Characterization of L-Methionine in a Peptide α-Helix by Far-infrared Spectroscopy. Synthesis and Examination of Sequential Polypeptides containing L-Methionine

Ryoichi Katakai* and Yasuko Iizuka
Department of Chemistry, College of Technology, Gunma University, Tenjin-cho, Kiryu-shi 376, Japan

Sequential polypeptides containing L-methionine, *viz.* $(Met\text{-}Ala\text{-}Ala)_n$, $(Met\text{-}Met\text{-}Ala\text{-}Ala)_n$, $(Met\text{-}Met\text{-}Ala)_n$, $(Leu\text{-}Met\text{-}Leu)_n$, $(Leu\text{-}Met\text{-}Met\text{-}Leu)_n$, and $(Met\text{-}Met\text{-}Leu)_n$, were synthesized by polycondensation of peptide ONSu esters with corresponding sequences of amino acids; the ONSu esters were prepared stepwise by the method for peptide synthesis using Nps-amino acids and dicyclohexylcarbodi-imide. α-Helical samples of all the polypeptides showed a common far-i.r. band at 409 cm^{-1}, the strength of which changed with the proportion of L-methionine residue in the polypeptides. Thus, this band was assigned to L-methionine in an α-helical polypeptide.

Recent studies of i.r. spectra in the far-i.r. region of a number of polypeptides showed that there are some far-i.r. bands characteristic of amino acid residues in an α-helix and of those in a β structure.[1–5] Thus, far-i.r. spectroscopy is a very useful tool for characterizing the conformations of polypeptides. We have found a far-i.r. band at 416 cm^{-1} characteristic of L-methionine in an α-helix by examining some oligopeptides containing this amino acid residue.[6] In this study we applied the assignment to a number of co-polypeptides containing specifically designed amino acid sequences in order to demonstrate the validity of the assignment of the far-i.r. band.

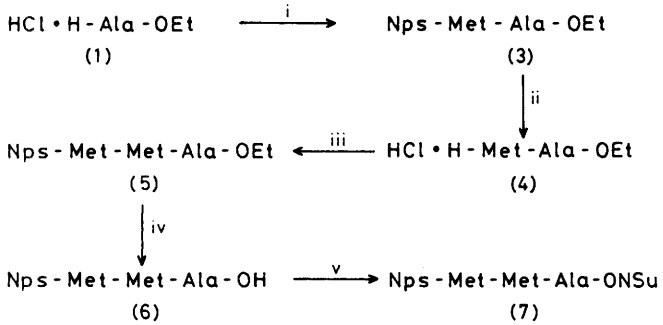
L-Alanine and L-leucine were chosen as amino acids to be incorporated into the co-polypeptides with L-methionine because they have the strongest tendency to form the α-helix,[7] and the far-i.r. bands characteristic of these amino acids in an α-helix have been clearly assigned.[3]

Polycondensation of peptide active esters[8] having defined sequences of these amino acids was used to obtain the co-polypeptides containing L-methionine. An increasing proportion of L-methionine to L-alanine or L-leucine was incorporated for the sequence of the amino acids in the co-polypeptides: Met-Ala-Ala, Met-Met-Ala-Ala, Met-Met-Ala, Leu-Met-Leu, Leu-Met-Met-Leu, and Met-Met-Leu. If our assignment of the far-i.r. band at 416 cm^{-1} to L-methionine in the α-helix is valid, a change in the strength of this band in comparison with those of L-alanine and L-leucine should be observed with changing proportions of L-methionine in the polypeptides.

Results and Discussion

The peptide active esters required as monomers for polycondensation were prepared by a solution method for peptide synthesis using Nps-amino acids† and dicyclohexylcarbodi-imide (DCC) as a condensation reagent.[9] The typical synthesis of *N*-protected monomers is illustrated by the preparation of Nps-Met-Met-Ala-ONSu (Scheme). L-Alanine ethyl ester hydrochloride (**1**) was allowed to react with Nps-L-methionine dicyclohexylammonium salt (**2**) in the presence of DCC to give a dipeptide derivative Nps-Met-Ala-OEt (**3**). The Nps-protecting group of compound (**3**) was removed by treatment with hydrochloric acid in dioxane to give the dipeptide ester hydrochloride HCl·H-Met-Ala-OEt (**4**), which was allowed to react with compound (**2**) with the presence of DCC to give a tripeptide derivative Nps-Met-Met-Ala-OEt (**5**).

† Abbreviations for protecting groups are given in *Pure Appl. Chem.*, 1984, **56**, 595.



Scheme. Synthesis of an *N*-protected monomer. *Reagents:* i, Nps-Met-OH·DCHA (**2**), DCC; ii, HCl; iii, (**2**), DCC; iv, NaOH, then H^+; v, HONSu, DCC

The ethyl ester group in compound (**5**) was removed by saponification with sodium hydroxide to give a free acid, Nps-Met-Met-Ala-OH (**6**), which was esterified with *N*-hydroxysuccinimide (HONSu) and DCC to give a protected monomer Nps-Met-Met-Ala-ONSu (**7**). The intermediates (**3**)—(**6**) obtained in each reaction step and the final product were highly crystalline and easily purified by recrystallization to be chromatographically pure. Other *N*-protected monomers were prepared by the same method described above. Results of the syntheses of the *N*-protected monomers are summarized in Table 1.

Sequential co-polyptides were obtained by polycondensation of monomer hydrochlorides which were prepared by treatment of the *N*-protected monomers with hydrochloric acid. The polycondensation was started by addition of triethylamine to a concentrated solution of the monomer hydrochlorides in dimethyl sulphoxide (DMSO) and was allowed to proceed for 2 days at room temperature. Polypeptides were precipitated by dilution of the polymerization system with methanol and collected by filtration. The polypeptides thus isolated were reprecipitated by dissolution in dichloroacetic acid followed by dilution with diethyl ether. The yields, intrinsic viscosities, and elemental analyses of the polypeptides are given in Table 2.

The polypeptides thus obtained were found to adopt an α-helical conformation in the solid state as shown by the amide I and II regions in the i.r. spectra and *X*-ray powder diffraction measurements. All the samples showed i.r. bands at 1 655 and 1 540 cm^{-1}, characteristic of the α-helical conformation.[10] A prominent peak which can be assigned to the (100) plane of the hexagonal unit cell of the peptides with the α-helical

Table 1. Analytical data for the *N*-protected monomers

Compound	Yield[a] (%)	M.p. (°C)	R_F[b]	$[\alpha]_D$[c]	Found (%) (Required) C	H	N
Nps-Met-Ala-Ala-ONSu	68	140—141 (decomp.)	0.21	−74.0	46.3 (46.6)	5.1 (5.0)	12.8 (12.9)
Nps-Met-Met-Ala-Ala-ONSu	59	148—150 (decomp.)	0.67[d]	−33.0[e]	44.8 (44.3)	5.4 (5.2)	11.6 (12.1)
Nps-Met-Met-Ala-ONSu	65	146—149 (decomp.)	0.32	−67.3	46.2 (46.8)	6.8 (6.9)	12.4 (12.6)
Nps-Leu-Met-Leu-ONSu	63	142—143	0.57	−83.5	52.4 (51.8)	6.5 (6.3)	11.2 (11.2)
Nps-Leu-Met-Met-Leu-ONSu	52	174—176 (decomp.)	0.35	−72.6	48.6 (48.5)	5.7 (5.8)	10.7 (10.9)

[a] The yields are the values from the starting amino acid esters. [b] Eluant: THF–benzene (3:7). [c] (*c* 0.1 in THF). [d] Eluant: THF–benzene (7:3). [e] (*c* 0.1 in *N*,*N*-dimethylformamide).

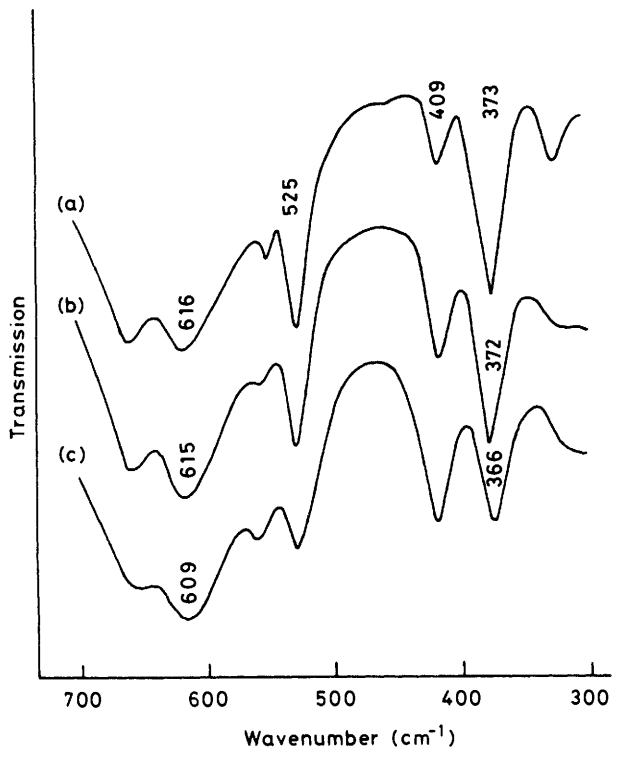


Figure 1. Far-i.r. spectra of (L-Met-L-Ala-L-Ala)$_n$ (a), (L-Met-L-Met-L-Ala-L-Ala)$_n$ (b), and (L-Met-L-Met-L-Ala)$_n$ (c)

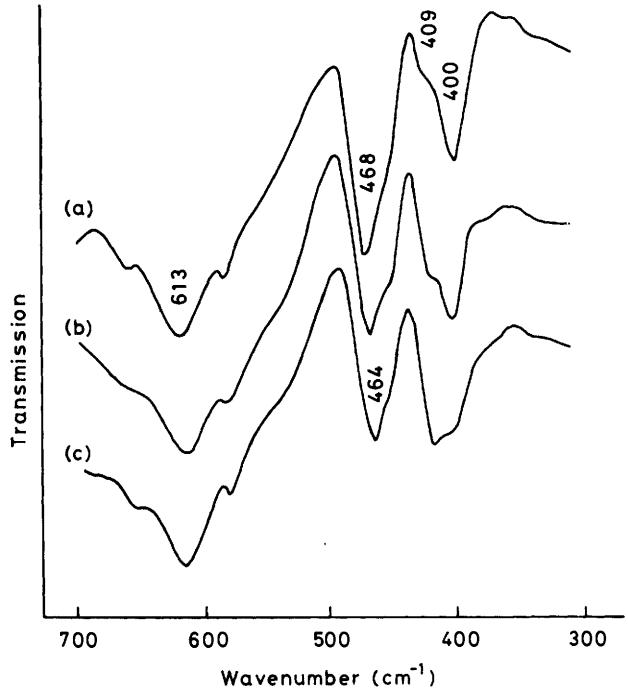


Figure 2. Far-i.r. spectra of (L-Leu-L-Met-L-Leu)$_n$ (a), (L-Leu-L-Met-L-Met-L-Leu)$_n$ (b), and (L-Met-L-Met-L-Leu)$_n$ (c)

conformation was found at 2θ *ca.* 9.5 and 8.5° for the polypeptides containing L-methionine and L-alanine and those containing L-methionine and L-leucine, respectively.[11]

Far-i.r. spectra of the α-helical polypeptides containing L-methionine and L-alanine (Met-Ala-Ala)$_n$, (Met-Met-Ala-Ala)$_n$, and (Met-Met-Ala)$_n$ are shown in Figure 1. All the polypeptides showed common bands near 610, 525, 410, and 370 cm^{-1}. The rather broad band at *ca.* 610 cm^{-1} can be assigned to the amide V band characteristic of the α-helical conformation.[12] The bands at 525 and 370 cm^{-1} are characteristic of L-alanine in an α-helix.[3] These bands of L-alanine are very strong for the polypeptide (Met-Ala-Ala)$_n$. The strength of these bands decreases with a decreasing proportion of L-alanine residues in the polypeptides (Met-Met-Ala-Ala)$_n$ and (Met-Met-Ala)$_n$. In contrast to the decreasing strength of the bands of L-alanine, the band at 409 cm^{-1} becomes stronger with an increasing proportion of L-methionine residues from (Met-Ala-Ala)$_n$ to (Met-Met-Ala)$_n$.

The same spectral changes were obtained for the sequential co-polypeptides containing L-methionine and L-leucine. Figure 2 shows the far-i.r. spectra of (Leu-Met-Leu)$_n$, (Leu-Met-Met-Leu)$_n$, and (Met-Met-Leu)$_n$. For these polypeptides, far-i.r. bands were commonly observed at *ca.* 610, 470, 410, and 400 cm^{-1}. The band near 610 cm^{-1} is characteristic of the amide V band for an α-helical peptide.[12] The bands at *ca.* 470 and 400 cm^{-1} are characteristic of L-leucine in an α-helix.[3] The strength of these bands decreases with a decreasing proportion of L-leucine residue from (Leu-Met-Leu)$_n$ to (Met-Met-Leu)$_n$. In contrast to this, the band at 409 cm^{-1} becomes stronger with an increasing proportion of L-methionine residue in the polypeptides.

Table 2. Analytical data for the polypeptides

Polypeptide	Yield (%)	[η] in DCA*	Found (%) (Required) C	H	N
(Met-Ala-Ala)$_n$	85	0.35	47.8 (48.3)	6.9 (6.7)	14.3 (15.4)
(Met-Met-Ala-Ala)$_n$	90	0.19	47.0 (47.5)	6.7 (6.9)	12.9 (13.8)
(Met-Met-Ala)$_n$	89	0.28	46.2 (46.8)	6.8 (6.9)	12.1 (12.6)
(Leu-Met-Leu)$_n$	92	0.19	57.0 (57.1)	8.8 (8.8)	11.0 (11.8)
(Leu-Met-Met-Leu)$_n$	95	0.41	53.9 (54.0)	8.1 (8.2)	10.8 (11.5)
(Met-Met-Leu)$_n$	88	0.13	50.0 (51.1)	7.6 (7.7)	10.6 (11.2)

*DCA = dichloroacetic acid,

The facts that the band at 409 cm^{-1} is commonly observed for polypeptides containing L-methionine with L-alanine or L-leucine, and that the strength of this band changes with a change in the proportion of L-methionine residue, clearly show that it is diagnostic for L-methionine in an α-helix. This result is consistent with that obtained by a preliminary study on oligopeptides containing L-methionine.[6]

Experimental

M.p.s are uncorrected and were determined using a Yamato MP-21 apparatus. Optical rotations at the sodium D line were measured with a Jasco DIP-SL polarimeter. *X*-Ray powder diffractions were recorded with a Rigaku GF-2012 *X*-ray diffractometer. I.r. spectra in the amide I and II and far-i.r. regions were recorded for KBr disks with a Jasco A-702 spectrophotometer controlled by a Jasco DP-A330 microcomputer.

The general procedure for the preparation of *N*-protected monomers is illustrated by the synthesis of Nps-Met-Met-Ala-ONSu.

Nps-Met-Ala-OEt.—L-Alanine ethyl ester hydrochloride (15.4 g, 0.1 mol) and 2-nitrophenylsulphenyl-L-methionine dicyclohexylammonium salt (48.4 g, 0.1 mol) were dissolved in chloroform (200 ml). The solution was cooled to −10 °C. Then DCC (22.7 g, 0.11 mol) was added to the stirred solution. The mixture was stirred for 2 h at −10 °C and for 2 h at room temperature. The solution was diluted with ethyl acetate (300 ml) and the resulting crystals were filtered off. The filtrate was concentrated under reduced pressure to give an oil, which was dissolved in ethyl acetate (400 ml). The solution was washed in turn with 5% aqueous citric acid, water, 5% aqueous $NaHCO_3$, and water, and dried over Na_2SO_4. Evaporation of the solvent gave an oily product, which was crystallized by addition of hexane. The crude product was recrystallized from ethyl acetate to give the *title dipeptide* (34.5 g, 86%), m.p. 126—127 °C; R_F 0.63 [silica gel; ethyl acetate–benzene (1:1)]; $[\alpha]_D$ − 43.7 °C (*c* 1.0 in tetrahydrofuran) (Found: C, 48.5; H, 5.9; N, 10.5. $C_{16}H_{23}N_3O_5S_2$ requires C, 47.9; H, 5.8; N, 10.5%).

Nps-Met-Met-Ala-OEt.—Nps-Met-Ala-OEt (32.1 g, 0.08 mol) was dissolved in a solution of 3M-hydrochloric acid in dioxane (54 ml). To the solution were added diethyl ether (200 ml) and hexane (200 ml). The resulting precipitate was extracted with diethyl ether and hexane until its yellow colour was discharged. Then the precipitate was dissolved in chloroform (300 ml) and 2-nitrophenylsulphenyl-L-methionine dicyclohexylammonium salt (38.7 g, 0.08 mol) was added. The solution was cooled at −10 °C and DCC (16.5 g, 0.08 mol) was added. The mixture was stirred for 3 h at −10 °C and for 2 h at room temperature. The resulting crystals were filtered off and the filtrate was washed in turn with 5% aqueous citric acid, water, 5% aqueous $NaHCO_3$, and water, and dried over Na_2SO_4. Evaporation of the solvent gave a solid, which was dissolved in tetrahydrofuran (THF) (100 ml). Insoluble crystals were filtered off. Addition of hexane to the filtrate gave the *title peptide* as a crystalline product (33.2 g, 78%), m.p. 146—147 °C; R_F 0.31 [silica gel; ethyl acetate–benzene (1:1)]; $[\alpha]_D$ −51.1 °C (*c* 1.0 in THF) (Found: C, 47.6; H, 6.1; N, 10.5. $C_{21}H_{32}N_4O_6S_3$ requires C, 47.35; H, 6.1; N, 10.5%).

Nps-Met-Met-Ala-OH.—Nps-Met-Met-Ala-OEt (26.6 g, 0.05 mol) was dissolved in a mixture of THF (70 ml) and methanol (30 ml). To the solution was added 1M-sodium hydroxide (50 ml). The solution was stirred for 1 h at room temperature. After the addition of diethyl ether (50 ml), the organic layer was separated. The aqueous layer was acidified with 10% citric acid and extracted twice with ethyl acetate (2 × 70 ml). The combined extracts were washed with water and dried over $MgSO_4$. Evaporation of the solvent gave an oil, which was crystallized by the addition of hexane. The crude product was recrystallized from ethyl acetate–hexane (23.4 g, 93%), m.p. 100—102 °C; R_F 0.28 [silica gel; ethyl acetate–methanol (5:2)]; $[\alpha]_D$ −44.5 °C (*c* 1.0 in THF).

Nps-Met-Met-Ala-ONSu.—Nps-Met-Met-Ala-OH (15.1 g, 0.03 mol) and *N*-hydroxysuccinimide (6.9 g, 0.06 mol) were dissolved in THF (100 ml). The solution was cooled to −10 °C and DCC (6.6 g, 0.032 mol) was added to the stirred mixture. The reaction was allowed to proceed for 3 h at −10 °C and then overnight at 0 °C. The solution was diluted with ethyl acetate (200 ml). The resulting crystals were filtered off and the filtrate was washed rapidly in turn with 5% aqueous $NaHCO_3$ and water, and dried over Na_2SO_4. Evaporation of the solvent gave a solid, which was recrystallized from THF–hexane.

Polycondensation of Monomers.—The Nps-peptide-ONSu derivative (0.01 mol) was dissolved in THF (50 ml) and 3M-hydrochloric acid (7 ml) was added. Addition of diethyl ether (300 ml) then gave a precipitate, which was collected on a glass filter and washed with diethyl ether until its yellow colour was discharged. The solid was dissolved in DMSO (10 ml). To the vigorously stirred solution was added triethylamine (1.68 ml, 0.012 mol) and the mixture was stirred for 2 days at room temperature. The system was diluted with methanol (200 ml) to precipitate polymer. The precipitate was collected on a glass filter and washed with methanol and diethyl ether. Then the polymer was dissolved in dichloroacetic acid (5 ml) and reprecipitated by the addition of diethyl ether (100 ml). The precipitate was collected on a glass filter, washed with diethyl ether, and dried *in vacuo*.

References

1 Y. Koyama and T. Shimanouchi, *Biopolymers*, 1968, **6**, 1037.
2 K. Itoh, T. Nakahara, T. Shimanouchi, M. Oya, K. Uno, and Y. Iwakura, *Biopolymers*, 1968, **6**, 1759.
3 K. Itoh and H. Katabuchi, *Biopolymers*, 1973, **12**, 921.
4 R. Katakai, *J. Am. Chem. Soc.*, 1977, **99**, 232.
5 R. Katakai, *J. Chem. Soc., Perkin Trans. 1*, 1977, 1193.
6 R. Katakai and Y. Iizu... *J. Chem. Soc., Chem. Commun.*, 1982, 1027.
7 P. Y. Chou and G. D. Fasman, *Biochemistry*, 1974, **13**, 222.

8 D. F. DeTar, W. Honsberg, U. Honsberg, A. Wieland, M. Gouge, H. Bach, A. Tahara, W. S. Brinigar, and T. F. Rogers, *J. Am. Chem. Soc.*, 1963, **85**, 2873.
9 R. Katakai, H. Shida, and T. Takada, *Biopolymers*, 1984, **23**, 1397.
10 T. Miyazawa, 'Poly-α-Amino Acids,' ed. G. D. Fasman, Marcel Dekker, New York, 1967, p. 69.
11 T. Komoto, K. Y. Kim, M. Oya, and T. Kawai, *Makromol. Chem.*, 1974, **175**, 283.
12 T. Miyazawa, Y. Masuda, and K. Fukushima, *J. Polym. Sci.*, 1962, **62**, S62.

Received 26*th April* 1984; *Paper* 4/680