New developments in enzymatic peptide synthesis

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Abstract. This review article describes new enzymatic methods developed for the efficient and irreversible synthesis of peptides based on native and modified proteases, and for the synthesis of polypeptides containing D- and/or unnatural amino acids. Potential opportunities for future developments in the field based on new enzymes, tailor-made catalytic antibodies, and on the technique of in vitro mutagenesis are also described.
Key words. Enzymes; peptide synthesis; enzyme modification.

Synthetic peptide chemistry has been well developed for the laboratory synthesis of various sizes of peptides[7, 18, 26]. The synthesis of polypeptides greater than 100 amino acids in length based on the solid phase method[26], or on the strategy of segment condensation[18], however, often suffers from problems such as racemization, low yield, and poor solubility. The biological method based on recombinant DNA technology has been the choice for the synthesis of large protein molecules and modified analogs with altered characteristics for structure-function study. Enzymatic methods based on proteases[20] combined with chemical synthetic methods are attractive alternatives because enzymatic reactions are catalytic, regio- and stereoselective, racemization-free, and require minimal side-chain protection. Such reactions capture the maximum benefits of the molecular recognition between substrate and enzyme. Further, the reactions can be carried out in a mixture of organic solvent and water to improve the solubility of peptide segments. With these advantages, however, come the disadvantages that the amidase activity of proteases causes secondary hydrolysis of the growing peptide chain and that the substrates of proteases are generally limited to natural L-amino acids, consistent with the normal recognition properties of proteases. This article reviews new enzymatic methods which have been developed for peptide synthesis and describes possible future development in this field.

$$RCO_2^- + \overset{+}{H_3}N\text{-}R' \rightleftharpoons RCO_2H + H_2N\text{-}R' \rightleftharpoons RCOHNR' + H_2O \quad (1)$$

$$\underset{(S)}{RCO_2R'} + \underset{(E)}{\text{Enzyme}} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} [E\cdot S] \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} R\text{-}\overset{O}{\overset{\|}{C}}\text{-}E \xrightarrow[H_2O]{k_3} RCO_2H + E \quad (2)$$

$$k_4' \uparrow\downarrow k_4 \; R''\text{-}NH_2$$

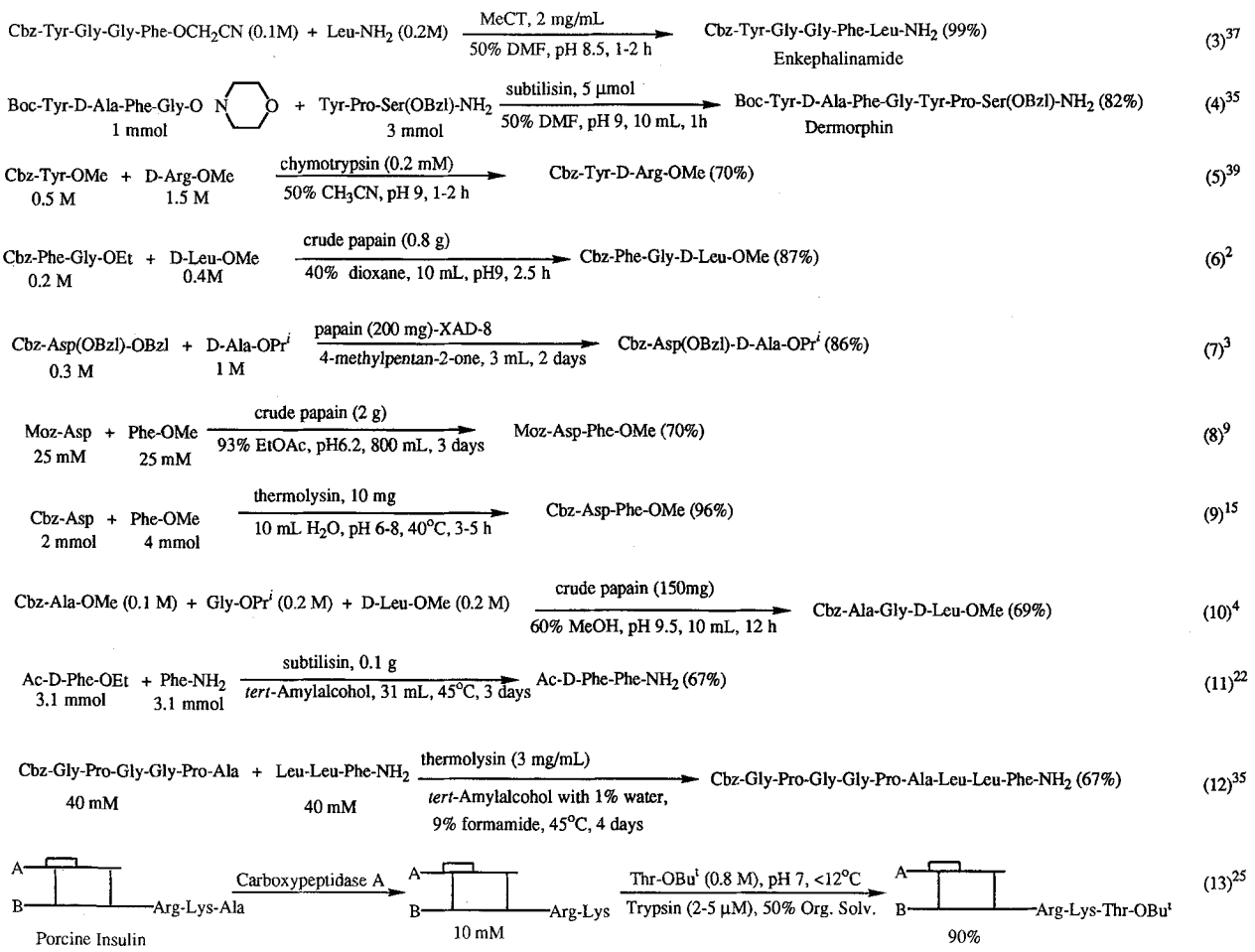
$$[\, R\text{-}\overset{O}{\overset{\|}{C}}\text{-}E\cdot H_2N\text{-}R''] \xrightarrow{k_5} RCOHNR'' + E$$

Strategies of protease-catalyzed peptide coupling

Two strategies are often used in protease-catalyzed peptide synthesis[16]: one is the direct reversal of the catalytic hydrolysis of peptides (i.e. thermodynamic approach, Eq. 1) and the other is the aminolysis of N-protected amino acid or peptide esters (i.e kinetic approach, Eq. 2). The thermodynamic approach is an endergonic process, and manipulation of reaction conditions is required to increase the product yield. The addition of water-miscible organic solvents to increase the pK value of the carboxyl component (thus increasing the substrate concentration[13]), the use of a biphasic system[24], reverse micelles[21], anhydrous media containing a minimal amount of water[30], water mimics[19], or the selection of appropriate N- or C-protecting groups to reduce the solubility of products are often employed. The kinetic approach is faster, and the product yield can be improved by manipulating the reaction conditions as used in the thermodynamic approach. Aminolysis, however, requires the use of esters as substrates and is limited to those enzymes (e.g. chymotrypsin, trypsin, papain, and subtilisin) which form an acyl intermediate[2]. The synthesis of ester substrates for enzymatic peptide coupling can be accomplished by chemical (by solid phase or solution phase) or enzymatic methods as illustrated in a recent report on subtilisin-catalyzed peptide segment coupling[35]. Examples of successful enzyme-catalyzed peptide syntheses are shown in equations 3–13.

Control of amide cleavage activity in serine protease catalyzed aminolysis

The hydrolyses of esters and amides catalyzed by serine or thiol proteases have similar mechanisms but different rate-determining steps. Formation of an acyl intermediate in amide hydrolysis is rate-determining and pH independent, while deacylation of the acyl intermediate in ester hydrolysis is the rate-determining step of general base catalysis and thus is pH dependent. The significant enhancement of esterase versus amidase activity of such proteases at higher pH has been utilized in the stepwise synthesis and fragment coupling of peptides via aminolysis, where hydrolysis of the growing polypeptide chain



was inhibited [3]. Alternatively, esterases without amidase activities (such as lipases) can be used as catalysts for aminolysis [23, 37]. The rate, however, is very slow. Serine and thiol proteases [2, 39] have been found to behave similarly as nonproteolytic esterases when water-miscible organic solvents such as dimethylformamide (DMF), dioxane, or acetonitrile are added to the aqueous enzyme solution. The different effect of water-miscible organic solvents on the esterase and amidase activities of trypsin has been known for some time [11]. The application of such a solvent effect to the kinetically controlled peptide synthesis, however, has only recently been exploited [2, 39]. Further investigation of this phenomenon by reducing the water content in a subtilisin-catalyzed aminolysis revealed that the enzymatic activity decreased with decrease in water content and became catalytically inactive in anhydrous DMF [2]. Although the enzyme is reasonably stable in anhydrous DMF, the aminolysis reaction is too slow to be useful for practical synthesis. In the presence of 50–70% DMF, the enzyme is quite stable and active for aminolysis, while the amidase activity is insignificant [2].

To further investigate the effect of organic solvents on catalysis, the kinetic parameters (k_{cat} and K_m) for chymotrypsin [35] and subtilisin [3] catalyzed hydrolysis of ester and amide substrates were determined. It was found that organic solvents affect both catalysis and binding. Both k_{cat} and k_{cat}/K_m for the amide as well as the ester hydrolysis decrease as the content of organic solvent increases. The rate of decrease for the amide hydrolysis, however, is faster than that for the ester hydrolysis. Amides are generally more tightly bound to the enzyme as the organic solvent is added to the solution except in the case of hydrophobic amides. The binding of ester substrates, however, is affected less by organic solvents (tables 1 and 2). A typical free energy diagram for chymotrypsin catalysis in an aqueous solution and in a 50% DMF solution is shown in figure 1. The transition-state energy for both the ester and amide hydrolysis increases when the organic solvent is added to the solution. The acyl-intermediate, however, is less stable in the presence of DMF compared to that in the aqueous solution. The energy barrier for the formation of an acyl-enzyme-nucleophile complex in the presence of DMF is lower, and

Table 1. Kinetic parameters for native, 50% DMF modified chymotrypsin, and methylchymotrypsin catalyzed hydrolyses of Suc-Ala-Ala-Pro-Phe-X (Where X = pNA and SBzl)[a].

	k_{cat} (s^{-1})	K_m (μM)	k_{cat}/K_m ($M^{-1}s^{-1}$)	k_2 (s^{-1})	K_S (M^{-1})
Native					
Suc-AAPF-pNA	46 ± 2	86 ± 9	5.35×10^5	76.5	1.43×10^{-4}
in 50% DMF	8.5 ± 2	270 ± 34	3.15×10^4	9.3	2.96×10^{-4}
Suc-AAPF-SBzl	116 ± 1	30 ± 2	3.92×10^6	–	–
in 50% DMF	98.2 ± 16	2280 ± 400	4.31×10^4	–	–
MeCT					
Suc-AAPF-pNA	0.020 ± 0.005	270 ± 15	75	0.0312	4.15×10^{-4}
SUC-AAPF-SBzl	0.059 ± 0.002	415 ± 75	142	–	–

k_2 is the rate constant from ES complex to the acyl intermediate and K_S is the dissociation constant of enzyme-substrate complex.

Table 2. Free energy values for native, 50% DMF modified chymotrypsin and methylchymotrypsin action on Suc-Ala-Ala-Pro-Phe-X (Where X = pNA and SBzl).

Enzyme	Relative free energies, [a] kcal/mol ΔG° (ES)	$\Delta G^{\neq}$ (k_2)	$\Delta G^{\neq}$ (k_3)	$\Delta G^{\neq}$ (k_{cat}/K_m)[b]	ΔG° (ES')
Native	2.94	14.87	14.62	16.63	2.01
in 50% DMF	3.36	16.12	14.72	19.30	4.58
MeCT	3.56	19.49	19.11	22.68	3.57

[a] All free energies calculated are relative to the energy of the free enzyme assigned 0 kcal/mol. [b] For the ester hydrolysis.

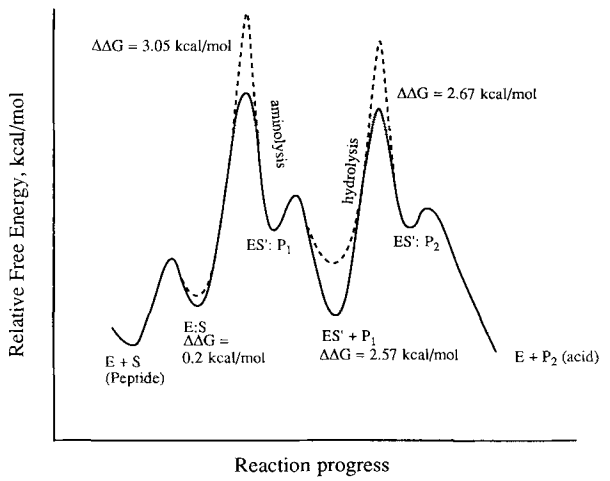


Figure 1. Free energy diagrams for the chymotrypsin reactions in aqueous (solid line) and 50% DMF solution (dashed line).

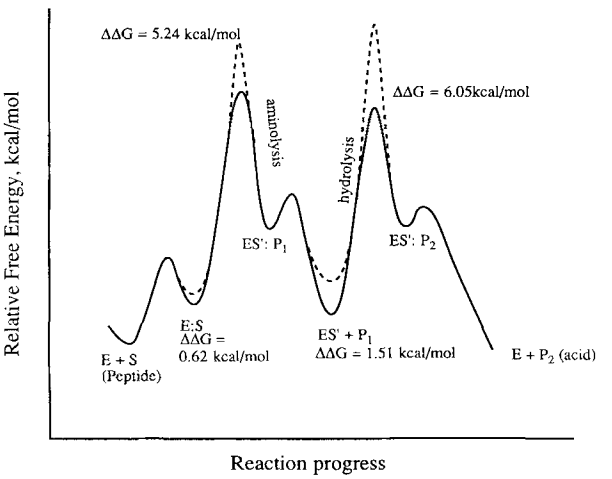


Figure 2. Free energy diagrams for the native (solid line) and methylated (dashed line) chymotrypsin reactions.

aminolysis is thereby more favorable than hydrolysis. In the direction of amide cleavage, the rate-determining energy barrier for the formation of an acyl intermediate is substantially high, accounting for the irreversible nature of the aminolysis process.

Introduction of a methyl group to the ε-2 N of the active-site His of chymotrypsin resulted in a significant change in the enzymatic catalysis[36]. The methylated enzyme (MeCT) favors aminolysis over hydrolysis (Eq. 3). The effects of methylation on the enzyme kinetics (tables 1 and 2) are very similar to that of organic solvents as indicated in the free-energy diagram (fig. 2)[35]. Previous studies on the acylation and deacylation reactions catalyzed by MeCT indicated that a functional group with $pK_a = 7$ was involved as a general base[12]. Our recent ^{13}C-NMR study indicated the presence of an O-acyl intermediate[36] in the MeCT-catalyzed ester hydrolysis. All these results suggest that the unmethylated N (δ-1) of the active site His acts as a general base for aminolysis (fig. 3). This process may require ring-flipping of the methylated imidazole, a process first suggested by Henderson[12] and later supported by studies on solvent isotope effects[8], proton inventories[34], and model systems[33]. The slight change in the orientation of the active-site groups Asp, His, Ser after methylation and ring-flipping may account for the favorable aminolysis versus hydrolysis for MeCT. Other serine proteases may be modified similarly to have new enzymatic activities

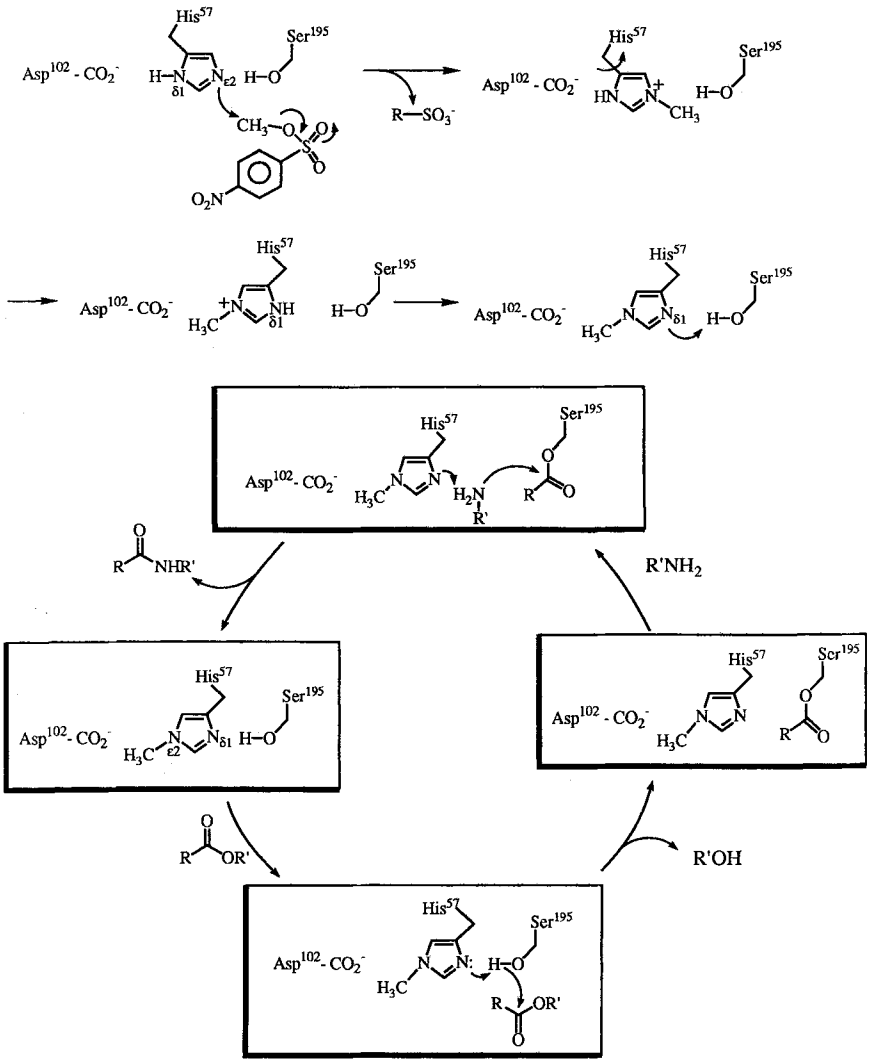
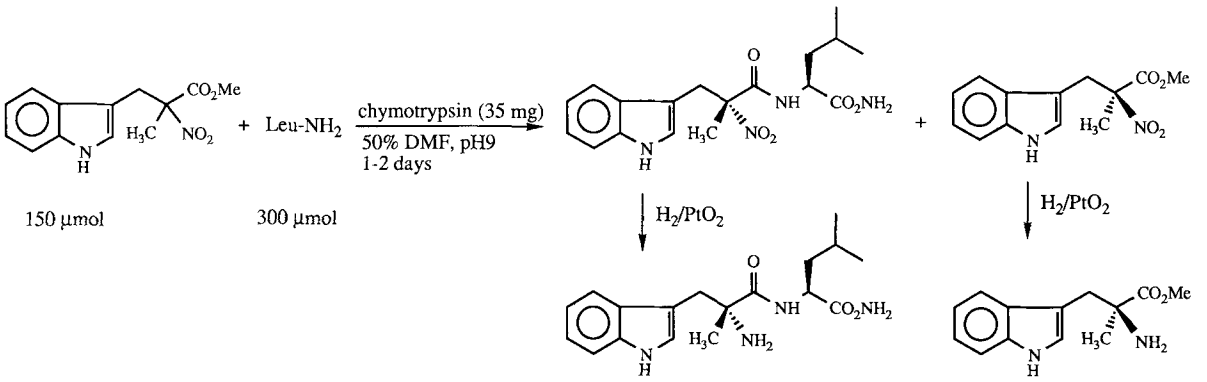


Figure 3. Mechanisms for the active-site methylation of chymotrypsin (top) and aminolysis catalyzed by the methylated enzyme (bottom).

useful for peptide segment coupling. It is worth noting that subtilisin can also be converted to an acyl transferase via modification of the active-site serine to cysteine[28] or selenocysteine[41]. An alternative approach to avoid the problem associated with the endopeptidase activity of proteases is to use exopeptidases as catalysts for peptide coupling. Carboxypeptidase Y, for example, has been successfully used in the stepwise synthesis of peptides from the N to C terminus[40]. Nonproteases involved in protein synthesis also hold potential. These enzymes require ATP or GTP to activate the carboxyl group of an amino acid, and seem to accept various amino acid nucleophiles for amide bond formation[27].

Introduction of D- and unnatural amino acids to polypeptides

In the protease-catalyzed peptide coupling, the enzyme is very specific for L-amino acids as the P1 residue (the acyl donor group). The P1′ residue (the nucleophile) is more flexible, and both L- and D-amino acids are acceptable. D-amino acids usually react about one tenth (1–5 μmol/min/mg enzyme) as fast as the L-counterparts[31,38]. Proline and other secondary amines are usually not accepted as donor or acceptor for serine proteases due to stereoelectronic effects[6]. Peptide segments containing D- or unnatural amino acids at the position remote from the P1 or P1′ site, however, can be used as substrates for enzymatic peptide segment coupling[2,39] (Eqs 5–7). Nonproteolytic enzymes such as esterases and lipases accept both D- and L-amino acid derivatives as weak acyl donors or acceptors[37]. The enantioselectivity of enzyme catalysis can be altered by solvent engineering. The enantioselectivity of subtilisin, for example, changed dramatically upon a transition from aqueous solution to anhydrous *tert*-amyl alcohol as measured by the rate ratio (V_L/V_D) of the enzyme catalyzed hydrolysis and aminolysis of N-acetyl-L- and D-phenylalanine



Scheme 1.

chloroethyl ester[22] (Eq. 11). This observation led to the incorporation of D-amino acids as the P1 residue into the N-terminal position of peptide bond. The reaction rate, however, is very slow (i.e. < 0.1 µmol/min/mg enzyme). The D-isomer selectivity of chymotrypsin in the hydrolysis of α-methyl-α-nitro carboxyl esters enabled us to develop a new strategy for the synthesis of pseudodipeptides containing D-α-methyl-α-amino acids at the N-terminal position. α-chymotrypsin-catalyzed enantioselective aminolysis of butyl 3-(3-indolyl)-2-methyl-2-nitropropionate with L-leucine amide, for example, gave a peptide which upon catalytic hydrogenation yielded α-methyl-D-tryptophanyl-L-leucine amide[35] (Scheme 1). The above methods provide satisfactory methods for the incorporation of unusual amino acids in small peptides because of the relaxed recognition requirements of subsites. However for protein synthesis, more subtle techniques are required.

A new method recently developed for the site-specific incorporation of unnatural amino acids into proteins involves a chemically acylated suppressor tRNA that inserts the amino acid into a position where the codon is substituted with a nonsense codon[29].

New enzymatic catalysts for peptide synthesis

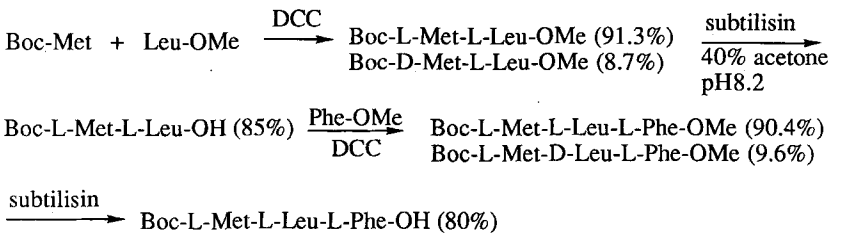
Although some proteases can be manipulated to accept D-amino acids as weak acyl donors, their utility for the synthesis of D-peptides is still quite limited. New enzymes are required for this purpose. A new thiol protease specific for D-alanine amide was recently isolated[1]. The enzyme possess both esterase and amidase activities for certain D-amino acids and has been used in the synthesis of D-alanyl dipeptides via aminolysis. Other proteases specific for the D-configuration may exist in nature, but remain to be explored. The technique of site-directed mutagenesis has so far not been able to change an enzyme specific for L-substrates to that for D-substrates. The recent development in catalytic antibodies, however, seems to be promising in this regard. Antibodies raised against a phosphonamidate hapten catalyze an aminolysis reaction to form a peptide bond[17]. The reaction was believed to proceed through a tetrahedral transition state which resembled the hapten. Phosphonate haptens were also used to elicit antibodies for bimolecular amide formation[5] and D-ester hydrolysis[32]. Major problems in the antibody catalysis that remain to be solved are to overcome product inhibition and to increase the catalytic efficiency for large-scale synthesis. It may be possible in the future to develop tailor-made catalytic antibodies for the synthesis of specific peptide bonds.

Issues of stability

As proteases and other enzymatic catalysts become useful for peptide synthesis, their stability becomes a major concern. One limitation to the usefulness of most enzymes in synthesis is their intrinsic instability in many unnatural environments required for organic reactions. Many techniques such as immobilization have been developed to improve the stability of enzymes for large-scale reactions. Another solution to this problem is to create a stable protein catalyst via amino acid substitution. More stable enzymes can be obtained via selection from thermophilic species or via site-directed mutagenesis. A subtilisin mutant derived from subtilisin BPN' via six site-specific mutations was 100 times more stable than the wild-type enzyme in aqueous solution and 50 times more stable than the wild-type in anhydrous DMF, and was applied to the synthesis of peptides, chiral amino acids and sugar derivatives[39] (Eq. 4).

Synthesis of racemization-free peptides

The high enantio- and diastereoselectivity in protease-catalyzed peptide coupling in various conditions enables us to synthesize peptide bond without racemization at the P1 position. Recently, the stereospecificity of



Scheme 2.

protease reactions has been further investigated at the P2 and P3 positions, and it has been found that subtilisin reactions in water-organic cosolvents are highly specific for L-amino acids at the P1 and P2 positions [10, 39]. It is flexible for both D- and L-amino acids at position P3 and beyond. A new strategy based on this finding has been developed for the synthesis of racemization-free peptides from the N to C terminus [10]. The procedures involve the chemical coupling of peptide bonds and subtilisin-catalyzed deprotection of the carboxyl ester. The enzymatic deprotection reaction only hydrolyzes the ester group with an L-amino acid at the P1 site; the racemized product is not hydrolyzed and can be easily separated. Repeating this procedure will therefore extend the peptide chain with no contamination of racemized products. Scheme 2 illustrates the synthesis of a tripeptide based on this strategy. Similarly, stepwise synthesis of peptides from the N to C terminus using carboxypeptidase Y [40] or from the C to N terminus using papain [3] or other proteases will provide racemization-free peptides. Eqs 3–13 illustrate examples of enzymatic peptide coupling.

Prospects

It seems clear that many biological catalysts including those from nature and from rational design will continuously be explored for peptide synthesis. The technology of recombinant DNA and site-directed mutagenesis together with the advanced instrumentation available for structure determination, molecular modeling, and design will play very important roles in the rational development of catalysts for organic reactions. The recent breakthrough in the field of catalytic antibody based on a phage vector system [14] has made possible the production of tailor-made Fab fragments without going through the hybridoma technology. As more and more new enzymatic catalysts and new biocatalytic reactions are developed, organic synthesis based on biocatalysis is expected to combine with various chemical methods to tackle the new generation of synthetic problems associated with peptides, particularly those containing unnatural amino acids and peptide mimetics and conjugates.

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1 Asano, Y., Nakazawa, A., Kato, Y., and Kondo, K., Discovery of a D-stereospecific aminopeptidase and its use as a catalyst in organic synthesis. Angew. Chem. Int. Ed. Engl. *28* (1989) 450–451.
2 Barbas, C. F. III, Matos, J. R., West, J. B., and Wong, C.-H., A search for peptide ligase: cosolvent-mediated conversion of proteases to esterases for irreversible synthesis of peptides. J. Am. chem. Soc. *110* (1988) 5162–5166.
3 Barbas, C. F. III, and Wong, C.-H., Papain catalyzed peptide synthesis: control of amidase activity and the introduction of unusual amino acids. J. chem. Soc. chem. Comm. (1987) 532–534.
4 Barbas, C. F. III, and Wong, C.-H., One-pot tripeptide syntheses from three single amino acid derivatives catalyzed by papain. Tetrahedron Lett. *29* (1988) 2907–2910.
5 Benkovic, S. J., Napper, A. D., and Lerner, R. A., Catalysis of a stereospecific bimolecular amide synthesis by an antibody. Proc. natl Acad. Sci. *85* (1988) 5355–5358.
6 Bizzozero, S. A., and Butler, H., Stereochemical aspects of peptide hydrolysis catalyzed by serine proteases of the chymotrypsin type. Bioorg. Chem. *10* (1981) 46–62.
7 Bodanszky, M., Principles of Peptide Synthesis. Springer-Verlag, Berlin 1984.
8 Byers, L. D., and Koshland, D. E. Jr, On the mechanism of action of methyl chymotrypsin. Bioorg. Chem. *7* (1978) 15–33.
9 Chen, S. T., and Wang, K. T., Papain catalyzed synthesis of aspartame precursor in biphasic system. J. org. Chem. *53* (1988) 4589–4590.
10 Chen, S.-T., Wu, S.-H., and Wang, K.-T., Diastereoselective hydrolysis of peptide esters by alkaline protease: preparation of racemization-free peptides. Int. J. Peptide Protein. Res. *37* (1991) 347–350.
11 Coletti-Previero, M.-A., Previero, A., and Zuckerkandl, E., Separation of the proteolytic and esterasic activities of trypsin by reversible structural modifications. J. molec. Biol. *39* (1969) 493–501.
12 Henderson, R., Catalytic activity of α-chymotrypsin in which histidine-57 has been methylated. Biochem. J. *124* (1971) 13–18.
13 Homandberg, G. A., Mattis, J. A., and Laskowski, M. Jr, Synthesis of peptide bonds by proteinases. Addition of organic cosolvents shifts peptide bond equilibria toward synthesis. Biochemistry *17* (1978) 5220–5227.
14 Huse, W. D., Sastry, L., Iverson, S. A., Kang, A. S., Alting-Mees, M., Burton, D. R., Benkovic, S. J., and Lerner, R. A., Generation of a large combinatorial library of the immunoglobulin repertoire in phage lambda. Science *246* (1989) 1275–1281.
15 Isowa, Y., Ohmori, M., Ichikawa, T., Mori, K., Nonaka, Y., Kihara, K. I., and Oyama, K., The thermolysin-catalyzed condensation reactions of N-substituted aspartic and glutamic acids with phenylalanine esters. Tetrahedron Lett. *20* (1979) 2611–2612.
16 Jakubke, H.-D., Kuhl, P., and Konnecke, A., Basic principles of protease-catalyzed peptide bond formation. Angew. Chem. Int. Ed. Engl. *24* (1985) 85–93.
17 Janda, K. D., Lerner, R. A., and Tramontano, A., Antibody catalysis of bimolecular amide formation. J. Am. chem. Soc. *110* (1988) 4835–4837.
18 Kaiser, E. T., Mihara, H., Laforet, G. A., Kelly, J. W., Walters, L., Findeis, M. A., and Sasaki, T., Peptide and protein synthesis by segment synthesis-condensation. Science *243* (1989) 187–192.
19 Kitaguchi, H., and Klibanov, A. M., Enzymatic peptide synthesis via segment condensation in the presence of water mimics. J. Am. chem. Soc. *111* (1989) 9272–9273.
20 Kullmann, W., Enzymatic peptide synthesis. CRC Press, 1987.
21 Luthi, P., and Luisi, P. L., Enzymatic synthesis of hydrocarbon-soluble peptides with reverse micelles. J. Am. chem. Soc. *106* (1984) 7285–7286.

22 Margolin, A. L., Tai, D.-F., and Klibanov, A. M., Incorporation of D-amino acids into peptides via enzymatic condensation in organic solvents. J. Am. chem. Soc. *109* (1987) 7885–7887.
23 Margolin, A. L., and Klibanov, A. M., Peptide synthesis catalyzed by lipases in anhydrous organic solvents. J. Am. chem. Soc. *109* (1987) 3802–3804.
24 Martinek, K., Semenov, A., and Berezin, I. V., Enzymatic synthesis in biphasic aqueous-organic systems. Biochim. biophys. Acta *658* (1981) 76–89.
25 Morihara, K., Using proteases in peptide synthesis. TIBTECH *5* (1987) 164–170.
26 Merrifield, R. B., Solid phase synthesis. Angew. Chem. Int. Ed. Engl. *24* (1985) 799-810.
27 Nakajima, H., Kitabatake, S., Tsurutani, R., Yamamoto, K., Tomioka, I., and Imahori, K., Peptide synthesis catalzyed by aminoacyl-tRNA synthetases from Bacillus stearothermophilus. Int. J. Pept. Prot. Res. *28* (1986) 179–185.
28 Nakatsuka, T., Sasaki, T., and Kaiser, E. T., Peptide segment coupling catalyzed by the semisynthetic enzyme subtilisin. J. Am. chem. Soc. *109* (1987) 3808–3810.
29 Noren, C. J., Anthony-Cahill, S. J., Griffith, M. C., and Schultz, P. G., A general method for site-specific incorporation of unnatural amino acids into proteins. Science *244* (1989) 182–188.
30 Oyama, K., Nishimura, S., Sonaka, Y., Kihara, K., and Hashimoto, T., Synthesis of an aspartame precursor by immobilized thermolysin in an organic solvent. J. org. Chem. *46* (1981) 5242–5244.
31 Petkov, D. D., and Stoineva, I. B., Enzyme peptide synthesis by an iterative procedure in a nucleophile pool. Tetrahedron Lett. *25* (1984) 3751–3754.
32 Pollack, S. J., Hsiun, P., and Schultz, P. G., Stereospecific hydrolysis of alkyl esters by antibodies. J. Am. chem. Soc. *111* (1989) 5961–5962.
33 Rebek, J. Jr, Recognition and catalysis using molecular clefts. Chemtracts – Organic chemistry *2* (1989) 337–352.
34 Scholten, J. D., Hogg, J. L., and Raushel, F. M., Methyl chymotrypsin catalyzed hydrolyses of specific substrate esters indicate multiple proton catalysis is possible with a modified charge relay triad. J. Am. chem. Soc. *110* (1988) 8246–8247.
35 West, J. B., Hennen, W. J., Lalonde, J. L., Bibbs, J. A., Zhong, Z., Meyer, E. F., and Wong, C.-H., Enzymes as synthetic catalysts: mechanistic and active-site considerations of natural and modified chymotrypsin. J. Am. chem. Soc. *112* (1990) 5314–5320.
36 West, J. B., Scholten, J., Stolowich, N. J., Hogg, J. L., Scott, A. I., and Wong, C.-H., Modification of proteases to esterases for peptide synthesis: methylchymotrypsin. J. Am. chem. Soc. *110* (1988) 3709–3710.
37 West, J. B., and Wong, C.-H., Use of nonproteases in peptide synthesis. Tetrahedron Lett. *28* (1987) 1629–1632.
38 West, J. B., and Wong, C.-H., Enzyme-catalyzed irreversible formation of peptides containing D-amino acids. J. org. Chem. *51* (1986) 2728–2735.
39 Wong, C.-H., Chen, S.-T., Hennen, W. J., Bibbs, J. A., Wang, Y.-F., Liu, J. L.-C., Pantoliano, M. W., Whitlow, M., and Bryan, P. N., Enzymes in organic synthesis: use of subtilisin and a highly stable mutant derived from multiple site-specific mutations. J. Am. chem. Soc. *112* (1990) 945–953.
40 Widmer, F., Breddam, K., and Johansen, J. T., Carboxypeptidase Y catalyzed peptide synthesis using amino acid alkyl esters as amine components. Carlsberg Res. Commun. *45* (1980) 453–463.
41 Wu, Z.-P., and Hilvert, D., Conversion of a protease into an acyl transferase: selenosubtilisin. J. Am. chem. Soc. *111* (1989) 4513–4514.

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Molecular recognition in antibodies and its application

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Abstract. The structure and function of immunoglobulins, and the nature of the antibody – antigen interaction are described. Applications of the molecular recognition properties of antibodies are discussed in the areas of immunotherapy, immunoassay, immunotargeting and catalytic antibodies.
Key words. Immunoglobulin; monoclonal antibody; antigen; immunotargeting; cytotoxic agent; immunotoxin; immunoassay; enzyme-linked-immunosorbent-assay (ELISA); catalytic antibody; hapten; conjugate.

Introduction

Ever since Kohler and Milstein [39, 41] developed the cell hybridisation method for producing continuous cell lines secreting a homogeneous (monoclonal) antibody of predefined specificity, there has been a rapid growth in the knowledge and understanding of immunoglobulin and antibody structure, function and production at the molecular level. This has arisen mainly through contributions from X-ray crystallography, NMR, genetic engineering and immunochemical studies, and there is no doubt that the area will continue to develop and expand during the 1990s. As a consequence, society will shortly begin to reap the benefits of newly developed antibody technologies, particularly through the areas of immunotherapy, catalytic antibodies, antibody-targeting and immunodiagnostics.

Immunoglobulin structure and function

Antibody production is induced in vivo when the hosts' lymphoid system comes into contact with a foreign substance, micro-organism, or other infectious agent. Antibodies bind specifically to the antigen that induced their synthesis whereas the term immunoglobulin is used to designate molecules having the same physical characteristics as antibodies but where their antigen specificity is unknown.

In the early part of the 20th century it was widely accepted that the antigen must instruct the specificity of the antibody by providing a template. However, the discovery by Sanger and Thompson [64] in 1953 that proteins had predefined sequences, and the realisation that the primary amino acid sequence was sufficient to specify all the biological activity of a protein [2] led to the demise of this