1) The abbreviations for amino acids are those recommended by the IUPAC-IUB Commission on Biochemical

Nomenclature, *J. Biol. Chem.*, **247**, 977 (1972). Amino acid symbols except for Gly denote the L-configuration. Additional abbreviations used are the following: DMSO, dimethyl sulfoxide; DMF, *N,N*-dimethylformamide; HMPA, hexamethylphosphoric triamide; HFIP, 1,1,1,3,3,3-hexafluoro-2-propanol; TFE, 2,2,2-trifluoroethanol; NMP, *N*-methyl-2-pyrrolidinone; Mts, 2-mesitylenesulfonyl; Boc, *t*-butoxycarbonyl; Pac, phenacyl; Bzl, benzyl; OBzl, benzyl ester; Z, benzyloxycarbonyl; IR, infrared; DCC, dicyclohexylcarbodiimide; HOBt, 1*H*-1,2,3-benzotriazol-1-ol.

2) M. Narita, K. Ishikawa, J.-Y. Chen, and Y. Kim, *Int. J. Peptide Protein Res.*, **24**, 580 (1984).

3) M. Narita, M. Doi, T. Nakai, and H. Takegahara, *Int. J. Peptide Protein Res.*, **32**, 200 (1988).

4) M. Narita, H. Umeyama, and C. Sasaki, *Int. J. Peptide Protein Res.*, **33**, 340 (1989).

5) M. Narita, S. Honda, and S. Obana, *Bull. Chem. Soc. Jpn.*, **62**, 342 (1989).

6) M. Narita, S. Isokawa, S. Honda, H. Umeyama, H. Kakei, and S. Obana, *Bull. Chem. Soc. Jpn.*, **62**, 773 (1989).

7) M. Narita, H. Umeyama, S. Isokawa, S. Honda, C. Sasaki, and H. Kakei, *Bull. Chem. Soc. Jpn.*, **62**, 780 (1989).

8) M. Narita, H. Umeyama, and T. Yoshida, *Bull. Chem. Soc. Jpn.*, **62**, 3577, 3582 (1989).

9) M. Narita and Y. Kojima, *Bull. Chem. Soc. Jpn.*, **62**, 3572 (1989).

10) M. Narita, J.-S. Lee, Y. Murakawa, and Y. Kojima, *Bull. Chem. Soc. Jpn.*, **66**, 483 (1993).

11) A. Liljas, *Progr. Biophys. Mol. Biol.*, **40**, 161 (1982).

12) M. Leijonmarck and A. Liljas, *J. Mol. Biol.*, **195**, 555 (1987).

13) A. Liljas and A. T. Gudkov, *Biochime*, **69**, 1043 (1987).

14) M. Narita, T. Fukunaga, A. Wakabayashi, K. Ishikawa, and H. Nakano, *Int. J. Peptide Protein Res.*, **23**, 306 (1984).

15) M. Narita, J.-Y. Chen, H. Sato, and Y. Kim, *Bull. Chem. Soc. Jpn.*, **58**, 2494 (1985).

16) M. Narita, M. Doi, H. Sugasawa, and K. Ishikawa, *Bull. Chem. Soc. Jpn.*, **58**, 1473 (1985); M. Narita, K. Ishikawa, H. Sugasawa, and M. Doi, *Bull. Chem. Soc. Jpn.*, **58**, 1731 (1985).

17) M. Narita, M. Doi, K. Kudo, and Y. Terauchi, *Bull. Chem. Soc. Jpn.*, **59**, 3553 (1986).

18) M. Narita, S. Honda, and H. Umeyama, *Bull. Chem. Soc. Jpn.*, **60**, 4127 (1987).

19) W. König and R. Geiger, *Chem. Ber.*, **103**, 788 (1970).

20) M. Narita, S. Isokawa, Y. Tomotake, and S. Nagasawa, *Polym. J.*, **15**, 25 (1983).

21) M. Narita, S. Honda, H. Umeyama, and T. Ogura, *Bull. Chem. Soc. Jpn.*, **61**, 1201 (1988).

22) A. G. Ludwick, L. W. Jelinski, D. Live, A. Kintanar, and J. J. Dumais, *J. Am. Chem. Soc.*, **108**, 6493 (1986).

23) M. Narita, S. Isokawa, T. Matsuzawa, and T. Miyauchi, *Macromolecules*, **18**, 1363 (1985); M. Narita, S. Isokawa, S. Nagasawa, and T. Ishijima, *Macromolecules*, **20**, 2306 (1987).