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AN ATTEMPT ON ACCELERATION OF PEPTIDE SYNTHESIS USING THE ENZYME MODEL HAVING PREORGANIZED CATALYTIC GROUPS

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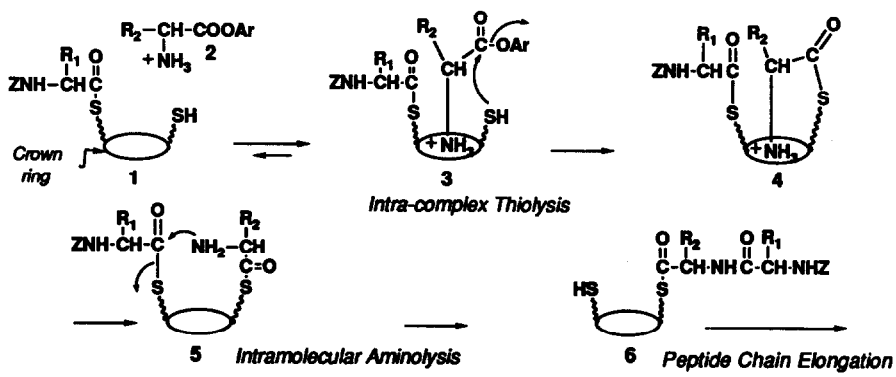
Summary: An attempt was made to accelerate intramolecular amide bond formation by introducing additional catalytic groups to the crown ether having two thiol groups as the enzyme model for the peptide synthesis. The host with methyl ester groups performed the reaction more effectively than that without them. It has been suggested that the ester groups can achieve acceleration through their preorganization in the crown host.

It has been of great interest to achieve intrinsic activity of the enzymes in artificial molecules. The multi-functionalized surface of the enzyme active site and the preorganized catalytic groups are believed to play an essential role in the catalysis.[2] During the last two decades, studies on macrocycles as enzyme models have been developed, and have realized some biomimetic reactions by mimicking the enzymatic process of capturing substrates into the active site, which is one of the most important properties of enzyme catalysis.[3] Recent interests have been focused on enzyme-mimetic catalysts with efficient turnover, especially for synthetic reactions, and additional introduction of catalytic groups on the artificial molecules has been expected as a useful method.[4]

We already developed the enzyme model for peptide synthesis using optically active crown ethers, where amino acids are bound by the crown host to form peptide bond (**Scheme 1**).[5] In order to make this method efficient enough to realize catalysis for peptide synthesis, the concept of preorganization has been applied to introducing additional catalytic groups, as well as fixing the host conformation. Here, we wish to report that the additional ester groups attached to the chiral crown ether have effected to accelerate the peptide synthesis.

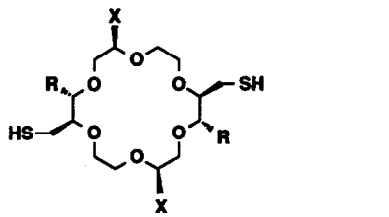
Scheme 1



The biomimetic peptide synthesis consists of the following three key steps; (i) *intra-complex thiolysis* of α-amino acid *p*-nitrophenyl ester salt forming the corresponding thioester (**1** to **4**), (ii) *intramolecular aminolysis* to form peptide bond (**4** to **6**), and (iii) *peptide chain elongation* by the repeat of the above two process. A formal turnover of the enzyme model has been demonstrated by the synthesis of a tetrapeptide derivative.[5] Although the

intra-complex thiolysis was rapid enough, the intramolecular aminolysis was not efficient enough to apply this method to effective elongation of a peptide chain. We attempted to design a new host to improve the intramolecular aminolysis.

It is reported that the aminolysis of the thioester proceeds most effectively in the presence of the acid catalyst in combination with the equimolar base catalyst, and that the macrocyclic dipolar intermediate in the aminolysis with the host 7[6], whose rate-determining proton transfer is catalyzed by the acid and the base, occurs on the crown ether surface, as shown in **Fig.1**.[5c] It also appeared that the facility in proximity between this intermediate and the catalysts affected the aminolysis rate.[7] Thus, we expected that such rate-determining proton transfer would be mediated by carboxyl-related groups attached to the crown ring in such a manner as shown by arrows in **Fig.1**.



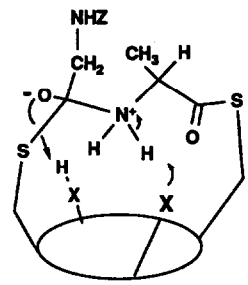
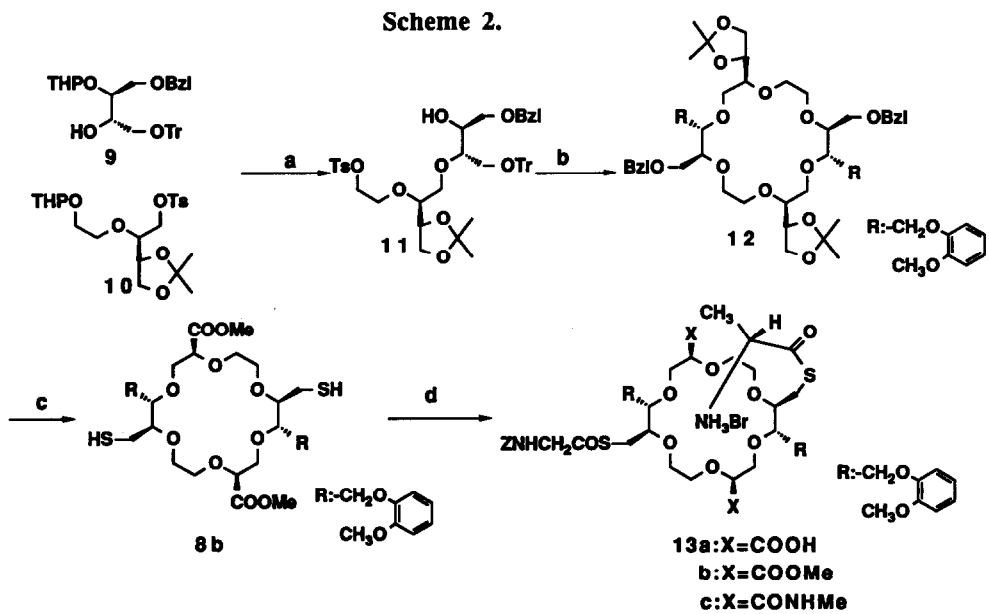


Fig 1. Expected catalysis for proton transfer of the dipolar intermediate

Scheme 2.

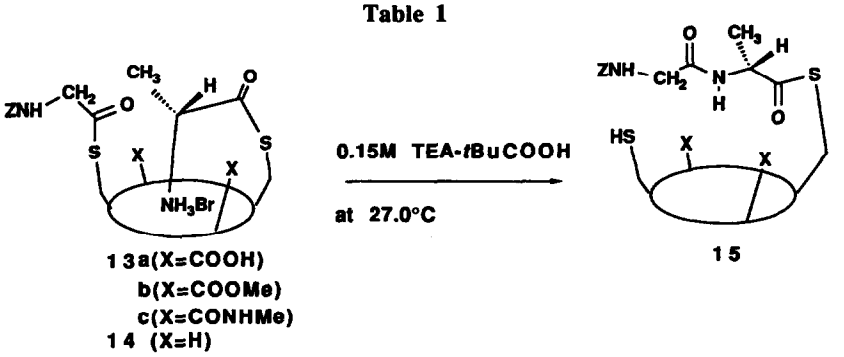


a (i) NaH, DMF; (ii) PPTS, acetone-MeOH; (iii) TsCl, iPr_2NEt, $CHCl_3$; b (i) NaH, DMF; (ii) HCl-MeOH; (iii) TsOH, acetone; (iv) TsCl, pyridine; (v) *o*-methoxyphenol, NaH, DMSO; c (i) 1N HCl, THF; (ii) $NaIO_4$, aq. $NaHCO_3$, THF; (iii) PDC, DMF; (iv) CH_2N_2, Et_2O; (v) H_2/Pd-C, MeOH; (vi) TsCl, pyridine; (vii) KSCOPh, THF, (viii) K_2CO_3, MeOH; d **13a**: (i) 1N NaOH, THF; (ii) NaH, Z-Gly-ONp (0.7eq), THF; (iii)D-Ala-ONp·HBr, TEA, CH_2Cl_2; **13b**: (i)Z-Gly-OH, DEPC, TEA, DMF; (ii) D-Ala-ONp·HBr, pyridine, CH_2Cl_2; **13c**: (i) 1N NaOH, THF; (ii) NaH, Z-Gly-ONp (2eq), THF; (iii) $MeNH_2$, DEPC, TEA, DMF; (iv) 1N NaOH (0.5eq), THF; (v) D-Ala-ONp·HBr, pyridine, CH_2Cl_2.

The introduction of three kinds of carboxyl-related functional groups (**8**, X=COOH, COOMe, CONHMe) were designed by expecting as follows: carboxyl groups would act as intramolecular acid catalysts, and ester and amide groups would form hydrogen bondings with the dipole intermediate to facilitate its proton transfer with the catalysts in the media. **Scheme 2** shows the synthetic route of the novel multi-functionalized crown ethers, starting from the optically active alcohols.[8] The crown ethers (**13**) having thioesters with *N*-carbobenzyl-oxyglycine and D-alanine units were synthesized by taking advantage of the rapid intra-complex thiolysis.

The intramolecular peptide bond formation was performed in the presence of 0.15M triethyl amine-*t*BuCOOH catalysts at 27°C, and the disappearance of the starting material was followed by TLC densitometer. The first-order rate constants thus obtained were summarized in **Table 1.**

Table 1



13a(X=COOH)
b(X=COOMe)
c(X=CONHMe)
14 (X=H)

15

entry	host (X=)[a]	solvent	k_1 (mM/min)	$t_{1/2}$ (min)
1[b]	H	benzene	12.2	57
2	H	AcOEt	4.0	174
3	H	DMF	decomposition	-
4	COOH	benzene	no reaction	-
5[b]	COOMe	benzene	20.8	34
6	CONHMe	benzene	2.0	354

a) 1mM solution was used. b) After 4h, the products were isolated and purified by silica gel column to give 60-70% yields.

It is shown that the host with carboxyl groups (**13a**) does not bring about the intramolecular reaction (entry 4), and that **13c** with carboxamide groups gives only a slow reaction rate (entry 6). The carboxyl groups might protonate the nucleophilic amine and inhibit its attack to the thioester rather than have effect on the dipole intermediate. Amide groups might form too stable hydrogen bondings with the dipole intermediate to enhance its proton transfer. However, the accelerated reaction was attained in the reaction of **13b** (X=COOMe), (entries 1 vs 5). The rate constant of 20.8 mM/min ($t_{1/2}$=34 min) is the fastest one so far obtained by us.[5]

The rate of the intramolecular aminolysis is much affected by the polarity of solvents used. That is, the more polar solvent causes the slower reaction rate (entries 1, 2, 3). As a rate determining step is the transformation of the dipolar intermediate to the neutral one,[9] it is a reasonable explanation that the polar intermediate becomes too stable in the polar solvent to be transformed further. Considering that the solvent effect of ethyl acetate is disadvantageous for the reaction (entries 1 vs 2), it is quite interesting that the methyl ester groups attached to the crown ring accelerate the reaction.[10] It seems that the ester groups form hydrogen bondings with the dipolar intermediate, which are probably less tight than those formed by the amide groups, and mediate its proton transfer

with the catalysts in the media. Although the rate enhancement by the ester groups was not very large, it has been demonstrated that its preorganized introduction to the host can realize catalytic effect.

For functional groups to exhibit efficient catalysis in the enzyme active site, they must be optimally oriented to the substrate as well as to each other, therefore, preorganized prior to the reaction. Although the mechanism of the enzyme catalysis in peptide synthesis is still unclear, the present study has demonstrated that ester group can exhibit catalysis in peptide synthesis. Optimal preorganization of catalytic groups including carboxylic acid will be the future subject.

References and Notes

(1) Present address: Faculty of Pharmaceutical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812, Japan.

(2) Alan Fersht, "*Enzyme Structure and Mechanism*", W. H. Freeman and Company, New York, 1985.

(3) Recent examples; Cyclophanes: (a) F. Diederich, H.-D. Lutter, *J. Amer. Chem. Soc*, **1989**, *111*, 8438; Cyclodextrins: (b) E. Anslyn, R. Breslow, *ibid*, **1989**, *111*, 8931; Multidentate ligands: (c) J. T. Groves, L. A. Baron, *ibid*, **1989**, *111*, 5442; (d) M. W. Hosseini, A.J. Blacker, J.-M. Lehn, *ibid*, **1990**, *112*, 3896, and references cited therein.

(4) (a) K. D. Crammer, S. C. Zimmerman, *J. Amer. Chem. Soc.*, **1990**, *112*, 3680, (b) J. Rebek, Jr., *Angew. Chem. Int. Ed. Engl.* **1990**, *29*, 245, (c) D. R. Benson, R. Valentekovich, F. Diederich, *ibid*, **1990**, *29*, 191, (d) D. J. Cram, P. Y.-S. Lam, S. P. Ho, *J. Amer. Chem. Soc*, **1986**, *108*, 839, and references cited therein.

(5) (a) S. Sasaki, M. Shionoya, K. Koga, *J. Amer. Chem. Soc*, **1985**, *107*, 3552; (b) K. Koga, S. Sasaki, *Pure and Appl. Chem.*, **1988**, *60*, 539, (c) S. Sasaki, K. Koga, *J. Inclusion Phenom. Mol. Recognit. Chem.*, **1989**, *7*, 267; (d) *idem*, *Chem. Pharm. Bull.*, **1989**, *39*, 2531.

(6) The structure of **7**, which was designed to have dithiol on the same surface of the crown and to force the bound substrates into close proximity, has been shown to be preorganized to some extent for both thiolysis and aminolysis.[5c)]

(7) For example, the rate enhancement by diisopropylethylamine as a bulky base was about half that by triethylamine as a less bulky base in the aminolysis catalyzed by pivalic acid. Unpublished result.

(8) Satisfactory analytical and/or spectroscopic data were obtained for the synthetic intermediates.

(9) William P. Jencks, "*Catalysis in Chemistry and Enzymology*", McGraw-Hill Inc., New York, 1969, Chapter 10.

(10) In the recent study, where peptide synthesis was carried out in the similar manner to our method by the use of acyclic model compounds bearing dithioester with α-amino acid derivatives, covalently-bound acid catalyst accelerated the intramolecular aminolysis by the factor of 4-8 compared to the intramolecular acid catalyst, whereas the corresponding covalently-bound ester group showed no catalytic effect; C. Gennari, F. Molinari, U. Piarulli, *Tetrahedron Lett.*, **1990**, *31*, 2929.

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