A REMARKABLE CATALYTIC EFFECT OF THE POTASSIUM SALT OF 1-HYDROXYBENZOTRIAZOLE ON PEPTIDE BOND FORMATION[1]

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Abstract — A semiquantitative investigation was done on the catalytic effects of 1-hydroxybenzotriazole (HOBt) (5) and the potassium salt 6 of HOBt (5) on peptide bond formation by the active ester method. HOBt (5) was found to catalyze the aminolysis reaction of two different types of active esters, benzyloxycarbonyl-L-phenylalanine 2,4,5-trichlorophenyl ester (1) and benzyloxycarbonyl-L-phenylalanine 1-succinimidyl ester (2), in tetrahydrofuran (THF) and in a dipolar aprotic dimethylformamide (DMF) solvent. A large rate enhancement was also observed in the aminolysis reaction of 1 in THF when the potassium salt 6 of HOBt (5) was added in the presence of dicyclohexyl-18-crown-6 (7). The rate acceleration in the aminolysis reaction of the active esters by 5 and 6 may reasonably be explained in terms of nucleophilic catalysis.

Our continuing interest in the properties of N-hydroxy compounds in peptide synthesis and related reactions led us to investigate the mechanism of a catalytic effect of 1-hydroxybenzotriazole (HOBt) (5) on peptide bond formation by the active ester method, a phenomenon originally discovered by König and Geiger.[2] They observed a pronounced rate enhancement by HOBt (5) for the aminolysis of active esters in a dipolar aprotic solvent, such as dimethylformamide (DMF) and dimethylacetamide (DMA) but retardation of the reaction in a nonpolar tetrahydrofuran (THF) solvent. Although they[3] also observed an ester exchange between *p*-nitrophenyl acetate and HOBt (5) in dimethyl sulfoxide (DMSO) by the addition of N-ethylmorpholine, this was not necessarily evidence for the nucleophilic catalysis of HOBt (5) in peptide synthesis. Furthermore, their proposed mechanism,[2] as shown in Figure 1, raises the question of what roles the solvent plays in the formation of complexes, because the solvent itself is not a part of the complexes.

Rebek et al.[4] have shown by their elegantly developed three-phase test that the aminolysis reaction of polymeric active ester proceeds through nucleophilic catalysis of HOBt (5), but the reaction using polymeric

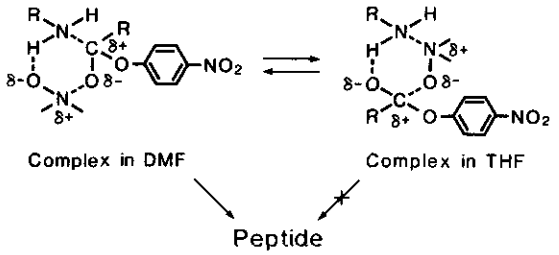

Figure 1. Mechanism proposed by König and Geiger[2]

reagents occasionally proceeds in a different manner from that using monomeric reagents.[5] In addition, the three-phase test, although a very useful technique for detecting an unstable intermediate in the elucidation of reaction mechanisms in general, does not necessarily provide the quantitative catalytic effect of HOBt (5) on peptide bond formation.

Since the finding of König and Geiger[2,3] seems interesting and very useful for peptide synthesis, we conducted semiquantitative kinetic studies on the effect of HOBt (5) on peptide bond formation according to a slightly modified procedure of König and Geiger.[3] Two kinds of active esters, such as benzyloxycarbonyl-L-phenylalanine 2,4,5-trichlorophenyl ester (1)[6] and benzyloxycarbonyl-L-phenylalanine 1-hydroxysuccinimidyl ester (2),[6] were prepared and the commercially available L-alanine *p*-nitroanilide (3)[7] and L-valine *o*-nitroanilide (4)[8] synthesized in this laboratory were used as amino components.

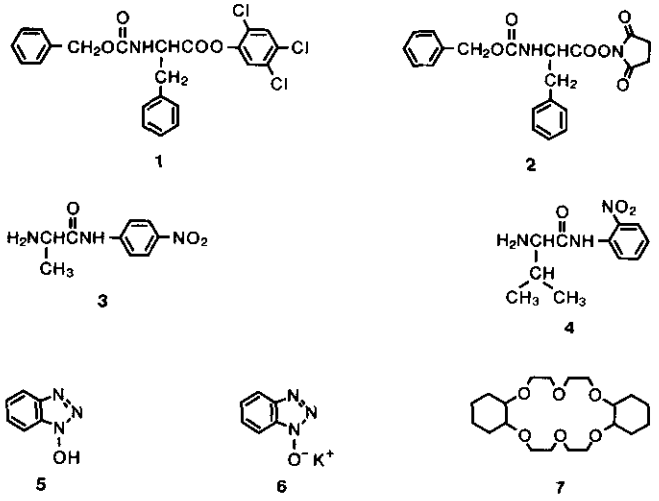

Figure 2. Structures of compounds described in the present study

Aminolysis[3,9] of trichlorophenyl ester (1) with the amine (3) in THF at 25°C was carried out under pseudo-first-order conditions with variation of the amine concentration from 20 to 50 times in excess and the results are shown in Table 1.

Table 1. Rate constants for aminolysis of 1 with 3 in THF at 25.0°C[a]

Amine (3)×10² [M]	HOBt (5)×10³ [M]	k_{obsd}×10² (min^{-1})
3.0	---	1.48
	1.7	1.87
	3.4	2.10
	5.1	2.39
	b	29.0
	c	1.60
4.5	--	2.13
6.0	--	2.81
7.5	--	4.50

[a] Initinal concentration of the ester (1) = 1.5×10^{-3} M, [b] Performed in the presence of potassium salt 6 of HOBt (5) (1.5×10^{-3} M) and dicyclohexyl-18-crown-6 (7) (6.0×10^{-3} M), [c] Performed in the presence of dicyclohexyl-18-crown-6 (7) (6.0×10^{-3} M), but without potassium salt 6 of HOBt (5)

When k_{obsd} for the reaction of 1 with 3 in the absence of HOBt (5) was plotted against the amine concentration, a deviation from the straight line at a higher amine concentration (50-fold excess) was seen as shown in Figure 3, which suggests the second-order in amine might be involved.

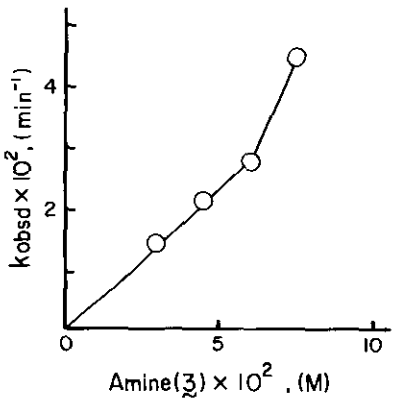

Figure 3. Plot of k_{obsd} vs. amine (3) concentration for the reaction of 1 with 3 in THF at 25.0°C

The best fit equation was obtained as shown below by the method of least squares by forcing k_{obsd} to pass the origin when the amine (3) was absent.

$$k_{obsd} = 0.316\,[Amine] + 3.47\,[Amine]^2$$

The pseudo-first-order rate constant (k_{obsd}) of aminolysis reaction in the presence of catalyst in aprotic solvents can be generally expressed by the following equation:[10]

$$k_{obsd} = k_2[Amine] + k_3[Amine]^2 + k_{cat}[Cat][Amine]$$

$$k_{obsd}/[Amine] = k_2 + k_3[Amine] + k_{cat}[Cat]$$

Under a constant amine concentration, the plot of the values of k_{obsd} divided by the amine concentration against the concentration of catalyst would afford a straight line because the first two terms, (k_2 + k_3[Amine]), should be given as an intercept.

Rate enhancements, although small, were observed by increasing the HOBt (5) concentration from 1.7 to 5.1 x 10^{-3} M when the amine concentration was kept constant at 3 x 10^{-2} M (20 molar equivalents of the ester (1)), as shown in Table 1. A plot of k_{obsd} / [Amine] as a function of HOBt (5) concentration (not shown) afforded a fairly good straight line, which gave a catalytic rate constant (k_{cat}) of 58 $M^{-2}\,min^{-1}$.

The reaction of trichlorophenyl ester (1) with an equimolar amount of the amine (3) under second-order conditions in the absence and presence of 1.13 equimolar amounts of HOBt (5) in THF at 25°C was also carried out and the time-conversion profile is shown in Figure 4. Addition of HOBt (5) brought about a small rate enhancement and did not retard the reaction rate — results which are very different from those observed by König and Geiger.[2,3]

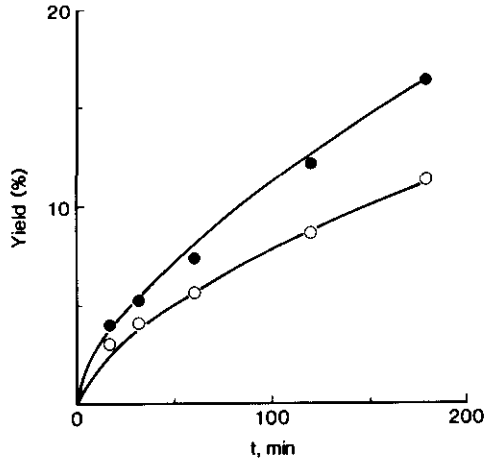

Figure 4. Time-conversion profile in the reaction of 1 with 3 in THF at 25.0°C
Initial concentration: 1 = 3 = 1.5 × 10^{-3} M
—●—: presence of 1.13 equiv. of 5, —○—: absence of 5

The reaction of 1-succinimidyl ester (2) with an equimolar amount of amine (3) in THF at 25°C was carried out but the reaction was not completed (yield: up to 67%). The time-conversion profile in the absence and presence of HOBt (5) is shown in Figure 5: the presence of 1.13 molar equivalents of HOBt (5) again increased the rate and these results sharply contrasted with those of König and Geiger.[2,3] For comparison, we conducted the reaction of 1-succinimidyl ester (2) with an equimolar amount of the same amine (4) that they used. However, the rate retardation was not observed in the presence of HOBt (5), although the rate was slower compared to the reaction of 2 with alanine *p*-nitroanilide (3), probably due to the bulkiness of the

amine (4) (Figure 5). The reason for the discrepancy between the results of König and Geiger and those of the present studies is not clear at present and closer studies are required in order to elucidate the difference. Table 2 shows the second-order rate constants for 1-succinimidyl ester (2) and the roughly estimated rates of trichlorophenyl ester (1) in the reaction of an equimolar concentration of amine (3) in DMF at 25°C. A large rate acceleration for the reaction of 1 by HOBt (5) was observed, which was similar to that observed by König and Geiger.[2,3] The dependence of the rates on the amount of HOBt (5) as seen in Table 1 suggests either bifunctional catalysis or a nucleophilic pathway[11,12] as shown in Figure 6.

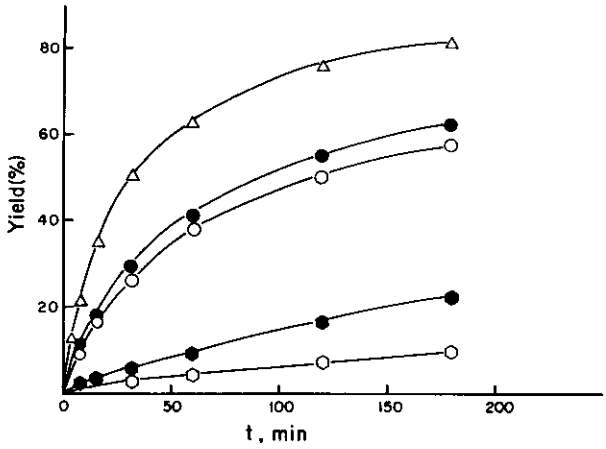

Figure 5. Time-conversion curve in the reaction of 2 with 3 or 4 at 25.0°C
Initial concentration: 2 = 3 = 1.5×10^{-3} M, 2 = 4 = 6×10^{-3} M
—△—: 3 in DMF, —●—: 3 and 1.13 equiv. of 5 in THF,
—○—: 3 in THF, —●—: 4 and 1 equiv. of 5 in THF,
—○—: 4 in THF

Table 2. Rate constants for aminolysis of 1 or 2 with 3 in DMF at 25.0°C

Ester[a]	HOBt (5)$\times10^3$ [M]	k_2[b] (M^{-1} min^{-1})
1	---	5.6 (4.1)
	1.7	~3×10^2 (~2×10^2)
2	---	26 (18)
	1.7	44 (28)

[a] Initial concentration of 1 = 2 = 3: 1.5×10^{-3} M
[b] Figures in parentheses were calculated on the assumption of 100% completion of the reaction

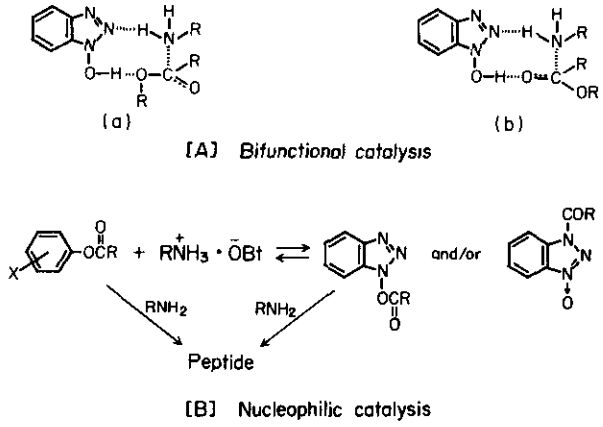

Figure 6. Two alternative possibilities of HOBt catalysis

In order to discriminate the two pathways in Figure 6, the aminolysis reaction of 1 with 3 in THF was done in the presence of potassium salt 6[13] of HOBt (5), in which no hydroxyl proton exists, that was equivalent to the amount of the ester together with a dicyclohexyl-18-crown-6 (7).[14] Here, the crown ether 7 was used *only for* the dissolution of the potassium salt 6 of HOBt (5) in THF. A large rate enhancement (ca. 20-fold rate enhancement) was observed, as shown in Table 1. Gandour et al.[15] reported that several kinds of crown ether containing 2,6-pyridine subunits linked by carbon-oxygen bonds have catalytic effects on the aminolysis reaction in chlorobenzene, but in the control experiment in the present studies, the crown ether 7 had no effect as seen in Table 1. This might be attributed to the difference in the crown ether from those of Gandour et al. or the effect, if present, *might be overshadowed by the THF solvent.* Thus, the bifunctional pathway may be eliminated.

Based on the above experiment with the potassium salt 6 of HOBt (5) and the very high reactivity of 1-hydroxybenzotriazolyl ester,[2] the nucleophilic pathway in Figure 6 [B][16] seems to be the most plausible one for DMF and THF solutions. Therefore, the more effective catalysis of HOBt (5) in DMF than in THF in the reaction of trichlorophenyl ester (1) and amine (3) as seen in Tables 1 and 2 may be ascribed to the higher concentration of the extremely reactive benzotriazolyl oxyanion ($^{-}$OBt) resulted from the *dissociation of the ion pair*[11,16] of HOBt (5) and amine (3) in such a polar DMF solvent. Studies are underway to examine the usefulness of the potassium salt 6 of HOBt (5) as a catalyst or a mediator[17] in the synthesis of more complicated peptides.

ACKNOWLEDGMENT

We are grateful to Professor R. D. Gandour of Louisiana State University for his helpful discussions.

REFERENCES AND NOTES

1. This paper was presented at the 17th Symposium on Peptide Chemistry, Tokyo, 1979.
2. W. König and R. Geiger, 'Chemistry and Biology of Peptides, Proceedings of the 3rd American Peptide Symposium,' ed. by J. Meienhofer, Ann Arbor Science Publishers Inc., Ann Arbor, Michigan, 1972, pp. 343-350.
3. W. König and R. Geiger, Chem. Ber., 1973, **106**, 3626.
4. J. Rebek, Jr., Tetrahedron, 1979, **35**, 723; J. Rebek, D. Brown, and S. Zimmerman, J. Am. Chem. Soc., 1975, **97**, 454.
5. For example, see C. Yaroslavsky, A. Patchornik, and E. Katchalski, Tetrahedron Lett., 1970, 3629; W. R. Roush, D. Feitler, and J. Rebek, Tetrahedron Lett., 1974, 1391.
6. Benzyloxycarbonyl-L-phenylalanine 2,4,5-trichlorophenyl ester (1) was prepared by allowing benzyloxycarbonyl-L-phenylalanine, 2,4,5-trichlorophenol and dicyclohexylcarbodiimide (DCC) to react together in ethyl acetate; the usual treatment gave crystals, mp 139.5-140.5°C. Anal. Calcd for $C_{23}H_{18}NO_4Cl_3$: C, 57.70; H, 3.79; N, 2.93; Cl, 22.22. Found: C, 57.89; H, 3.77; N, 2.92; Cl, 22.05. Benzyloxycarbonyl-L-phenylalanine 1-succinimidyl ester (2) was similarly prepared by condensing benzyloxycarbonyl-L-phenylalanine and 1-hydroxysuccinimide with DCC in dioxane; the usual treatment afforded crystals, mp 138-139°C. Anal. Calcd for $C_{21}H_{20}N_2O_6$: C, 63.63; H, 5.09; N, 7.07. Found: C, 63.85; H, 5.15; N, 7.09.
7. L-Alanine *p*-nitroanilide (3) was purchased from Protein Research Foundation, Minoh, Osaka and was used without further purification.
8. L-Valine *o*-nitroanilide (4) was prepared by a reported procedure[3]: mp 49-51°C, $[\alpha]^{22.0}_D$ −76.3° (c 0.984, DMA); see also K. Horiki, A. Murakami, and N. Chomei, React. Polymers, 1987, **6**, 127. [lit. values[3]: mp 74°C, $[\alpha]^{20}_D$ −75.6° (c 1, DMA)].
9. Amount of the conversion at appropriate time intervals was determined spectrophotometrically at 317 nm for the reaction of 1 or 2 with 3 and at 352 nm for the reaction of 2 with 4, respectively.
10. This rate expression is generally obtained for general base-catalyzed aminolyses of aryl esters in aprotic solvents. For a review on the mechanism of peptide coupling reactions, see K. Horiki, J. Synth. Org. Chem. Jpn., 1977, **35**, 814 and pertinent references contained therein.
11. Ion-pair formation was confirmed by isolation of the salt of 3 and 5 when the reaction of the ester (1) (1.5×10^{-3} M) with 20 molar equivalents of amine (3) was carried out in the presence of 5.1×10^{-3} M of 5. The salt (mp 207-209.5°C), precipitated after the reaction mixture was allowed to stand for a prolonged period of time, was characterized by its elemental analysis: Anal. Calcd for $C_{15}H_{16}N_6O_4$ [$C_9H_{11}N_3O_3 \cdot C_6H_5N_3O$]: C, 52.32; H, 4.68; N, 24.41. Found: C, 52.50; H, 4.59; N, 24.29.

Here we used the names of salt and ion pair for simplicity but strictly speaking, they would be in more complicated forms, especially in solutions.

12. For discussions on nucleophilic catalysis, see A. Fersht, 'Enzyme Structure and Mechanism,' W. H. Freeman and Company, Reading and San Francisco, 1977; A. J. Kirby, 'Comprehensive Chemical Kinetics, Vol. 10, Ester formation and hydrolysis, and related reactions,' ed. by C. H. Bamford and C. F. H. Tipper, Elsevier Publishing Company, Amsterdam, London, New York, 1972, pp. 57-207; M. F. Dunn and S. A. Bernhard, 'Techniques of Chemistry, Vol. 6, Investigation of rates and mechanisms of reactions,' ed. by E. S. Lewis, Part 1, A Wiley-Interscience Publication, New York, London, Sydney, Toronto, 1974, pp. 619-691; M. L. Bender, R. J. Bergeron, and M. Komiyama, 'The Bioorganic Chemistry of Enzymatic Catalysis,' A Wiley-Interscience Publication, John Wiley & Sons, New York, Chichester, Brisbane, Toronto, Singapore, 1984.

13. HOBt (**5**), obtained from Protein Research Foundation, was added to a small volume of aqueous methanolic solution of potassium carbonate of less than an equivalent of the HOBt (**5**). After carbon dioxide gas evolution had ceased, the solvent was evaporated to dryness *in vacuo* to give a crystalline solid; the solid was dissolved in hot methanol followed by addition of ether to give a pure solid **6**: Anal. Calcd for $C_6H_4N_3OK$: C, 41.60; H, 2.33; N, 24.26. Found: C, 41.80; H, 2.43; N, 24.64.

14. This is a conventional name. Perhydrodibenzo [18] crown-6 would be correct as suggested by J. Smid [Angew. Chem. Int. Ed. Engl., 1972, **11**, 112]. The compound **7** was purchased from Nakarai Chemicals Ltd., Kyoto and was used without further purification.

15. R. D. Gandour, D. A. Walker, A. Nayak, and G. R. Newkome, J. Am. Chem. Soc., 1978, **100**, 3608.

16. A general base-catalyzed mechanism by the oxyanion ($^-$OBt) produced by dissociation of either the ion pair[11] of HOBt (**5**) and an amine or the potassium salt **6** of HOBt (**5**) cannot be excluded within a limited number of experiments. But we feel that the dissociation of the ion pair seems unlikely to occur in such a nonpolar THF solvent. This will be discussed in a forthcoming paper.

17. Y. Shai, K. A. Jacobson, and A. Patchornik, J. Am. Chem. Soc., 1985, **107**, 4249.

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