Solubility and Coupling Reactivity of Protected Peptides in Highly Polar Solvents

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Synopsis. The solubility of protected peptides as well as the coupling rate was quantitatively examined in highly polar solvents. Symmetrical anhydrides in dimethyl sulfoxide (DMSO) containing *N*,*N*-dimethylformamide (DMF) or DMF solution of lithium chloride and diethyl phosphorocyanidate with triethylamine in DMSO-DMF were proved to be efficient coupling reagents for solid phase synthesis of hardly soluble peptides.

The demand for chemical synthesis of peptides is ever increasing in relation to peptides with non-natural sequences of amino acids. Kaiser et al. designed and synthesized non-natural amphiphilic peptides[1,2] possessing biological activities displayed by melittin and β-endorphin[3] whereas Ho et al. synthesized the 4-bundle helices[4] of peptides. However, syntheses of large peptides with regular sequences are often difficult probably because of the low solubility of the peptides. Narita et al. have investigated[5,6] the solubility and the conformation of the synthetic intermediates in organic solvents and suggested that the aggregation by the β-sheet formation causes the low solubility. In order to prevent the β-sheet formation, they recommended the use of *N*-substituted or α,α-disubstituted amino acid residues. However, when designed peptide sequence does not contain these residues destroying β-sheet structure, the use of highly polar solvents seems effective. Actually, DMF as well as DMF-DMSO[7] has been used in the fragment condensation to solubilize component peptides. DMF and NMP are also effective in the solid phase synthesis. Relatively high yields were obtained by using a DMF-DMSO mixed solvent.[4] The descriptions about the solubility of the protected peptides in highly polar solvents are qualitative in most papers and there are only few data available for the selection of coupling solvents.[8] During the course of our studies on non-natural long chain peptides, optimization of coupling conditions has been required. This paper deals with the more detailed examination of solubility of several types of peptides in highly polar solvents and the reactivity of several coupling reagents in these solvents.

Experimental

Materials[9] and Methods. The organic solvents were dried over Molecular Sieves 4A, and LiCl was dealt under dry conditions. TLC was performed on silica gel (Kieselgel $60F_{254}$, Merck). R_f values refer to the following solvent systems: R_f^1 ethyl acetate-MeOH (5:1 v/v), R_f^2 dioxane-MeOH (1:1), R_f^3 1-butanol-acetic acid-water (4:1:1), R_f^4 chloroform-MeOH (9:1). The optical rotations were determined with a Horiba SEPA-200 polarimeter at 24 °C. Elemental analyses were performed with a Yanaco MT-3 CHN Corder.

Synthesis. Boc-Asn-Asn-Phe-OBzl (Ia). This peptide was prepared stepwise by the standard solution technique.[10] R_f^2 0.86; R_f^3 0.80; R_f^4 0.10; $[\alpha]_D$ −11.3° (*c* 1.0, DMSO).

Boc-Asn-Asn-Phe-Ala-Asn-Asn-Phe-OBzl (Ib). This compound was prepared by the fragment condensation of Boc-Asn-Asn-Phe-OH with H-Ala-Asn-Asn-Phe-OBzl using WSC and HOBt. Mp 260—261 °C (decomp); $[\alpha]_D$ −20.6° (*c* 1.0, DMSO). Calcd for $C_{49}H_{63}N_{11}O_{14}\cdot H_2O$: C, 56.2; H, 6.3; N, 14.7%; Found: C, 56.0; H, 6.3; N, 15.1%.

Boc-Glu(OBzl)-Glu(OBzl)-Phe-Ala-Glu(OBzl)-Ala-Phe-Gly-OBzl (IIa). The peptide **IIa** was synthesized by a stepwise solid-phase procedure employing oxime resin described by Kaiser et al.[11] Mp 184—187 °C (decomp). Calcd for $C_{74}H_{86}N_8O_{17}\cdot H_2O$: C, 64.5; H, 6.4; N, 8.1%; Found: C, 64.5; H, 6.5; N, 8.1%.

Boc-Glu(OBzl)-Glu(OBzl)-Phe-Ala-Glu(OBzl)-Ala-Phe-Glu(OBzl)-Glu(OBzl)-Phe-Ala-Glu(OBzl)-Ala-Phe-Gly-OBzl (IIb). The fragment Boc-Glu(OBzl)-Glu(OBzl)-Phe-Ala-Glu(OBzl)-Ala-Phe-N_2H_3 was prepared by the cleavage of the heptapeptide resin with hydrazine according to the method of Kaiser et al.[11] Yield, 52%; R_f^1 0.78; R_f^2 0.86; R_f^4 0.58. Another fragment H-Glu(OBzl)-Glu(OBzl)-Phe-Ala-Glu(OBzl)-Ala-Phe-Gly-OBzl was obtained from compound **IIa** in 90% yield. The compound **IIb** was prepared with these two fragments according to the azide method. Mp 254—257 °C (decomp); $[\alpha]_D$ −5.0° (*c* 1.0, DMSO). Calcd for $C_{134}H_{153}N_{15}O_{30}\cdot 3H_2O$: C, 64.2; H, 6.4; N, 8.4%; Found: C, 64.4; H, 6.5; N, 8.8%.

Boc-Phe-Phe-Leu-Leu-Phe-Phe-Leu-Leu-Ala-OBzl (III). This compound was prepared in a similar manner as for the peptide **IIa**. Yield, 65%; mp 299—302 °C (decomp); $[\alpha]_D$ −23.2° (*c* 1.0, DMSO). Calcd for $C_{75}H_{101}N_9O_{12}\cdot H_2O$: C, 67.3; H, 7.8; N, 9.4%; Found: C, 67.6; H, 8.0; N, 9.5%.

To suppress racemization and side reactions in the fragment condensations, phenylalanine was chosen as C-terminus of carboxyl components. Little racemization was detected by ^1H NMR for compound **Ib**.

Solubility. The peptide was weighed precisely in a small tube and one or more drops of solvent was added to it. The suspension was stirred magnetically for one minute. By direct observation of the precipitant, it was judged whether an insoluble part was remained or not. The addition of the solvent and the judgement were repeated.

Determination of Coupling Rate. Five coupling reagents, i.e., DCC, HOBt-DCC, DEPC, DEPC-TEA, and symmetrical anhydride, were examined in the following way. Deprotected [4-(glycyloxymethyl)phenylacetamido]-methyl-resin (200 mg)[12] was placed in a test tube and suspended in a solvent. Boc-phenylalanine (2 equiv) was added subsequently. The coupling reaction was initiated with the addition of the coupling reagent such as DCC or DEPC. The reaction was pursued in 2.00 g of solvent. In the case of HOBt-DCC, HOBt (4 equiv) was added in advance. For DEPC-TEA, TEA was added one minute later after the addition of DEPC. Symmetrical anhydride was pre-formed with Boc-phenylalanine (4 equiv) and DCC (2 equiv) in dichloromethane and used as DMF solution. After specified times, the aliquots of the reaction mixture were removed and immediately washed with ethanol. The content of unreacted amino groups on the resin was deter-

mined by the method of Gisin,[13] which employs the salt formation with picric acid.

Results and Discussion

The amino acid residues in synthetic intermediates can be classified as follows: (i) unprotected residues which tend to form hydrogen bond, such as asparagine; (ii) hydrophilic residues with hydrophobic protecting groups; (iii) hydrophobic residues. The peptides **Ia** and **Ib** were designed to have an abundance of type-(i) residues. The peptides **IIa** and **IIb** were designed to be rich in type-(ii) residues and the peptide **III** was constructed with only type-(iii) residues. In common with the peptides **Ia—IIb** alanine and phenylalanine were used as the other residues.

The highly polar solvents such as HMPA, NMP, DMA, DMF, and DMSO are strong electron donors as well as strong electron acceptors on the basis of donor-acceptor concept.[14] HMPA is the strongest donor, and DMSO is the strongest acceptor among these solvents. Therefore, three representative solvents, i.e., HMPA, DMSO, and DMF, were examined in this study. DMF solution of LiCl was also examined because it is well-known as a good solvent for hydrogen-bonded compounds.

The characteristic solubility of the peptides in various solvent systems was obtained as shown in Fig. 1. The weights of peptide and solvent are represented as W_p and W_s, respectively. DMF has low solubilizing power for the peptides **Ia**, **Ib**, **IIb**, and **III**. These critical values of $\log(W_p/W_s)$ were in the range of -3 to -2. Only the peptide **IIa** was readily soluble in DMF. On the other hand, HMPA was a good solvent for the peptides **IIa**, **IIb**, and **III**. However, asparagine-rich peptides **Ia** and **Ib** had remarkably low solubility in HMPA. This result presumably relates to high donor character of HMPA. DMSO was quite a good solvent for the all peptides examined here. The critical values of $\log(W_p/W_s)$ were larger than -1.5. In comparison with the peptide **Ia** the peptide **Ib** had rather high solubility in DMSO in spite of the long chain length. This seems to be attributed to intramolecular hydrogen bonds.

DMF solution of LiCl was also a good solvent. The solubility of **Ib**, **IIa**, and **IIb** was comparable to that in DMSO. The peptides **Ia** and **III** were more soluble in DMF-LiCl than in DMSO. The solubility of these peptides was scarcely dependent on the content of LiCl in the range of 4% to 7%. In the mixed solvent systems, the solubility changes toward the mixing ratios were not simple. The peptide **III** had almost a constant solubility in DMF-HMPA system containing 0—80% of HMPA, though it had higher solubility in HMPA. It was also the case of DMF-DMSO system.

Further experiment suggested that the solubility in a mixed solvent depended on the order of mixing. For example, although the peptide **III** was insoluble in a mixture of DMF and DMSO at $W_p/W_s=10^{-2}$, the solubilization could be realized by dissolving it in pure DMSO at first and subsequently diluting with DMF to the same concentration. The procedure involving the devided addition of the solvents seems practically useful because mixed solvent systems give better coupling efficiency as described later.

For the solid-phase peptide synthesis, a rapid coupling reaction is desirable as a high efficiency can be achieved in a short time. Therefore, the coupling methods employing DCC, DCC-HOBt, DEPC, DEPC-TEA, and symmetrical anhydride were examined. The azide method and the pre-formed active ester methods were excluded because the former with diphenyl phosphorazidate was reported to be slower than DEPC method in DMF[15] and the latter reactions take place very slow in general.

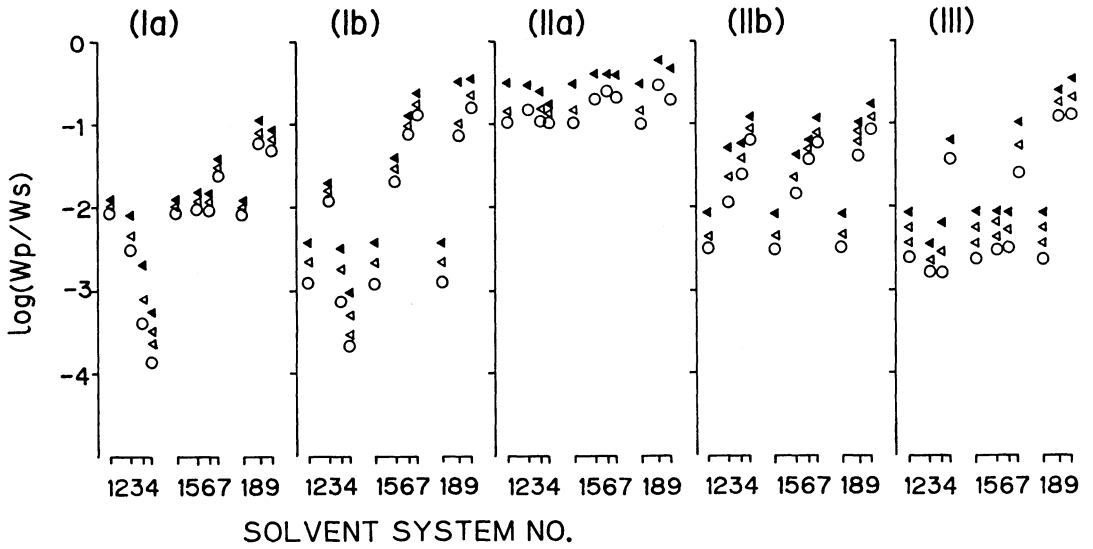

Fig. 1. Solubility of the peptides. The following solvent systems were used; 1: DMF, 2: HMPA-DMF (50 : 50 v/v), 3: HMPA-DMF (80 : 20), 4: HMPA, 5: DMSO-DMF (50 : 50), 6: DMSO-DMF (80 : 20), 7: DMSO, 8: DMF-LiCl (4%), 9: DMF-LiCl (7%). The open circles, the open triangles, and the closed triangles represent 'soluble', 'slightly insoluble', and 'apparantly insoluble', respectively.

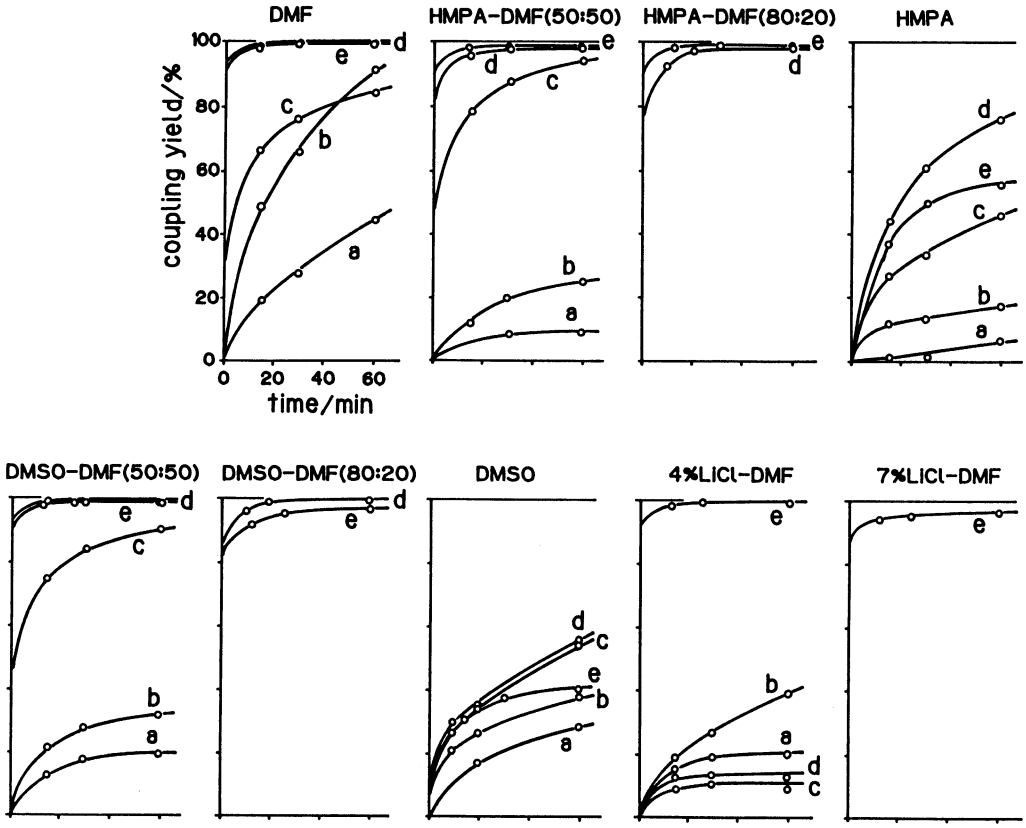

Fig. 2. Conversion curves of coupling reactions using DCC(a), DCC-HOBt(b), DEPC(c), DEPC-TEA(d) and symmetrical anhydride(e).

The conversion curves of those coupling reactions in various solvent systems are shown in Fig. 2. When DMF was used as a solvent, the coupling rate with DEPC-TEA and symmetrical anhydride were remarkably high. The result for DEPC-TEA is in line with the previous report.[15] On the other hand, DCC method is very slow. The coupling rates were considerably reduced for all the methods in HMPA as well as DMSO in comparison with those in DMF. This result means that the use of HMPA or DMSO alone is inadequete to realize both high solubilization and rapid coupling rate. Therefore, the coupling rates were examined in mixed solvent systems. In any mixed solvent containg DMF, the coupling reactions with DEPC-TEA and symmetrical anhydride method were as rapid as in DMF. The role of DMF seems to be essential for these rapid coupling reactions.

In conclusion, for the highly efficient synthesis of peptides the following methods can be recommended, i.e., symmetrical anhydride and DEPC-TEA in DMSO containing a small amount of DMF and the former method in DMF-LiCl.

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9) The following abbreviations are used; Boc, *t*-butoxycarbonyl; OBzl, benzyl ester; DCC, dicyclohexylcarbodiimide; HOBt, 1-hydroxybenzotriazole; DEPC, diethyl phosphorocyanidate; TEA, triethylamine; MeOH, methanol; DMF, *N*,*N*-dimethylformamide; DMSO, dimethyl sulfoxide; HMPA, hexamethylphosphoric triamide; NMP, *N*-methylpyrrolidone; DMA, *N*,*N*-dimethylacetamide; WSC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride.
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