The β-Sheet Structure-Disrupting Potential of Electron-Donor and -Acceptor Solvents and Role of Mixed Solvents in Solvation of Peptides[1)]

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Synopsis. The β-sheet structure-disrupting potential of hydrogen-donor and -acceptor solvents was in the good relationship with their electron-acceptor and -donor numbers (AN and DN), respectively. As AN and DN of solvents are larger, their β-sheet structure-disrupting potential becomes higher. Based on the results of the β-sheet structure disruption in the three component solvent system, it can also be clarified that combinations of homogeneous solvents are suitable for peptide and protein synthesis, but those of heterogeneous ones are not effective. The results of this study are of quite significance for the search for effective solvents in peptide and protein synthesis.

The elucidation of dissolution mechanism of peptides in organic solvents is of practical significance because the insolubility of peptide intermediates, protected peptides, is a serious obstacle in peptide and protein synthesis.[2,3)] In previous papers,[4–10)] we showed that the insolubility of peptides in high-polar organic solvents such as DMF, NMP, DMSO, and HMPA was caused by a β-sheet aggregation formed by peptide chains of equal to or larger than an octa- or nonapeptide sequence. Furthermore, in a recent paper,[11)] we demonstrated that the hydrogen donating and accepting ability of solvent played an important role in disrupting a β-sheet aggregation, and that strongly hydrogen-donating HFIP had a high peptide-solubilizing potential.

In this paper, using a solvent-titration technique,[12)] we first demonstrate that the β-sheet structure-disrupting potential of hydrogen-donor and -acceptor solvents is in the good relationship with their electron-acceptor and -donor numbers (AN and DN),[13)] respectively. Next, we examine the β-sheet structure disruption in the three component solvent system and discuss the criteria to search for effective solvents in peptide and protein synthesis.

Experimental

Materials. The sample of Boc-Val-Gly-Phe-Gly-Leu-Ile-Leu_2-OBzl is that prepared before.[4)] The purity of the peptide was confirmed by elemental and amino acid analyses. It gave a single peak on high-performance liquid chromatography.

IR Measurements. The IR absorption spectra of the sample in CH_2Cl_2 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 peptide was kept 1.0×10^{-3} M (1 M=1 mol dm^{-3}).

Results and Discussion

The β-Sheet Structure-Disrupting Potential of Electron-Donor and -Acceptor Solvents. As a β-sheet structure of peptides is disrupted by hydrogen bonding between solvents and peptide main chains such as N-H···O=C, N-H···O=S, N-H···O=P, and C=O···H-O, the β-sheet structure-disrupting potential of hydrogen-acceptor solvents such as DMF, NMP, DMSO, and HMPA is expected to correlate with their DN. Actually, as shown in Fig. 1, plots of log $[S_{0.5}]_0$ vs. DN for HMPA, DMSO, TMP, and PC gave a straight line. The apparent molarity of titrating solvents, $[S_{0.5}]_0$, corresponds to that at the half value of the initial intensity of 1630-cm^{-1} band in the solvent-titration curves of Boc-Val-Gly-Phe-Gly-Leu-Ile-Leu_2-OBzl[11)] when molarity of titrating solvents is used as abscissa instead of vol-%.

In this paper, we further investigated the relationship between the β-sheet structure-disrupting potential and AN of electron-acceptor solvents. As expected from the result of electron-donor solvents, the fine straight line relationship between log$[S_{0.5}]_0$ and AN for AcOH, MeOH, EtOH and *i*-PrOH was also obtained by the solvent titration curves of the peptide in CH_2Cl_2 using electron-acceptor solvents as titrating solvents (Fig. 1). As AN and DN of titrating solvents are larger, their β-sheet structure-disrupting potential clearly becomes higher. The straight lines observed between log$[S_{0.5}]_0$ and AN or DN indicate that AN and DN of solvents are a measure of their solvating power for peptide C=O and N-H bonds, respectively, and that the hardness of a peptide C=O bond as a base and a

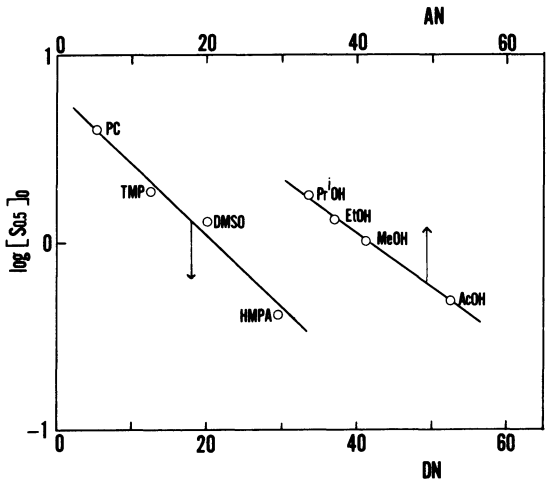

Fig. 1. Plots of log$[S_{0.5}]_0$ vs. AN for AcOH, MeOH, EtOH, and *i*-PrOH and DN for HMPA, DMSO, TMP, and PC.

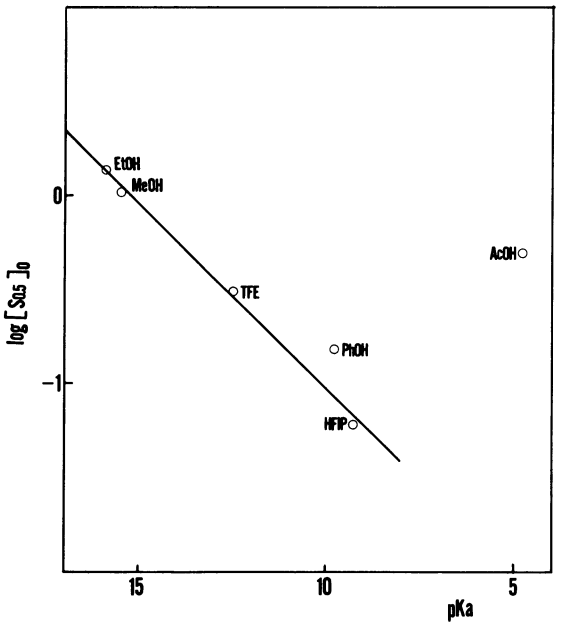

Fig. 2. The linear relationship between $\log[S_{0.5}]_0$ and pK_a values of hydrogen-donor solvents.

peptide N-H bond as an acid is similar to that of triethylphosphine oxide and antimonyl(V) chloride, respectively.[13] Furthermore, although the AN values using triethyphosphine oxide as an electron donor are not reported for HFIP, PhOH, TFE, and *n*-BuOH, the linear relationship observed by AcOH, MeOH, EtOH, and *i*-PrOH suggests that the values of $[S_{0.5}]_0$ for HFIP (0.05), PhOH (0.14), TFE (0.33), and *n*-BuOH (1.5) give their AN values in the case that peptide C=O bonds act as an electron donor. Thus, from Fig. 1, the AN values are estimated to be 88 for HFIP, 70 for PhOH, 59 for TFE, and 36 for *n*-BuOH, respectively.

Figure 2 shows the fine correlation of the β-sheet structure-disrupting potential of hydrogen donor solvents with their pK_a values. The β-sheet structure-disrupting potential of hydrogen-donor solvents except AcOH has the linear relationship with their pK_a values, being in the following order: HFIP>PhOH>TFE>AcOH>MeOH>EtOH>*n*-BuOH>*i*-PrOH, and the lower the pK_a value of hydrogen-donor solvents is, the higher their β-sheet structure-disrupting potential becomes. The straight line observed between $\log[S_{0.5}]_0$ and pK_a values also indicates that pK_a values of hydrogen-donor solvents are a measure of their solvating power for a peptide C=O bond. Since AcOH is predominantly present in the dimeric form in CH_2Cl_2, AcOH has the potential lower than that expected from its pK_a value. In fact, the IR spectrum of AcOH in CH_2Cl_2 shows bands stronger at 1720—1708 cm^{-1} (dimer) than at 1760 cm^{-1} (monomer).

The β-Sheet Structure Disruption in the Three Component Solvent System. In peptide and protein synthesis, a mixture of aprotic high-polar solvents such as NMP, DMSO, and HMPA is generally used for coupling reactions between large peptide fragments because the solubilizing potential of their mixture is higher than that of them alone. Therefore, it is of practical significance to clarify why mixed solvents were more effective in peptide and protein synthesis.

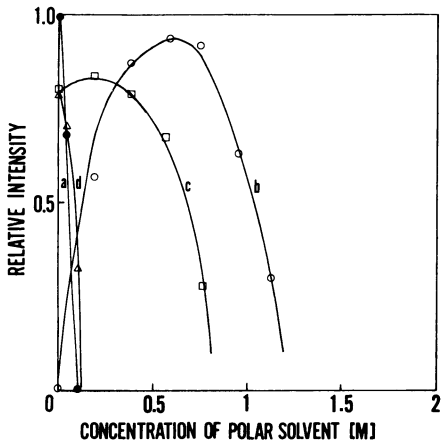

Fig. 3. The solvent-titration curves of the peptide in CH_2Cl_2 (a), HMPA-CH_2Cl_2 (b), DMSO-CH_2Cl_2 (c), and EtOH-CH_2Cl_2 (d) using HFIP as a titrating solvent. Each molar concentration of DMSO, HMPA, and EtOH in CH_2Cl_2 was 0.7 M.

On the other hand, the fine correlation of the β-sheet structure-disrupting potential of hydrogen-donor and -acceptor solvents with their AN and DN strongly suggests that combinations of homogeneous solvents are suitable for peptide and protein synthesis but those of heterogeneous ones are not effective. For example, in CH_2Cl_2, HMPA functions as a strong electron donor and HFIP, as a strong electron acceptor. Thus, in a mixture of HMPA and CH_2Cl_2, addition of HFIP is estimated to interrupt hydrogen bonding of HMPA to a peptide N-H bond and to reduce the peptide-solubilizing potential of a mixture of HMPA and CH_2Cl_2. Therefore, the investigation of the β-sheet structure-disrupting behavior in the three component solvent system is expected to offer significant information for the search for effective solvents in peptide and protein synthesis. Thus, we examined the β-sheet structure-disrupting behavior of the peptide in the three component solvent systems. Figure 3 shows the solvent-titration curves of the peptide in DMSO-CH_2Cl_2, HMPA-CH_2Cl_2, and EtOH-CH_2Cl_2 using HFIP as a titrating solvent. Figure 4 presents the solvent-titration curves of the peptide in DMSO-CH_2Cl_2 and EtOH-CH_2Cl_2 using HMPA as a titrating solvent. In Figs. 3 and 4, the molar concentration of each high-polar solvent was 0.7 M.

In Fig. 3, the curve b gives a clear-cut role of HMPA and HFIP in the β-sheet structure disruption. In the absence of HFIP, the peptide is completely solvated by HMPA in the HMPA-CH_2Cl_2 solution, being free from the β-sheet aggregation, and successive addition of HFIP induces the onset and increase in the β-sheet aggregation. These phenomena signify that HFIP counteracts hydrogen bonding between peptide N-H bonds and HMPA P=O bonds, and that HFIP functions as a strong hydrogen donor not for peptide C=O bonds but for HMPA P=O bonds since the DN of

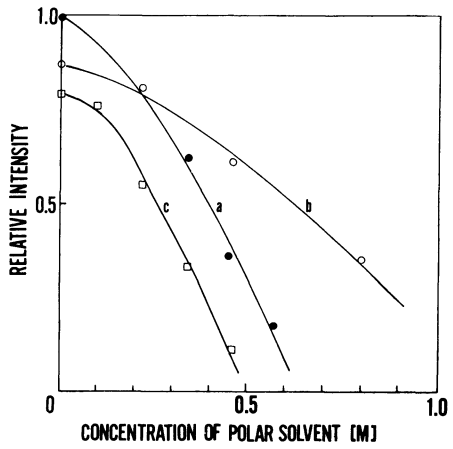

Fig. 4. The solvent-titration curves of the peptide in CH_2Cl_2 (a), EtOH-CH_2Cl_2 (b), and DMSO-CH_2Cl_2 (c) using HMPA as a titrating solvent. Each molar concentration of EtOH and DMSO in CH_2Cl_2 was 0.7 M.

a HMPA P=O bond is higher than that of amide C=O bonds.[13] Further addition of HFIP clearly causes the β-sheet structure disruption. In the DMSO-CH_2Cl_2 solution, HFIP also functions as a strong hydrogen donor for DMSO S=O bonds (Fig. 3, c). These results show that a mixture of electron-donor and -acceptor solvents is not suitable for disruption of the β-sheet structure in peptide and protein synthesis. On the other hand, successive addition of HFIP to the EtOH-CH_2Cl_2 solution induces a remarkable decrease in the 1630-cm^{-1} band due to disruption of the β-sheet structure (Fig. 3, d). In this case, HFIP and EtOH act independently as hydrogen donors for peptide C=O bonds since the DN of EtOH and HFIP is estimated to be lower than that of peptide C=O bonds. Similarly, a combination of HMPA and DMSO, which are strong hydrogen acceptors for peptide N-H bonds, showed effective disruption (Fig. 4, c) since no competition between HMPA and DMSO takes place by mixing. In addition, in the HFIP-EtOH-CH_2Cl_2 and HMPA-DMSO-CH_2Cl_2 solvent systems, each solvent can independently play an important role in effective solvation of peptide side chains in peptide and protein synthesis. We have the view that the results in this study clarify the criteria of the search for effective solvents in peptide and protein synthesis.

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References

1) This paper forms Part XI of "Designs of Three-dimensional Structures of Peptides and Proteins and the Synthetic Route for Peptides and Proteins" series. For part X of this series, see Ref. 11. The abbreviations for amino acid residues are the following: Val, L-valine; Gly, glycine; Phe, L-phenylalanine; Leu, L-leucine; Ile, L-isoleucine. Additional abbrevitions used are the following: Boc, *t*-butoxycarbonyl; OBzl, benzyl ester; HFIP, 1,1,1,3,3,3-hexafluoro-2-propanol; IR; infrared; DMF, *N*,*N*-dimethylformamide; NMP, *N*-methylpyrrolidone; DMSO, dimethyl sulfoxide; HMPA, hexamethylphosphoric triamide; TMP, trimethyl phosphate; PC, propylene carbonate; AcOH, acetic acid; MeOH, methanol; EtOH, ethanol; *i*-PrOH, 2-propanol; PhOH, phenol; TFE, 2,2,2-trifluoroethanol; *n*-BuOH, 1-butanol.

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