The Solubility of Peptide Intermediates in Organic Solvents.[1] Solubilizing Potential of Hexafluoro-2-propanol

Mitsuaki NARITA,* Shinya HONDA, Hiroshi UMEYAMA, and Satoshi OBANA
Department of Industrial Chemistry, Faculty of Technology, Tokyo University of Agriculture and Technology, Koganei 184
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The solubility of peptides to be insoluble in EtOH, MeOH, DMF, DMA, NMP, DMSO, and HMPA was examined in HFIP, sulfolane, TMP, PC, and a mixture of 5—10% HFIP-CH_2Cl_2 (v/v). HFIP has the highest solubilizing potential, followed by HMPA and DMSO, while the solubilizing potential of sulfolane, TMP, and PC is lower than that of HMPA, DMSO, DMF, and NMP and is close to that of MeOH and EtOH. The solubility of the peptides in the mixture of HFIP-CH_2Cl_2 marvelously is as high as that in HFIP alone. The solvent-titration study in CH_2Cl_2–polar solvents clearly indicated that the solubility of peptides having a β-sheet structure in organic solvents was independent of the polarity of the solvents but was strongly dependent on their hydrogen donating and accepting ability. The conformation of the peptides in the mixture of HFIP-CH_2Cl_2 and in HFIP alone was examined using the IR and CD spectroscopies, respectively.

The search for effective solvents for peptide and protein synthesis is of practical significance since the insolubility of peptide intermediates, protected peptides, is a serious problem for peptide and protein synthesis.[2] In many cases, the insolubility of peptide intemediates in high-polar organic solvents, namely, DMA, DMF, NMP, DMSO, and HMPA is caused by a β-sheet aggregation consisting of peptide chains equal to or larger than an octa- or nonapeptide sequence.[3-10] Therefore, it is of paramount significance to elucidate what plays an important role in disrupting a β-sheet aggregation.

1. Boc-Ser(Bzl)ValSer(Bzl)Thr(Bzl)ValLeu-OBzl
2. Boc-Leu6-OBzl
3. Boc-AlaSer(Bzl)LeuAsp(OBzl)Lys(Z)PheLeu-OPac
4. Boc-AlaSer(Bzl)ValSer(Bzl)Thr(Bzl)ValLeu-OPac
5. Boc-Glu(OBzl)AlaGlu(OBzl)Asp(OBzl)LeuGlnValGly-OPac
6. Boc-LeuAlaLeuGlu(OBzl)GlySer(Bzl)LeuGln-OPac
7. Boc-GlnValGlyGlnValGlu(OBzl)LeuGly-OPac
8. Boc-LeuAlaSer(Bzl)ValSer(Bzl)Thr(Bzl)ValLeu-OBzl
9. Boc-ValValLeuGlyAlaAlaIleVal-OBzl
10. Boc-Leu9-OBzl
11. Boc-Asp(OBzl)Lys(Z)PheLeuAlaSer(Bzl)ValSer(Bzl)Thr(Bzl)ValLeu-OBzl
12. Boc-ValAlaValLeuValValLeuGlyAlaAlaIleVal-OBzl
13. Boc-Leu12-OBzl
14. Boc-Glu(OBzl)AlaGlu(OBzl)Asp(OBzl)LeuGlnValGlyGlnValGlu(OBzl)LeuGly-OPac
15. Boc-AlaSer(Bzl)LeuAsp(OBzl)Lys(Z)PheLeuAlaSer(Bzl)ValSer(Bzl)Thr(Bzl)ValLeu-OBzl

Fig. 1. The peptides **1**—**15** used in this study.

Table 1. Solubility Properties[a] of the Peptides **1**—**15** (c=1.0 g dl^{-1})

Peptide ( )[c]	Solvent[b] HFIP	Sulfolane	TMP	PC	HMPA	DMSO	DMF DMA NMP	EtOH MeOH	AN	5% HFIP-CH_2Cl_2	10% HFIP-CH_2Cl_2	CH_2Cl_2
1 (6)	A	B	A	B	A	A	A	B	B	A	A	A
2 (6)	A	D	D	C	A	B	A	C, B	D	A	A	D
3 (7)	A	A	A	A	A	A	A	A	A	A	A	A
4 (7)	A	C	D	C	A	A	A	B	D	A	A	B
5 (8)	A	A	B	B	A	A	A	C	D	A	A	C
6 (8)	A	B	B	D	A	A	A	B	D	A	A	C
7 (8)	A	D	D	D	A	A	A	D	D	A	A	D
8 (8)	A	D	D	C	B	B	B	D	D	A	A	D
9 (8)	A	D	C	D	A	C	D	D	D	A	A	D
10 (9)	A	D	D	C	C	C	D	D	D	A	A	D
11(11)	A	D	D	D	C	D	D	D	D	D	A	D
12(12)	A	D	D	D	C	D	D	D	D	B	A	D
13(12)	C	D	D	D	D	D	D	D	D	D	D	D
14(13)	A	D	D	D	D	D	D	D	D	D	D	D
15(14)	C	D	D	D	C	D	D	D	D	D	D	D

a) Symbols of A, B, C, and D: see text. b) The dielectric constant of each solvent: HFIP, —; sulfolane, 43.3; TMP, —; PC, 65.1; HMPA, 30.0; DMSO, 46.7; DMF, 36.7; DMA, 37.8; NMP, 32.0; EtOH, 24.6; MeOH, 32.7; AN, 37.5; CH_2Cl_2, 8.9. c) Value in parenthesis: number of amino acid residues of peptides.

In this paper, we first examine the solubility of the peptides **1**—**15** (Fig. 1) in high-polar organic solvents shown in Table 1. Next using a solvent-titration technique, we exemplify that the hydrogen donating and accepting ability of solvent plays an important role in disrupting a β-sheet aggregation.

The peptides **1**—**15** have a β-sheet structure in the solid state, and their solubility was already examined in low-, medium-, and high-polar solvents such as benzene, carbon tetrachloride, ethyl acetate, tetrahydrofuran, dichloromethane, chloroform, AN, EtOH, MeOH, DMF, DMA, NMP, DMSO, and HMPA.[3-6] Generally, the solubility of peptides having a β-sheet structure in medium-polar solvents decreases extremely at a hexa- or heptapeptide level and their solubility in high-polar solvents, at an octa- or nonapeptide level.

Experimental

Materials. The samples of the peptides **1**—**15** are those prepared before.[3-6] The purity of the peptides was confirmed by elemental and amino acid analyses. The peptides soluble in DMF gave a single peak on high-performance liquid chromatography.

IR Measurements. The IR absorption spectra of the samples in dichloromethane were recorded at room temperature with a JEOL Model JIR-100 FT-IR spectrometer by employing 0.5 mm-path length cells with sodium chloride windows. The concentration of the peptides was kept 1.0×10^{-3} M (1 M=1 mol dm^{-3}).

CD Measurements. The CD spectra were recorded using a JASCO model J-40AS recording spectropolarimeter. Experimental details have been reported elsewhere.[11] The concentration of the peptides was kept 1.0 mg ml^{-1}.

Results and Discussion

The Solubility of the Peptides 1—15 in High-Polar Organic Solvents. The solubility of the peptides **1**—**15** was examined in the high-polar solvents shown in Table, namely, HFIP, sulfolane, PC, TMP, and a mixture of 5—10% HFIP-CH_2Cl_2 (v/v). The results are summarized in Table together with the solubility of the peptides **1**—**15** in HMPA, DMSO, DMF, DMA, NMP, EtOH, MeOH, AN, and CH_2Cl_2.[3-6] The solubility (c=1.0 g dl^{-1}) was divided into the following four classes: (A) completely soluble at room temperature, (B) completely soluble at 80°, (C) partially soluble at 80° and (D) nearly insoluble at 80°. In the cases of HFIP, EtOH, MeOH, 5% and 10% HFIP-CH_2Cl_2 mixtures, and CH_2Cl_2, the classes (B), (C), and (D) were decided at refluxing temperature. As shown in Table, among the solvents examined, HFIP has the highest solubilizing potential, followed by the second class of HMPA and DMSO and the third one of DMF, DMA, and NMP, while the solubilizing potential of sulfolane, TMP, and PC is lower than that of DMF, DMA, and NMP and is close to that of EtOH and MeOH. It is marvelous that the solubilizing potential of the mixture of HFIP-CH_2Cl_2 is higher than that of HMPA and DMSO and is close to that of HFIP alone. The last result is of great significance for peptide and protein synthesis since it suggests that we can use a mixture of HFIP and CH_2Cl_2 instead of quite expensive HFIP for further peptide-chain elongation.

Solvent-Titration Study of Boc-Val-Gly-Phe-Gly-Leu-Ile-Leu_2-OBzl 16. Octapeptides having a β-sheet structure in the solid state are usually insoluble

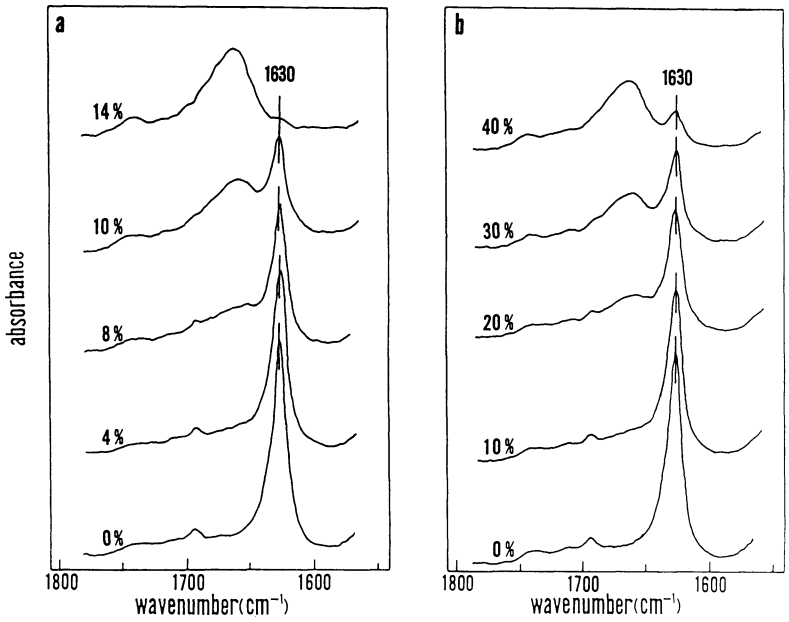

Fig. 2. Typical IR absorption spectra in the amide I region of the peptide **16** in CH_2Cl_2-DMSO (a) and CH_2Cl_2-TMP (b). The percentages in Fig. 2 indicate v/v-%.

in CH_2Cl_2,[3-6] but the octapeptide **16** is a novel one to be soluble in CH_2Cl_2. It has an antiparallel β-sheet structure in CH_2Cl_2 solution.[4] Figure 2 shows typical IR absorption spectra of the peptide **16** in CH_2Cl_2–DMSO and CH_2Cl_2–TMP solutions in the amide I region. Weak and strong bands at 1690 and 1630 cm^{-1} in CH_2Cl_2 solution, respectively, are assigned to an antiparallel β-sheet structure.[12] Successive addition of DMSO or TMP to the CH_2Cl_2 solution induces a dramatic decrease in the strong band at 1630 cm^{-1} and an increase in the broad band at 1660 cm^{-1}, indicating that the β-sheet aggregation of the peptide **16** is easily disrupted by increasing amounts of DMSO and TMP. Both solvents are known to function as hydrogen acceptor and to form effective hydrogen bonds with peptide N–H bonds, thereby disrupting the β-sheet aggregation. Figure 3 illustrates the solvent-titration curves of the peptide **16** in the CH_2Cl_2 solution. By adding increasing amounts of the high-polar solvents, namely, HFIP, HMPA, DMSO, TMP, and PC, similar trends as a function of solvent composition are observed among the high-polar solvents. These solvent-titration curves indicate that, among the solvents examined, HFIP has the highest potential for disrupting a β-sheet aggregation in the CH_2Cl_2 solution, followed by the second class of HMPA and DMSO, and the third one of TMP and PC. The similar trends were also observed among DMSO, HMPA, and TMP in the solvent-titration study of N-protected C-terminal sequences of substance P.[13] PC has the highest polarity (see footnotes in Table), but its solubilizing potential is low, while the polarity of HFIP is estimated to be similar to that of HMPA and DMSO, but its solubilizing potential is the highest. Similarly, AN with higher polarity than HMPA has remarkably lower solubilizing potential than HMPA. These results distinctly denote that the solubility of the peptides **1**—**15** in the high-polar solvents is independent of the polarity of solvent but is strongly dependent on the hydrogen donating and accepting ability of solvent.

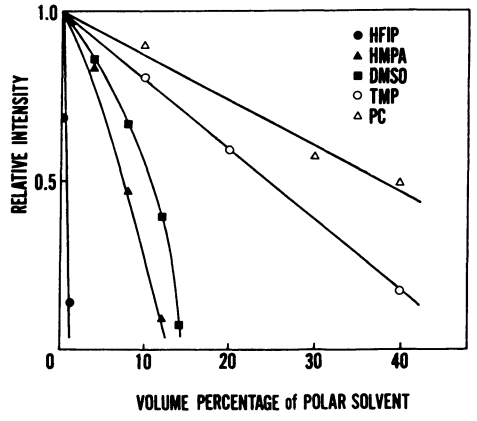

Fig. 3. Relative intensity of the amide I carbonyl stretching 1630-cm^{-1} band related to the β-sheet structure in the IR absorption spectra of the peptide **16** in CH_2Cl_2–HFIP (●) CH_2Cl_2–HMPA (▲), CH_2Cl_2–DMSO (■), CH_2Cl_2–TMP (○), and CH_2Cl_2–PC (△).

Conformation of the Peptides 6, 8—10, and 12 in 5% HFIP–CH_2Cl_2 (v/v) Solution and in HFIP Solution. Figure 4 presents the IR absorption spectra of the peptides **6**, **8—10**, and **12** in a 5% HFIP–CH_2Cl_2 solution in the amide I region. These spectra show that the 1630-cm^{-1} band disappears completely and the strong 1660-cm^{-1} band appears. These results distinctly indicate that HFIP having a strong proton donating ability effectively disrupts a β-sheet aggrega-

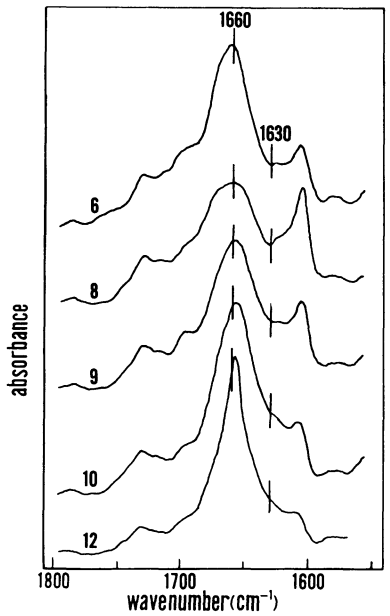

Fig. 4. IR absorption spectra in the amide I region of the peptides **6**, **8—10**, and **12** in 5% HFIP–CH_2Cl_2 solution.

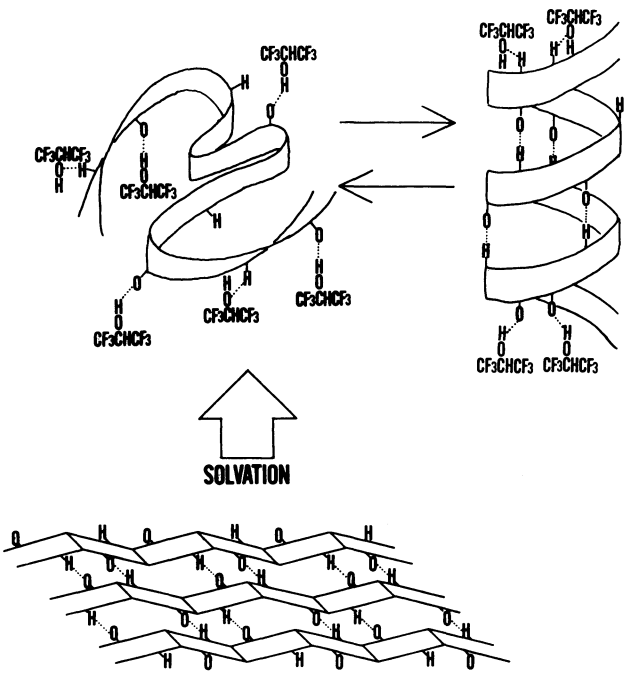

Fig. 5. Scheme in eqeuilibrium between unordered and helix structures.

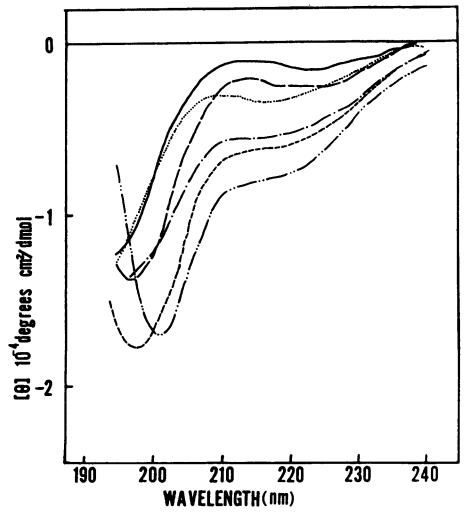

Fig. 6. CD spectra of the peptides **8**—**12** and **14** in HFIP solution. **8**, ——; **9**, ··; **10**, ------; **11**, —— **12**, —·—·—; **14**, —···—···—.

tion formed by peptide chains equal to or smaller than a dodecapeptide sequence. Since HFIP is known to promote helical folding of peptide chains, the conformation of the peptides **6**, **8**—**10**, and **12** in the 5% HFIP-CH_2Cl_2 solution might be in equilibrium between helical and unordered structures (Fig. 5), and the peptide to have unordered structures is justly subjected to solvation to form hydrogen bonds effectively shown in Fig. 5. As shown in Fig. 6, the CD spectra of the peptides **8**—**12** and **14** in HFIP practically indicate these peptides to be in equilibrium between helix and unordered structures. The equilibrium constant is properly dependent on the nature of amino acid sequences.

Conclusion

The solubilizing potential of high-polar organic solvents for peptide intermediates was in fairly good agreement with the hydrogen donating and accepting ability of solvents. Namely, the solubilizing potential was in the following order: HFIP≫HMPA and DMSO>DMF, DMA, and NMP≫TMP, PC, EtOH, and MeOH≫AN, while the hydrogen donating and accepting ability was also in the following order: HFIP≫HMPA>DMSO≫TMP>PC. The solubility of peptides equal to or larger than octapeptides having a β-sheet structure in the solid state in the mixture of 5—10% HFIP-CH_2Cl_2 (v/v) was marvelously close to their solubility in HFIP alone, indicating that HFIP behaves itself like a denaturant in CH_2Cl_2 solution although HFIP has a high potential as a promoter of helical folding of peptides. The results obtained in this paper afford the powerful basis for exploration of effective solvents for peptide and protein synthesis. Use of a mixture of HFIP and CH_2Cl_2 as a solvent in peptide chain elongation, in many cases, will also overcome the serious problem for peptide and protein synthesis, namely, the insolubility of peptide intermediates. Now, we are working on the peptide chain elongation in a mixture of HFIP-CH_2Cl_2.

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References

1) This paper forms Part X of "Designs of Three-dimensional Structures of Peptides and Proteins and the Synthetic Route for Peptides and Proteins" series. For part IX of this series, see M. Narita, S. Honda, and H. Umeyama, *Bull. Chem. Soc. Jpn.*, to be submitted. 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: EtOH, ethanol; MeOH, methanol; DMF, *N*,*N*-dimethylformamide; DMA, *N*,*N*-dimethylacetamide; NMP, *N*-methylpyrrolidone; DMSO, dimethyl sulfoxide; HMPA, hexamethylphosphoric triamide; HFIP, 1,1,1,3,3,3-hexafluoro-2-propanol; sulfolane, tetrahydrothiophene 1,1-dioxide; TMP, trimethyl phosphate; PC, propylene carbonate; AN, acetonitrile; IR; infrared; CD, circular dichroism; Boc, *t*-butoxycarbonyl; Bzl, benzyl; Z, benzyloxycarbonyl; Pac, phenacyl.

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