The Electron Donor-Acceptor Interaction between Mixed Solvents and Its Influence on Their β-Sheet Structure-Disrupting Potential[1)]

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Cross-linked polystyrene resin-bound peptides were useful for the investigation of the β-sheet structure-disrupting potential of mixed solvents although their β-sheet structure-disrupted behavior by mixed solvents was different from that of peptides free from a macromolecular protecting group due to the concentrating efficacy of a cross-linked resin. In mixed solvents, the electron-acceptor and -donor numbers (AN and DN) of organic solvents were one of the most important factors to estimate their β-sheet structure-disrupting potential. In a mixture of electron-acceptor solvents themselves or -donor solvents, the electron donor-acceptor interaction between solvents did not occur and electron-accepting or -donating property of each solvent was kept to disrupt the β-sheet aggregation in mixed solvents. On the other hand, in a mixture of electron-acceptor solvent and -donor solvent, the electron donor-acceptor interaction between solvents occurred predominantly to decrease the β-sheet structure-disrupting potential of mixed solvents. The present results clarify the criteria of the search for effective solvents in peptide and protein synthesis.

The β-sheet aggregation of peptides bound to a cross-linked polystyrene resin in the swollen state is one of the most serious obstacles in solid phase peptide synthesis.[2-6)] To elucidate the hydrogen-bonding behavior of resin-bound oligopeptides, we have investigated the conformational properties of oligo(Leu)s,[2)] human proinsulin C-peptide fragments,[3)] and a few of hydrophobic peptides[6)] which were bound to cross-linked polystyrene resins.

The β-sheet aggregation of peptides free from a macromolecular protecting group is also one of the most serious obstacles in classical peptide and protein synthesis since it causes the insolubility of peptide intermediates, protected peptides.[7-12)] In the study of the β-sheet structure-disruption of Boc-Val-Gly-Phe-Gly-Leu-Ile-Leu_2-OBzl in mixed solvents,[13)] we found that, in a mixture of organic solvents, their electron-acceptor and -donor numbers (AN and DN)[14)] were useful for estimating their solvating potential for peptide main chains, that is, their β-sheet structure-disrupting potential. As AN and DN of solvents were larger, their β-sheet structure-disrupting potential became higher. Moreover, on the basis of the results of the β-sheet structure-disruption of Boc-Val-Gly-Phe-Gly-Leu-Ile-Leu_2-OBzl in a three component solvent system, it could be concluded that the combination of electron-donor solvents themselves or -acceptor solvents was suitable in peptide and protein synthesis, but that of electron-donor solvent and -acceptor solvent was not effective due to the electron donor-acceptor interaction between them. The conclusion was obtained using Boc-Val-Gly-Phe-Gly-Leu-Ile-Leu_2-OBzl as a model peptide, which was a novel octapeptide to be soluble as a β-sheet structure in CH_2Cl_2. Thus, it is important to establish that the conclusion is generally available.

In this paper, we first demonstrate that the β-sheet structure-disruption of cross-linked polystyrene resin-bound peptides is more difficult than that of peptides free from a macromolecular protecting group due to the concentrating efficacy of a cross-linked resin. We next demonstrate the generality of the results of Boc-Val-Gly-Phe-Gly-Leu-Ile-Leu_2-OBzl and discussed the validity of AN and DN for the estimation of the electron donor-acceptor interaction between solvents as well as the hydrogen bonding interaction between a peptide and a solvent. The resin-bound peptides **1—5** used in this study are shown in Fig. 1.

Experimental

Materials. Copoly(styrene/1% divinylbenzene) beads of 200—400 mesh, Bio-Beads S-Xl, were purchased from Bio-Rad Laboratories. The resin-bound peptides except for **1b** and **1c** were prepared in previous papers,[3,6)] and the peptides **1b** and **1c** were also prepared by the method described previously.[6)] The graft ratios (grafted peptides per 100 styrene

Boc-ValGlyPheGlyLeuIleLeuLeu-Ala-OCH_2Pam-resin-a **1a**
Boc-ValGlyPheGlyLeuIleLeuLeu-Ala-OCH_2Pam-resin-b **1b**
Boc-ValGlyPheGlyLeuIleLeuLeu-Ala-OCH_2Pam-resin-c **1c**
Boc-ValAlaValAlaGly-OCH_2Pam-resin **2**
Boc-GlnValGlu(OBzl)LeuGly-$NHCH_2$-resin **3**
Boc-GlnValGlyGlnValGlu(OBzl)LeuGly-$NHCH_2$-resin **4**
Boc-Glu(OBzl)AlaGlu(OBzl)Asp(OBzl)LeuGlnValGlyGlnValGlu(OBzl)LeuGly-$NHCH_2$-resin **5**

Fig. 1. The resin-bound peptides **1—5** used in this study.

units) of the resin-bound peptides **1a—1c** are different with each other to be 0.95, 2.5, and 5.7%, respectively. Those of the resin-bound peptides **2—5** are 1.4, 1.2, 1.1, and 1.0%, respectively.

IR Measurements. The IR absorption spectra of resin-bound peptides were recorded at room temperature with a JEOL Model JIR-100 FT-IR spectrometer. IR measurements in the swollen state were performed by holding the samples between potassium bromide windows after resin-bound peptides were swollen overnight in various molar ratios of mixed solvents. The conformational analysis of the resin-bound peptides **1—5** was carried out using IR absorption spectroscopy as described in the previous papers.[3,6]

Results

The β-Sheet Structure-Disruption of Cross-Linked Polystyrene Resin-Bound Peptides by a Titrating Solvent. The β-sheet structure-disruption was investigated by a solvent-titration method.[15] It was monitored by the successive decrease in the intensity of the band around 1630 cm^{-1} due to the β-sheet structure together with the successive addition of a titrating solvent. Figure 2 shows the typical IR absorption spectra of the resin-bound peptide **1a** in a mixture of HFIP and CH_2Cl_2 at 0.19 M (1 M=1 mol dm^{-3}) and 0.38 M of HFIP. Figure 3 illustrates the solvent-titration curves of the resin-bound peptides **1a—1c**, which are different from each other in a peptide content, using HFIP and TFE as titrating solvents. Relative intensities in Fig. 3 as well as Figs. 4—10 were obtained using the band at 1603 cm^{-1} due to the aromatic ring of the resin support as a standard and were normalized to be 1.0 for each relative intensity in the absence of a titrating solvent. Figure 3 indicates that the more the loading amount of peptide chains on the matrices is, the more difficult the disruption of the β-sheet structure is. HFIP, a strong electron-acceptor solvent, has the high potential for the β-sheet structure disruption[13,16] and the β-sheet structure of the resin-bound peptides **1a—1c** swollen in CH_2Cl_2 is easily disrupted by adding increasing amounts of HFIP. However, in contrast with the fact that the β-sheet structure of Boc-Val-Gly-Phe-Gly-Leu-Ile-Leu_2-OBzl, which is free from a macromolecular protecting group, is completely disrupted at 0.19 M of HFIP,[13,16] that of the resin-bound peptides **1a—1c** is barely disrupted at the same concentration of HFIP. Moreover, the solvent-titration curves of the resin-bound peptides **1a—1c** with TFE show that the β-sheet structure of the resin-bound peptides **1a—1c** is incompletely disrupted even at 1.0 M of TFE, being in contrast with the fact that of Boc-Val-Gly-Phe-Gly-Leu-Ile-Leu_2-OBzl is completely disrupted below 1.0 M of TFE. The solvent-titration curves of the resin-bound peptide **1a** in CH_2Cl_2 using HMPA, DMSO, MeOH, and EtOH as titrating solvents (Fig. 4) are also quite different from those of Boc-Val-Gly-Phe-Gly-Leu-Ile-Leu_2-OBzl. The β-sheet structure of Boc-Val-Gly-Phe-Gly-Leu-Ile-Leu_2-OBzl in CH_2Cl_2, an electron-acceptor solvent, was easily disrupted by adding increasing amounts of strong electron-donor

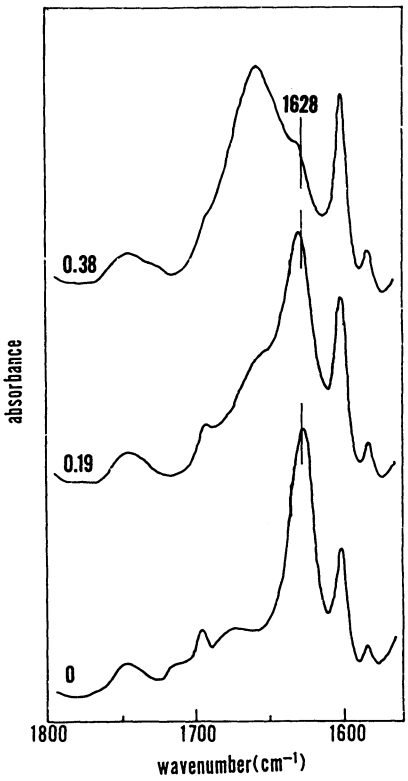

Fig. 2. Typical IR absorption spectra in the amide I region of the resin-bound peptides **1a** swollen in HFIP-CH_2Cl_2. The numerals in Fig. 2 indicate concentrations of HFIP (M).

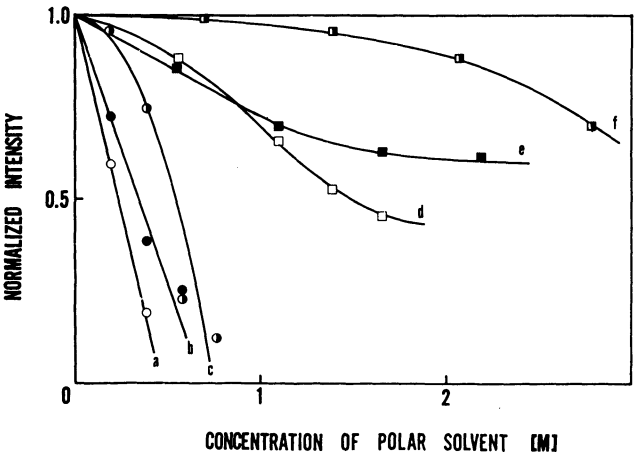

Fig. 3. The solvent-titration curves of the peptides **1a—1c** in CH_2Cl_2 using HFIP (a—c) and TFE (d—f) as titrating solvents. (a) and (d), the peptide **1a**; (b) and (e), the peptide **1b**; (c) and (f), the peptide **1c**. Relative intensities in Fig. 3 as well as Figs. 4—10 were obtained using the band at 1603 cm^{-1} due to the aromatic ring of the resin support as a standard and were normalized to be 1.0 for each relative intensity in the absence of a titrating solvent.

solvents, HMPA and DMSO, and -acceptor solvents, MeOH and EtOH, [13,17] while that of the resin-bound peptide **1a** could not be disrupted by the addition of these solvents. The solvent-titration curves in CH_2Cl_2 show that the larger the AN of titrating solvents is, the greater the β-sheet structure-disruption is, suggesting that AN of titrating solvents is the important factor to estimate their β-sheet structure-disrupting potential in CH_2Cl_2.

The Electron Donor-Acceptor Interaction between Mixed Solvents. Although the β-sheet structure-disruption of resin-bound peptides is more difficult than that of peptides free from a macromolecular protecting group, the fact that the β-sheet structure of resin-bound peptides is not disrupted in a mixture of electron-donor solvent and -acceptor solvent (Fig. 4) suggests that the electron donor–acceptor interaction occurs between mixed solvents. Thus, to certain the generality of the electron donor–acceptor interaction between electron-donor solvent and -acceptor solvent, the β-sheet structure-disruption of resin-bound peptides was examined using the peptides **2**—**5** having a variety of amino acid

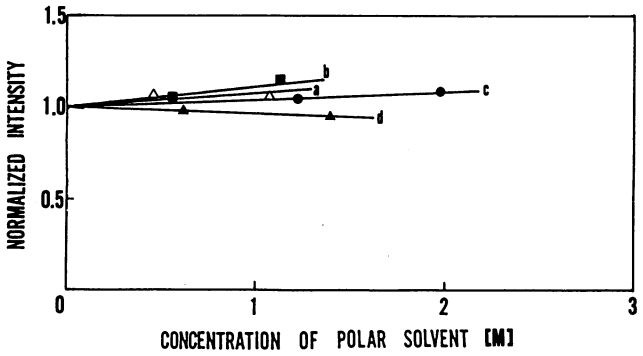

Fig. 4. The solvent-titration curves of the peptides **1a** in CH_2Cl_2 using HMPA (a), DMSO (b), MeOH (c), and EtOH (d) as titrating solvents.

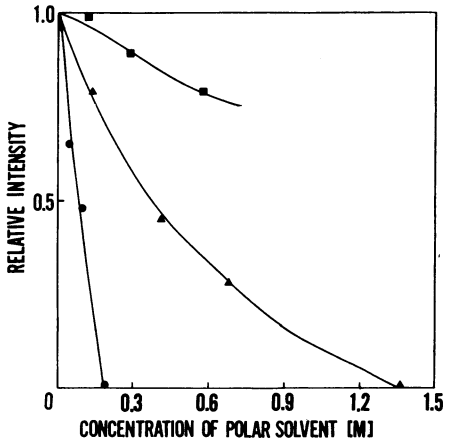

Fig. 5. The solvent-titration curves of the resin-bound peptide **2** in CH_2Cl_2 using HFIP (●), TFE (▲), and HMPA (■) as titrating solvents.

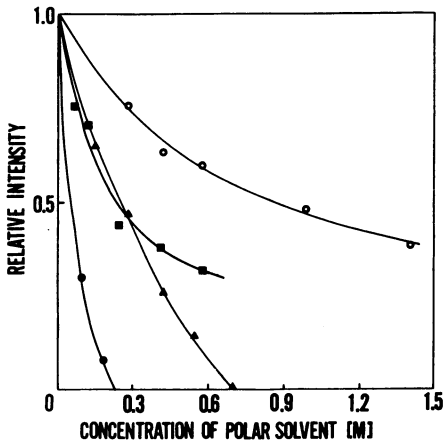

Fig. 6. The solvent-titration curves of the resin-bound peptide **3** in CH_2Cl_2 using HFIP (●), TFE (▲), HMPA (■), and DMSO (O) as titrating solvents.

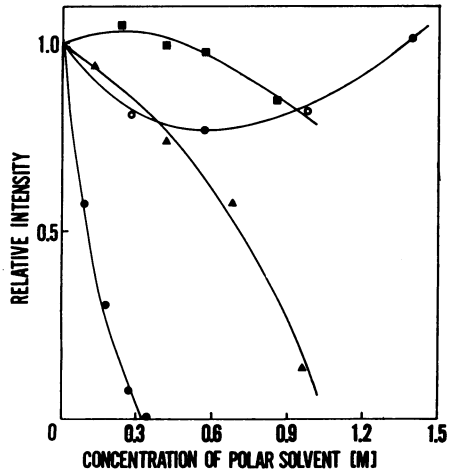

Fig. 7. The solvent-titration curves of the resin-bound peptide **4** in CH_2Cl_2 using HFIP (●), TFE (▲), HMPA (■), and DMSO (O) as titrating solvents.

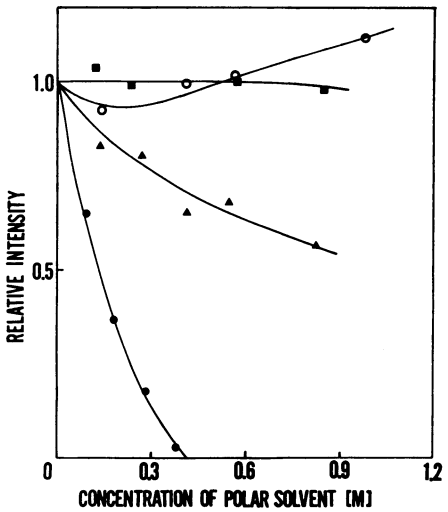

Fig. 8. The solvent-titration curves of the resin-bound peptide **5** in CH_2Cl_2 using HFIP (●), TFE (▲), HMPA (■), and DMSO (O) as titrating solvents.

sequences. It was monitored by the successive decrease in the intensity of the bands at 1632, 1630, 1628, and 1626 cm^{-1}, respectively. Figures 5—8 illustrate the solvent-titration curves of the resin-bound peptides **2—5** in CH_2Cl_2, an electron-acceptor solvent, using HFIP and TFE, electron-acceptor solvents, or DMSO and HMPA, electron-donor solvents, as, titrating solvents. The solvent titration curves in Figs. 5—8 also indicated that the combination of electron-acceptor solvents themselves effectively kept their β-sheet structure-disrupting potential and that of electron-donor solvent and -acceptor solvent did not, suggesting the electron donor-acceptor interaction between them. Furthermore, Figs. 5—8 exhibit that the β-sheet structure-disruption of peptides is strongly dependent on amino acid compositions and peptide chain lengths as discussed in the previous paper.[6]

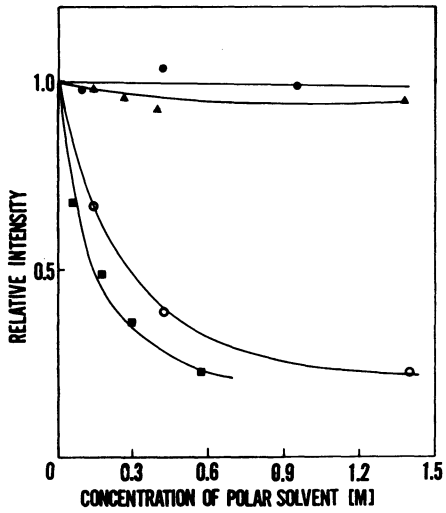

Fig. 9. The solvent-titration curves of the resin-bound peptide **2** in THF using HFIP (●), TFE (▲), HMPA (■), and DMSO (○) as titrating solvents.

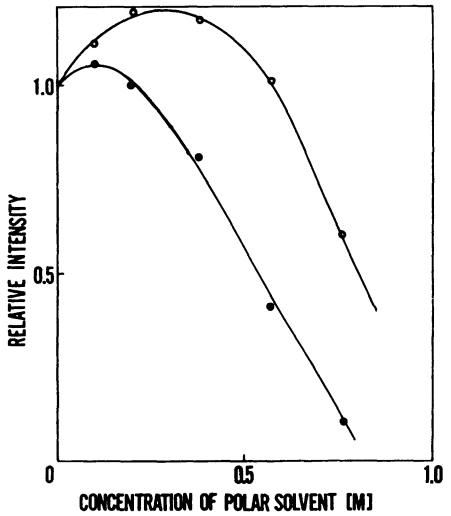

Fig. 10. The solvent-titration curves of the resin-bound peptide **4** in a mixture of CH_2Cl_2 and HMPA (●) and in that of CH_2Cl_2 and DMSO (○) using HFIP as a titrating solvent. Molar concentrations of HMPA and DMSO in CH_2Cl_2 were 0.17 M and 0.42 M, respectively.

Contrary to the results in an electron-acceptor solvent (Figs. 5—8), Fig. 9 illustrates the solvent titration curves of the resin-bound peptide **2** in an electron-donor solvent, THF. The results in Fig. 9 also indicate that the addition of heterogeneous electron-acceptor solvents (HFIP and TFE) decreases in their β-sheet structure-disrupting potential for peptides in THF, while that of homogeneous electron-donor solvents (HMPA and DMSO) does not influence on their β-sheet structure-disrupting potential. However, the β-sheet structure of Boc-(Val-Ala-Val-Ala)$_n$-Ile-OCH_2 Pam-resins (n=2 and 3)[6] in THF could little be disrupted by the successive addition of HMPA due to the low potential of HMPA for the β-sheet structure-disruption of the resin-bound nona- and tridecapeptides (not shown). The solvent titration curves in Fig. 10 illustrates the β-sheet structure-disruption of the peptide **4** in the three component solvent system. In a mixture of CH_2Cl_2 and HMPA or DMSO, the successive addition of HFIP changes the conformation of the resin-bound peptide **4**. The little increase in relative intensity together with the initial addition of HFIP denotes the increase in the β-sheet structure, also indicating the electron donor-acceptor interaction between HFIP and HMPA or HFIP and DMSO.

Discussion

Although peptides having a β-sheet structure in organic solvents usually could not be analyzed their conformation in solution due to their insolubility, anchoring of peptides to a cross-linked polystyrene-resin enabled their conformational analysis in various organic solvents in the swollen state. The solvent-titration curves of the resin-bound peptides **1a—1c** (Figs. 3 and 4) showed that their β-sheet-structure-disrupted behavior was quite different from that of Boc-Val-Gly-Phe-Gly-Leu-Ile-Leu_2-OBzl, which was free from a macromolecular protecting group, and strongly dependent on the loading amount of peptide chains on the matrices. The dependence on the loading amount clearly indicates that anchoring of a peptide to a cross-linked resin has a concentrating efficacy as pointed out before,[2,3] being contrary to the assumption that the high dilution principle would be achieved in solid-phase organic synthesis.[17]

The solvent-titration curves of the resin-bound peptides **1—5** (Figs. 3—10) also indicate that a mixture of heterogeneous solvents (combination of electron-acceptor solvent and -donor solvent) decreases in their β-sheet structure-disrupting potential due to the electron donor-acceptor interaction between them, while that of homogeneous solvents (Combination of electron-acceptor solvents themselves or -donor solvents) keeps electron-accepting or -donating property

Table 1. AN and DN of Organic Solvents Used in This Study[13,14]

Solvent	AN	DN
CH_2Cl_2	20.4	—
THF	8.0	20.0
HMPA	10.6	38.8
DMSO	19.3	29.8
HFIP	88	—
TFE	70	—
MeOH	41.3	19
EtOH	37.1	20

AN and DN of a peptide bond are not reported, but DN of *N*,*N*-dimethylacetamide is 27.8 and AN of formamide is 39.8.

of each solvent. Especially, the solvent-titration curves in Fig. 10 clearly show the electron donor–acceptor interaction between HFIP and HMPA or HFIP and DMSO. In the absence of HFIP, a small amount of HMPA or DMSO contributes solvation of peptide bonds and, by the initial addition of HFIP, the strong electron donor–acceptor interaction between HFIP and HMPA or HFIP and DMSO brings about to help the β-sheet formation of the resin-bound peptide **4**. By the further addition of HFIP, HFIP effectively solvates peptide bonds to disrupt the β-sheet aggregation. These conformational behaviors just correspond to those of Boc–Val–Gly–Phe–Gly–Leu–Ile–Leu_2–OBzl in a three component solvent system.[13] As discussed for the β-sheet structure-disruption of Boc–Val–Gly–Phe–Gly–Leu–Ile–Leu_2–OBzl in the three component solvent system,[13] the electron-accepting and -donating property of solvents indicated by AN and DN is one of the important factors to estimate their β-sheet structure-disrupting potential.

AN and DN of organic solvents used in this study were summarized in Table 1. AN of CH_2Cl_2 is 20.4, indicating that CH_2Cl_2 is a medium electron-acceptor solvent. DN of CH_2Cl_2 is not obtained, but is thought to be near zero since 1,2-dichloroethane is used as a standard for evaluation of DN of organic solvents.[14] In the medium electron-acceptor solvent, CH_2Cl_2, strong electron-acceptor solvents, HFIP and TFE, have a high potential for the β-sheet structure-disruption of the resin-bound peptides **1**—**5** having a variety of amino acid sequences (Figs. 3 and 5—8). On the other hand, strong electron-donor solvents, HMPA and DMSO, have no or little potential for their β-sheet structure-disruption (Figs. 4—8). These results clearly indicate that, in a mixture of homogeneous electron-acceptor solvents, electron donor–acceptor interaction does not occur and electron-accepting property of HFIP and TFE is kept to disrupt the β-sheet aggregation in CH_2Cl_2, while, in a mixture of heterogeneous solvents, electron donor–acceptor interaction occurs predominantly to lose electron-donating property of HMPA and DMSO for peptide N–H bonds.

Contrary to CH_2Cl_2, THF is a medium electron-donor solvent (DN, 20.0) and a weak electron-acceptor solvent (AN, 8.0). In THF, strong electron-donor solvents, HMPA and DMSO, have a high potential for the β-sheet structure-disruption of the resin-bound peptide **2**, while strong electron-acceptor solvents, HFIP and TFE, lose it. Namely, the electron donor–acceptor interaction does not occur in a mixture of homogeneous electron-donor solvents, and electron-donating property of HMPA and DMSO is kept to disrupt the β-sheet aggregation in THF, while, in a mixture of heterogeneous solvents, the electron donor–acceptor interaction occurs predominantly to lose electron-accepting property of HFIP and TFE for peptide C=O bonds. AN and DN of HMPA are 10.6 and 38.8, and those of DMSO, 19.3 and 29.8, respectively. These AN values suggest that the electron-donating property of HMPA and DMSO does not reduce in THF because the electron-accepting property of THF is smaller than that of HMPA and DMSO. HFIP has AN of 88 and strongly interacts with HMPA and DMSO through electron transfer, showing the solvent-titration curves presented in Fig. 10.

Conclusion

Cross-linked polystyrene resin-bound peptides were useful for the investigation of the β-sheet structure-disrupting potential of mixed solvents although their β-sheet structure-disruption by solvents was more difficult than that of peptides free from a macromolecular protecting group due to the concentrating efficacy of a cross-linked resin. As a results, the concentrating efficacy helped the observation of the electron donor–acceptor interaction between heterogeneous solvents which could not be observed for Boc–Val–Gly–Phe–Gly–Leu–Ile–Leu_2–OBzl. The β-sheet structure-disruption of resin-bound peptides having a variety of amino acid sequences indicates that the β-sheet structure disrupting potential of mixed solvents decreases in the case of the combination of heterogeneous solvents due to the electron donor–acceptor interaction. In peptide and protein synthesis, mixed solvents are commonly used in coupling reactions[18] since mixed solvents have a high solubilizing potential for large peptide intermediates probably due to the enhancement of solvation to peptide side chains by mixing. The results in this study clarify the criteria of the search for effective solvents in peptide and protein synthesis. The combination of homogeneous solvents should be selected in the direction that each solvent plays an important role in solvation of various kinds of peptide side-chains.

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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 follwing: Boc, *t*-butoxycarbonyl; Pam, phenylacetamidomethyl; OBzl, benzyl ester; IR, infrared; HFIP, 1,1,1,3,3,3-hexafluoro-2-propanol; TFE, 2,2,2-trifluoroethanol; DMSO, dimethyl sulfoxide; HMPA, hexamethylphosphoric triamide; MeOH, methanol; EtOH, ethanol; THF, tetrahydrofuran.

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