Efficient Amounts of Additives for Peptide Coupling Mediated by a Water-Soluble Carbodiimide in Aqueous Media

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The optimal amounts of HOBt, HOSu, and HONb for enhancement of peptide coupling mediated by EDC in aqueous media were found to be less than equimolar against the C-component or the carbodiimide. A combination of EDC and 0.1 equimolar amount of HOBt was shown to be an effective coupling reagent under aqueous conditions.

Peptide synthesis is often carried out in aqueous media,[1] and coupling in such media is a useful technique for partial synthesis of large peptides or proteins[2] and for immobilization of proteins.[3] Moreover, many advantages of such synthesis are known.[4] However, to our knowledge, the yields of coupling via direct activation of the C-terminal components under aqueous conditions are generally low. For efficient coupling under such conditions, active esters preformed in anhydrous solvents are usually employed;[1,2,5] yet studies on coupling reagents suitable for aqueous media seem to be few.[6] Previously we had found that yields of peptide coupling mediated by EDC[7,8] in DMF-H_2O (5/1,v/v) were improved by addition of some *N*-hydroxy compounds commonly used in peptide synthesis under anhydrous conditions.[9] In this communication are described quantitative studies on the additives in EDC-mediated peptide synthesis in water-containing solvents.

Table 1 shows the yields of Boc-Ala-Gly-OBzl prepared from the coupling of Boc-Ala-OH and H-Gly-OBzl with the aid of EDC in several solvents. Without additives, the yield was moderate when DMF was used as the solvent, but was low in the couplings carried out in water-containing media. Addition of equimolar HOBt[10] or HONb[11] against the C-component improved the yield as long as the concentrations of water in the solvent were low, but the effects of the additives diminished in the water-abundant media. HOSu[12] was practically ineffective in enhancement of the coupling in every solvent. Unexpectedly, the use of 0.05 - 0.2 equimolar amounts of the additives, including HOSu, much improved the coupling in DMF-H_2O (1/4)(Table 2). For every additive, the use of only 0.1 equimolar amount of the additive best enhanced the coupling under the water-enriched conditions, while the use of 1 or 2 equimolar amount was less effective. The use of 2 equimolar HONb slightly decreased the coupling rate. Data similar to those shown in Table 2 were obtained in the coupling carried out in water.

In the sterically hindered coupling between Boc-Val-OH and H-Ile-OBzl in DMF-H_2O (1/1), the yield of Boc-Val-Ile-OBzl was only 21% after coupling for 16 h when no additive was employed. Addition of the additives improved the yield again (Table 3). However, for the coupling, the optimal amounts of HOSu and HONb were around 0.5 equimolar against the C-component, respectively, and both additives seemed less effective activators. The use of 1 or 2 equimolar amounts of HOSu or HONb tended to decrease the coupling rate. On the other hand, HOBt in various amounts much improved the yield, showing it to be an effective activator under water-abundant conditions. The optimal amount of HOBt was around 0.1 equimolar, giving the desired peptide in the quantitative yield (coupling for 16 h). Data similar to those shown in Table 3 were observed in the coupling carried out in CH_3CN-H_2O (1/1 and 1/4).

Table 1. Coupling yield of Boc-Ala-Gly-OBzl (%)[a,b]

Solvent (v/v)	Additive None	HOBt	HOSu	HONb
DMF	77(75)	99(100)	67(68)	85(87)
DMF-H_2O (4/1)	64	97	72	93
DMF-H_2O (1 / 4)	52(52)	82 (82)	54(61)	81(81)
H_2O	52	84	56	76

[a]To a solution of Boc-Ala-OH, H-Gly-OBzl·TosOH, an additive, and Et_3N (1 mmol each) dissolved in a solvent (2.5ml), were added EDC (1 mmol) and the solvent (1 ml). The mixture was stirred for 2 h at room temperature. After addition of AcOH (1 ml), the mixture was diluted with MeOH to a known volume and an aliquot of the MeOH solution was quantitatively analyzed on a HPLC (TSK-gel ODS 80Ts, MeOH/1%$NaClO_4$, 2/1). [b]Figures in parentheses are the yields after coupling for 16 h.

Table 2. Yield of Boc-Ala-Gly-OBzl in DMF-H_2O (1/4) with use of different amounts of additives (%)[a,b]

Additive	Molar ratio of additive against Boc-Ala-OH 0.01	0.05	0.1	0.2	0.5	2
HOBt	80	94	100	90	85	78(82)
HOSu	65	90(93)	96(94)	85(89)	73(78)	37(41)
HONb	78	95	95	88	80	63(73)

[a]Conditions except amounts of additives were the same as described in Table 1. [b]Figures in parentheses are the yields after coupling for 16 h.

Table 4 shows the loss of optical purity during the segment condensation between Z-Gly-Phe-OH and H-Val-OMe[13] in DMF-H_2O (1/1) employing EDC and HOBt as the coupling reagent. The coupling using 0.1 equimolar HOBt against the C-component caused about a 3-fold excess formation of the D-isomer, Z-Gly-D-Phe-Val-OMe, in comparison with the corresponding coupling employing equimolar amount of the additive; although the coupling using 0.1 equimolar HOBt in the water-containing solvent gave Z-Gly-Phe-Val-OMe in a satisfactory chemical yield (94%) comparable to that (95%) observed in the condensation carried out in DMF employing equimolar HOBt.

The result indicating that the couplings in the aqueous media were best enhanced by the additives in less than equimolar

Table 3. Yield of Boc-Val-Ile-OBzl in DMF-H_2O (1/1) with use of different amounts of additives (%)[a,b]

	Molar ratio of additive against Boc-Val-OH						
Additive	0.02	0.05	0.1	0.2	0.5	1	2
HOBt	65 (73)	84 (96)	93 (100)	92 (99)	88 (98)	83 (92)	76 (82)
HOSu	27	38	50 (52)	58 (63)	61 (69)	38 (54)	20 (35)
HONb	32	42	57 (62)	69 (78)	74 (88)	58 (70)	47 (67)

[a] Boc-Val-OH and H-Ile-OBzl·TosOH were allowed to couple in a similar way described in Table 1. For the HPLC, MeOH/1%$NaClO_4$ (3.5/1) was used as the solvent. [b] Figures in parentheses are the yields after coupling for 16 h.

Table 4. Racemization during coupling between Z-Gly-Phe-OH and H-Val-OMe in DMF-H_2O (1/1)[a]

	Molar ratio of HOBt against Z-Gly-Phe-OH		
	0	0.1	1
Chemical yield of Z-Gly-Phe-Val-OMe (%)	44	94	74
Content of Z-Gly-D-Phe-Val-OMe (%)	18.0	2.75	0.82

[a] Couplings were carried out at 0° C for 2 h. Other conditions were silimar to those described in Table 1. After addition of AcOH, the mixture was diluted with AcOEt, and washed by 0.1 M HCl, water, 0.5 M $NaHCO_3$, and water. After removal of the solvent, the residue was diluted with MeOH to a known volume. An aliquot of the MeOH solution was quantitatively analyzed on the HPLC (MeOH/H_2O, 2/1).

amounts against the C-components or EDC may be partially explained by the fact that, since the *N*-hydroxy compounds are acidic ones, the pH values of the reaction mixtures containing different amounts of the additives differ slightly. The pH value of a mixture of Boc-Val-OH, H-Ile-OBzl·TosOH, and Et_3N (1 mmol each) in DMF-H_2O (1/1, 2.5 ml) was 6.6, while addition of 0.1 mmol HOBt to the mixture reduced the pH to 6.2, and addition of 1 mmol reduced it to 5.2. Further addition of 0.6 mmol excess Et_3N to the mixtures containing 1 equivalent HOBt increased the pH to 6.1. The coupling (2 h) of Boc-Val-OH and H-Ile-OBzl·TosOH (1 mmol each) in DMF/H_2O (1/1) containing Et_3N (1.6 mmol) with the aid of EDC and HOBt (1 mmol each) gave the dipeptide in a yield of 93%. These facts suggest that the subtle differences in the pH of the reaction mixture induced by alteration of the amounts of the additives caused alteration of the coupling yields. The alteration of the yields seems also to be ascribable to the fact that competitive hydrolysis of HOBt esters in water-containing media is accelerated by the presence of excess HOBt. A coupling (2 h) of preformed Boc-Val-OBt and H-Ile-OBzl in DMF gave Boc-Val-Ile-OBzl in a yield of 96%. The coupling carried out in DMF-H_2O (1/1) gave the dipeptide in a yield of 94%. The same coupling carried out in DMF-H_2O (1/1) in the presence of 0.5 or 1 equimolar HOBt gave the dipeptide in yields of 86% and 83%, respectively. Exposure of Boc-Val-OBt in DMF-H_2O (1/1) for 30 min prior to the addition of H-Ile-OBzl decreased the yields of the dipeptide to 79%, while the same treatment of the ester in the solvent containing 1 equimolar amount of HOBt further decreased the yield to 70%. Similar phenomena were also observed in the case of Boc-Ala-OBt, a more water-sensitive ester. However, Boc-Ala-OSu and Boc-Ala-ONb were quite stable in DMF-H_2O (1/1) and no marked decreases in the yields were observed in the similar experiments.

Insofar as was examined at this time, a combination of EDC and 0.1 equimolar HOBt was the most effective coupling reagent for peptide synthesis in aqueous solvents. Indeed care must be taken in employing the reagent in segment condensation for possibly increased racemization. However, this reagent did show the capability of giving efficient peptide-bond formation in water-abundant circumstances – which are rather unusual for peptide synthesis and have not been extensively examined. This coupling tool will permit broader selection of solvents in peptide synthesis.

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8 The following abbreviations were used herein: EDC = 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride; HOBt = 1-hydroxybenzotriazole; HONb = *N*-hydroxy-5-norbornene-2,3-dicarboximide; HOSu = *N*-hydroxy-succinimide; Boc = *t* -butoxycarbonyl; and Z = benzyloxycarbonyl.
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