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REVIEW

Use of Enzymes in Peptide Synthesis

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Abstract

Recent experiments in several laboratories have emphasized the benefits of proteolytic enzymes as effective catalysts for the formation of peptide bonds for synthesis and semisynthesis. This review summarizes successful applications in both stepwise synthesis for small peptides and fragment condensation to produce large polypeptides and proteins.

Index Entries: Peptide synthesis; proteases; peptidases; stepwise synthesis; fragment condensation; processing of intermediates; semisynthesis; recombinant DNA; immobilized enzymes.

Introduction

Peptide synthesis has become an approach applicable to the solution of many problems in the chemical and biological sciences. Its use spans such needs as the design of analogs of biologically active small peptides including hormones and neuropeptides, the production of peptide substrates for proteases and kinases, the synthesis of short sequences of viral proteins as immunogens for production of site-specific antibodies, and preparation of subtly modified protein analogs for studying

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the role of sequence in promoting the native conformation and function of species including enzymes, antibodies, heme proteins, and protein hormones. The methods of peptide synthesis most developed and widely used at present for constructing polypeptides are largely chemical [cf ref. (*1*) for several recent reviews]. These involve selective protection of side chain and specific α-amino or α-carboxyl groups of amino acid or peptide components, chemical coupling of an available set of α-carboxyl and α-amino groups to effect synthesis of the desired peptide bond, selective deprotection of groups to participate in further coupling reactions, and ultimate deprotection of final polypeptides of desired sequence. Both solution-phase and solid-phase methods devised to incorporate these basic steps have allowed an expanding symphony of syntheses to be accomplished. Yet, major problems systematically occur to restrict the successful completion of many syntheses. The need for protection of the many potentially reactive groups in peptide intermediates can lead to limited solubility of these species, ineffective coupling between these, and ultimately reduced yield of desired final polypeptides. Such problems of yield become most serious with larger protected polypeptide intermediates, a constraint that impedes total chemical synthesis of polypeptide chains of greater than 75–100 residues. Solid-phase synthesis (*2*) at least formally removes the problem of large-fragment coupling, but replaces it with less visible problems of coupling amino acids or peptides to large protected peptides on a solid phase and also with the thorny problem of purification at the end of the synthesis of desired finished polypeptide from a complex mixture of side-products of closely related but variant amino acid sequences. As a means of avoiding chemical synthesis of large polypeptides while still allowing the controlled modification of protein sequences afforded by synthesis, semisynthesis has been used (*3*). This involves chemical synthesis of limited segments of a polypeptide chain and subsequent reconstitution of the synthetic segment(s) with complementary fragments obtained from the native polypeptide to yield a functioning macromolecule. Yet the use of semisynthesis is restricted to cases for which one can achieve both favorable limited fragmentation of the native proteins and productive noncovalent or covalent reconstitution of synthetic and native pieces. What has become clear from the present usage of synthesis and semisynthesis is that, although successful in many cases, the general methodology is in need of innovations that will enhance its overall applicability and accomplishment.

Recent experiments in several laboratories have signaled the emergence of proteolytic enzymes as effective tools offering several advantages over chemical methods for the formation of peptide bonds for synthesis and semisynthesis. The capacity of proteases to effect peptide bond synthesis stereospecifically and without need of side chain protection was recognized early in the study of these enzymes as a natural result of their catalytic nature (*4*). And several specifically constructed small peptides, as well as nonspecific polymerizations to larger polypeptides or plasteins, were demonstrated. Although these early successes accrued largely from their being driven by precipitation of synthesized products, a more controlled type of protease-catalyzed peptide bond formation was observed in the ability of carboxypeptidases A and B, trypsin, and chymotrypsin to reform specific peptide bonds in trypsin inhibitors (*5*, *6*). To be sure, specific bond refor-

mation of the internal peptide bond of the inhibitor after it was cleaved by protease proceeded with significant yield largely because the restitched product was trapped by association of the protease itself. But, while this still represented a specialized case, the protease-inhibitor studies pointed out the benefits of sequence specificity, stereospecificity, and lack of need of side chain protection in protease-assisted peptide bond formation. Therein, they provided an important stimulus for the search and study, over the past few years, of more general ways to effect synthesis with proteases. The methods that have been developed as a result fall into two broad categories, defined in Fig. 1, namely, reversal of peptide bond hydrolysis and aminolysis of carboxylic acid alkyl esters. It is the purpose of this short review to summarize the successful use of these processes in peptide bond formation in both stepwise synthesis of small peptides and the fragment condensation of peptides to produce larger species, including proteins. The benefits of enzymatic coupling specificity without side chain protection, the expanded need of proteases with defined sequence recognition, and the limitations of enzymatic synthesis are dis-

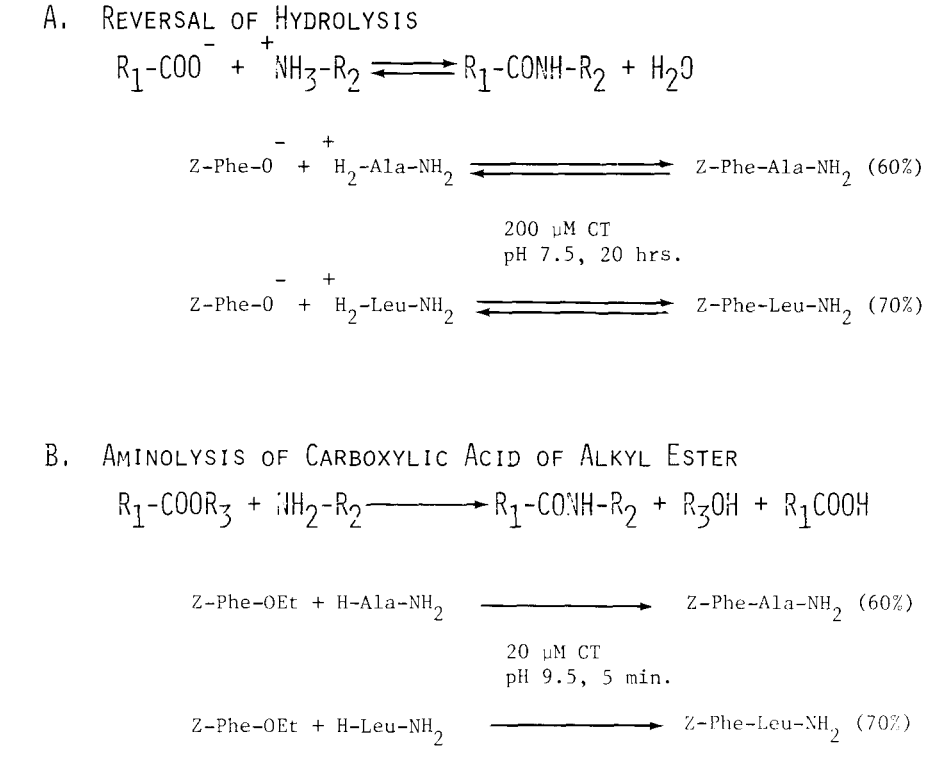

Fig. 1. Two major routes of enzymatic peptide synthesis. To date, route A has been most dominant in enzymatic fragment condensation, while route B has been most advantageously used in stepwise enzymatic synthesis. Examples show chymotrypsin-assisted formation of *N*-protected dipeptide amides using amino acide amides as amine component and either acyl amino acid (case A) or acyl amino acid ethyl ester (case B) as carboxyl component; stated coupling conditions refer to both examples of each set. Product yields are given, as percent, in parentheses. Z designates benzyloxycarbonyl; OEt, ethyl ester; CT, chymotrypsin. Data in examples are taken from Oka and Morihara (*11, 12*).

cussed. The uses of enzymes in other aspects of peptide synthesis also are summarized.

Stepwise Enzymatic Synthesis

Reversal of proteolytic reactions and various protease-catalyzed transacylation reactions for stepwise enzymatic peptide bond formation have been considered and exploited since 1937, when Bergmann, Fruton, and Fraenkel-Conrat demonstrated that papain and chymotrypsin catalyzed formation of an amide bond between acylamino acids and amino acid amides (*7, 8*). However, only over the past five years has interest in such reactions shifted from a mainly mechanistic one to one of using these reactions as a practical method of preparative peptide synthesis. Fruton (*4*) has extensively traced this historical development up to recent studies, while Morihara (*9*) and Brtnik and Jost (*10*) have presented reviews emphasizing recent work on enzymatic peptide synthesis.

The two most feasible approaches for stepwise synthesis are those in Fig. 1. Energetically, these two approaches are fundamentally different, a fact clearly manifested by the vastly different amounts of enzyme and time required for conversion of substrates to products. In reactions as those in Fig. 1A, the enzyme is used to speed up the attainment of equilibrium, which, however, lies far to the side of educts. Significant product yields therefore can be obtained only if the equilibrium is somehow altered to favor products. This was shown to be possible, for example, by (1) the presence of high concentrations of solvents such as glycol and glycerol (*13*); (2) constant removal of product from the reaction mixture by precipitation when the solubility of the product is below the equilibrium concentration; and (3) working in a biphasic organic/aqueous mixture where the product is much more soluble in the organic solvent and thus is continuously being removed from the equilibrium (*14*). Molecular traps, reaction-inert molecules that can chelate products, represent another vehicle for effecting synthesis by continual removal of product from equilibrium.

In reactions of the type exemplified in Fig. 1B, the enzyme rapidly reacts with an ester substrate to form the acyl–enzyme intermediate that reacts, in competition with water, with an amino acid-derived nucleophile to form a new peptide bond. When such aminolysis reactions are carried out at pH 8.5 and higher, the peptide products are usually quite stable, since at such high pH values the proteolytic activity of most proteases is low while the esterase activity remains high. This is of great advantage for preparative synthetic purposes since it allows for complete conversion of substrate before the product undergoes further reactions itself. Apart from this pH effect, the addition of certain solvents (*15*) also may be used to suppress proteolytic activity while leaving esterase activity unaffected. Peptide bond formation with esters as substrates thus has the clear advantages of proceeding at high speed (thus necessitating only low enzyme concentration) and being completely independent of product solubility.

The nature of the enzymes used with esters as substrates is limited to serine and thiol proteases, thus obviating the use of a large number of cheap metalloenzymes

of unique and useful specificity. However, the latter enzymes, together with serine and thiol proteases, can be used for peptide bond formation by reversal of hydrolysis (Fig. 1A). Examples of the use of various proteases in stepwise synthesis are given for aspartame, enkephalin, caerulein, and angiotensin in Table 1.

In addition to the peptide syntheses in Table 1, the potential usefulness of many proteases in peptide synthesis has been examined extensively in several model studies. For example, papain was used for synthesis of a wide range of protected di-, tri-, and tetrapeptides (*25*). Further, the same enzymes described for the angiotensin synthesis, together with several other ones of bacterial and fungal origin, were used by Isowa's group for preparation of small peptides (*26–28*). Morihara's group continues to investigate systematically the potential preparative use of all easily accessible proteolytic enzymes; detailed reports on thermolysin, subtilisin, papain, pepsin, chymotrypsin, and trypsin have been published (*11, 12, 29–32*).

Using the enzymes in immobilized form obviously would be a great advantage. This necessitates, however, that all reactants remain chiefly in solution, a condition that often requires working in mixed aqueous–organic systems (here for the sole purpose of solubility rather than for product formation by removal of product from equilibrium, as discussed earlier). A systematic study of the effect of various cosolvents and also of enzyme immobilization on the course of protease-assisted peptide synthesis was carried out by Jakubke's group (*33–35*). These studies also introduced the application of a novel thermophilic serine endoprotease (*36*).

Taking advantage of the stereospecificity and chemical simplicity of enzymatic peptide bond formation, pepsin and chymotrypsin have been used successfully in the preparation of chemically and chirally pure tryptophan-containing peptides used for spectroscopic and structural studies (*37–39*). In these experiments it also was demonstrated to be possible to obtain optically pure products from racemic substrates. This represents a notable advantage of the enzymatic method.

In spite of some difficulties in controlling transpeptidation reactions when using papain (*20, 40–42*), it is clear that endoproteases are very useful tools as catalysts for fragment coupling (see below) and for oligomerization of certain amino acids (*43*). However, for a general stepwise method of enzymatic peptide synthesis, one nonspecific or several specific serine-type exopeptidases could be used to great advantage. Although several serine-type carboxypeptidases are described in the literature (*44*), the carboxypeptidase from baker's yeast, carboxypeptidase Y (*45, 46*), is the only one available in large quantities and also stable enough towards solvents, salts, and extremes of pH to make it suitable as a reagent in preparative synthetic chemistry. The potential of this enzyme for stepwise synthesis was demonstrated by Widmer and Johansen (*47*). Its use since then has been explored systematically for this purpose (*21, 48–50*) as well as for amino acid ester oligomerization (*22*) and semisynthesis (*51, 52*). It has been applied to the total enzymatic synthesis of methionine enkephalin (*21, 22*).

Enzymatic methodology still is very much in its infancy and not yet as versatile as the classical, well-established methods. Yet, it is quite evident that enzymatic peptide synthesis is a method of significant potential and likely will be developed further with the advent of new synthetic objectives, such as synthetic vaccines (*53,*

TABLE 1
Peptides Synthesized by Stepwise Methods Using Various Proteases

Name and structure of peptide[a]		Enzymes used	Method of coupling[b]	Ref.
Aspartame	Asp-Phe-OMe	Thermolysin	A	*16–18*
Leu- and Met-enkephalin	Tyr-Gly-Gly-Phe-Leu Tyr-Gly-Gly-Phe-Met	Papain, chymotrypsin	A,B	*19,20*
Met-enkephalin	Tyr-Gly-Gly-Phe-Met	Carboxypeptidase Y, trypsin	B	*21,22*
Caerulein	Pyr-Gln-Asp-Tyr(SO_3H)-Thr-Gly-Trp-Met-Asp-Phe-NH_2	Thermolysin, papain, prolisin, subtilisin BPN, pepsin, chymotrypsin, acid proteinase No. 306	A,B	*23*
Angiotensin	Asn-Arg-Val-Tyr-Val-His-Pro-Phe-	Papain, subtilisin BPN′, microbial metalloenzyme	A	*24*

[a]In the synthesis of the last two peptides, not every peptide bond was formed enzymically; the two enkephalin syntheses, however, were totally enzymatic.

[b]Method A refers to the condensation reactions corresponding to a strict reversal of hydrolysis (Fig. 1A), while method B refers to reactions where the acyl component was used in the form of a carboxylic acid alkyl ester (Fig. 1B). "A,B" denotes use of both methods in combination.

54) and the consequent need for economically and ecologically sound methods to produce chemically and chirally pure peptides. Significant advances in the scope and versatility of enzymatic methodology can be expected, for example, from fully exploiting and manipulating the subsite specificity of the currently used enzymes (*55*). Furthermore, the collection of useful enzymes might be supplemented by suitably modified enzymes to obtain tailor-made catalysts for specific purposes. In other cases, the simple addition of a modifier molecule might suffice to obtain a desired effect on enzyme specificity. For example, it has been described that the addition of alkyl amines increases the activity of trypsin for esters of amino acid residues such as glycine, alanine, and valine (*56*). This observation is now exploited for synthetic purposes (*57*) and should considerably widen the scope of trypsin as a catalyst in peptide syntheses.

Enzymatic Fragment Condensation

The use of proteolytic enzymes for coupling synthetic as well as native protein fragments has gained renewed interest in recent years. The condensations reported to date can be classified into two general cases (Fig. 2). One is for fragments (often derived by limited proteolysis) that form a uniquely defined noncovalent complex in which the site of resynthesis is accessible to the proteolytic enzyme (Fig. 2A). The second case is for fragments that do not form a defined complex (Fig. 2B). In the former case, because of the stability of the complex formed, notable condensa-

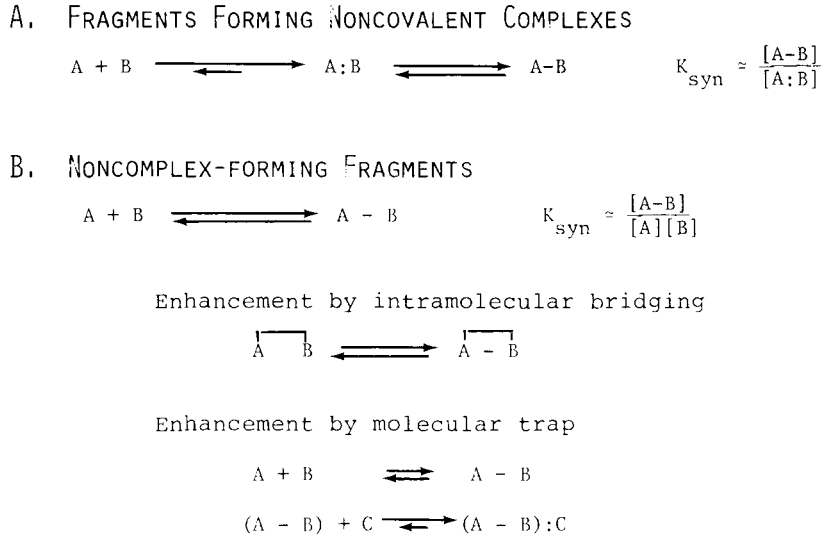

Fig. 2. Routes for enzymatic fragment condensation. Reactants A and B denote fragments to be condensed, A-B is the condensation product, A:B is a uniquely defined noncovalent complex between A and B, E is protease, and C is a molecule which binds specifically to the condensation product. K_{syn} defines the equilibrium constant for synthesis.

tion yields can be obtained at significantly lower fragment concentrations than in the latter case. Because of the existence of the stable noncovalent complex, condensation in the former case operationally appears to be an intramolecular reaction. To drive these systems toward synthesis, one can, aside from increasing the concentration of fragments, either remove the product from the equilibrium or change the equilibrium constant to favor synthesis. This latter can be accomplished by introducing an organic cosolvent into the reaction mixture. The effects of organic cosolvent on the equilibrium constant for synthesis can be recognized from a consideration of Fig. 1A. A cosolvent of low dielectric constant will increase the free energy of ionization of α-amino and α-carboxyl groups, resulting in a shift of the equilibrium of peptide bond formation toward synthesis. Homandberg et al. (*13*) investigated the effect of various cosolvents on enzymatic peptide bond hydrolysis and synthesis. They found that, taking the synthesis of N^{α}-benzyloxycarbonyl-Trp-Gly-NH_2 by chymotrypsin as a model, K_{syn} is 0.45 M^{-1} in water vs 2.5 and 38 M^{-1}, respectively, in 90% glycerol and in 85% (v/v) 1.4-butanediol. In terms of percent condensed product, 1,4-butanediol effects a greater synthesis than does 90% glycerol. However, since 1,4-butanediol has a greater tendency than glycerol to destabilize noncovalent complexes, enzymatic condensations of noncovalent fragment complexes such as ribonuclease S (*58, 59*), staphylococcal nuclease-T (59–61), cytochrome *c* (59, 62), and human somatotropin (63) (see Table 2) have utilized glycerol as cosolvent.

Although condensations of fragments locked in a stable noncovalent complex are advantageous, fragment condensations of the type in Fig. 2B represent the synthetic demand encountered most often. A successful example of this latter class is the enzymatic conversion of porcine to human insulin originally reported by Inouye et al. (*65*) (see Table 2). More recently, Leu B24 and Leu B25 analogs of human insulin have been prepared following similar methods (*66, 67*). The latter analogs have provided exciting information regarding the biological signaling mechanism of insulin. It is worth noting that recent work on insulin semisynthesis is carried out chiefly by the enzymatic method, reflecting in large part the advantages of this versus chemical methods. Nonetheless, since the condensation system here is the type in Fig. 2B, both high concentration of fragments and addition of cosolvent are needed to effect noticeable condensation yields. Typically, concentrations in the range of millimolar to molar are employed. Thus, the successes achieved in insulin semisynthesis notwithstanding, the need for rather high substrate concentration reflects important limiting factors of substrate cost and solubility for this and similar protease-catalyzed condensations.

To circumvent the above limitations for noncomplex-forming fragments, two alternatives have been explored. One approach is to convert an intermolecular condensation into one that is intramolecular by crosslinking the two fragment substrates (Fig. 2B). Chu et al. (*68*) reported a successful tryptic condensation of Arg B22 of desoctapeptide-insulin and Gly B23 of (B23-B30) in a molecule in which the α-amino group of glycine A1 is crosslinked to the ϵ-amino group of lysine B29 with the carbonyl-bis-methionyl group (*72*). After condensation, the crosslinking group was removed by reaction with cyanogen bromide. This case in fact is somewhat analogous to the manipulations used for reformation of the trypsin inhibitor

TABLE 2
Examples of Enzymatic Fragment Condensation for Preparing Biologically Active Peptides and Proteins

General case	Protein or peptide	Fragments condensed yielding active species	Enzyme used; solvent	Yield, %	References for process shown (for related processes)
A: Fragments forming noncovalent complexes	Bovine pancreatic ribonuclease	(1-20) + (21-124)	Subtilisin BPN′; 90% glycerol	50	*58* (*59*)
	Staphylococcal nuclease	[Gly 48](6-49) + (50-149)	Trypsin; 90% glycerol	20	*61* (*59, 60*)
	Horse heart cytochrome *c*	(1-38,heme)+(39-104)	Clostripain; 80% glycerol	25–30	*62* (*59, 64*)
	Human somatotropin	(1-134)+(135-191)	Thrombin; 90% glycerol	20	*63*
B: Noncomplex-forming fragments	Human insulin	[des B23-B30] insulin + (B23-B30)	Trypsin; 50% dimethyl-formamide	58	*65* (*52,66–70*)
	Ribonuclease *S*-peptide	(1-10)+(11-15) (product trapped by RNase-S-(21-124)	Clostripain; water	15	*71*

internal peptide bond by trypsin (*5*, *6*, *73*) where the fragments are held together by native disulfide bonds.

A second and more general approach developed to enhance condensation of noncomplex-forming fragments utilizes a molecular trap. The latter removes the product from the chemical equilibrium, as portrayed in Fig. 2B. Here the unfavorable synthesis reaction is coupled with a second, more favorable binding reaction. In such a condition, C takes the role of a molecular trap for the product (A-B). A successful execution of this scheme has been reported (*71*) for clostripain-catalyzed condensation of ribonuclease fragments (1–10) and (11–15). Ribonuclease-S-(21–124) was added to the reaction mixture as a trap for ribonuclease (1–15). Although only 0.03% of (1–15) subfragments was expected to be condensed in water at 1 m*M* concentration of substrates, a yield of up to 15% was achieved in the presence of the trap.

Use of Enzyme for Other Manipulations in Peptide Synthesis

In addition to the use of enzymes for catalyzing peptide bond formation, enzymes that hydrolyze amide bonds have been used for other manipulations of synthetic peptides and intermediates. Such reactions include enzymatic removal of protecting groups, site-specific cleavage for processing proteins produced by recombinant DNA methods, and synthesis of esters and hydrazide intermediates.

Enzymatic Processing of Intermediates and Removal of Protecting Groups

The first direct use of enzymes for manipulating synthetic peptide intermediates was in the synthesis of leucyl-leucine, for which Holley (*74*) prepared benzoyl-phenylalanyl-leucyl-leucine and digested it with chymotrypsin to remove benzoyl-phenylalanine. Similarly, benzyloxycarbonyl-arginine was introduced as a trypsin-removable α-amino protecting group (*75*, *76*). And more recently, in the enzymatic synthesis of enkephalin by Widmer et al. (*21*), benzoyl-arginine was incorporated chemically at the *N*-terminal end of methionine enkephalin during synthesis and subsequently removed by trypsin after completion of the synthesis steps. In the latter case, the added benzoyl-arginyl group enhanced the solubility of peptide intermediates in aqueous medium and therein made the handling of these easier during enzymatic coupling. Analogously, thermolysin has been used for specific cleavage of a supporting peptide Leu-Gly-Gly-ethyl ester in the synthesis of the protected, but C-terminal-free, octapeptide of staphylococcal nuclease sequence 43–50 (*77*). Here, as above, the peptide being synthesized cannot contain other peptide bonds susceptible to cleavage by the proteases used. Yet, when applicable, this use of proteases can be quite helpful for synthesis of peptides in an aqueous phase.

Highly sequence-specific proteases can be quite useful for processing intermediates. For example, collagenase recognizes the X-Gly bond in the sequence Pro-X-Gly-Pro, which is particularly rare in active peptides or proteins other than collagen. Wünsch et al. (*78*) synthesized des-pyroglutamyl-[15-leucine]human little gastrin I bearing 4-phenylazobenzyloxycarbonyl-Pro-Leu attached to the

N-terminal sequence and then specifically removed 4-phenylazobenzyloxycarbonyl-Pro-Leu by collagenase. Töpert et al. (*79*) showed that the X-Gly bond of an *N*-terminal Pro-X-Gly-Pro extension can be cleaved by clostridopeptidase A, with the remaining Gly-Pro dipeptide removable with postproline-dipeptidylaminopeptidase. This approach is useful, as they mentioned, for specific production of a desired protein using recombinant DNA techniques, by synthesis and subsequent proteolytic processing of fused protein product.

Enzymatically removable protecting groups from side chain carboxyl and ϵ-amino groups have been reported. For example, arginine methyl ester has been used for the γ-carboxyl of isoglutamine (*76, 80*). Here, deblocking is achieved by successive digestion with trypsin and carboxypeptidase B. In addition, Brtnik et al. (*81*) have used phenylacetyl for protecting the ϵ-amino group of Lys-8 in the synthesis of deamino-lysine vasopressin. The phenylacetyl group was removed specifically by penicillin amidohydrolase [EC 3.5.1.11] without hydrolysis of backbone peptide bonds.

Removal of esters on C-terminal α-carboxyl groups can be especially important for producing a free α-carboxyl in peptide intermediates after building a fully protected peptide chain. In such circumstances, alkaline saponification can cause racemization or other side reactions that should be avoided. Immobilized carboxypeptidase Y, which exhibits esterase activity in addition to peptidase activity (*45, 46*), especially at high pH, has been used for such C-terminal ester cleavage (*82, 83*). Other proteolytic enzymes bearing esterase activity can be employed, obviously under conditions appropriate to control peptide bond hydrolysis. Generally, hydrolysis of esters is much faster than hydrolysis of amides, making selective cleavage of the ester bond possible.

Synthesis of Esters and Hydrazides

Recently, enzymatic synthesis of esters has been reported, although only for short peptides, using carboxypeptidase Y (*51*) as well as two endoproteases (*84, 85*). Martinek et al. (*84*) showed that a biphasic system containing organic solvent such as chloroform is greatly favorable for esterification by chymotrypsin. Here, chloroform promotes ester formation by increasing the pK_a of the α-carboxyl group by over 3 pH units, an effect analogous to that used for enzymatic fragment condensation (see above). Yagisawa (*85*) studied the pH profile of tryptic coupling of C-terminal arginine and lysine with alcohols, hydrazine, and substituted hydrazines (tert-butyloxycarbonyl-$NHNH_2$ and benzyloxycarbonyl-$NHNH_2$) with and without organic solvent. Selective and almost quantitative synthesis of substituted hydrazides has particular promise, enabling the azide coupling reaction to be used for fragment condensation without protection of any other carboxyl groups.

Discussion

Progress so far on the use of enzymes in peptide synthesis indicates a promising role for this approach in making peptides and proteins of research, clinical, and industrial interest. Enzymatic peptide synthesis can proceed stereospecifically and

only minimal side chain protection is needed, particularly when esters are used as acyl components. Further, reactions can be carried out at ambient temperature and in an aqueous phase and they are chemically so unambiguous that excess reagents can be isolated and re-used. Thus, many of the problems of chemical synthesis, such as insolubility of protected substrates, side reactions during protection and deprotection, and low yields in condensing large protected fragments, can be circumvented by using enzymes. In cases for which chemical condensation still is preferred, the use of enzymes for protection, deprotection, and other manipulations of peptide intermediates could provide advantages. At present, the enzymatic peptide synthesis approach does suffer from several limitations. These include as yet incomplete evaluation of usefulness for a wide spectrum of systems, complexities in purifying products from mixtures in which heterogeneity may be magnified by enzymatic overreaction (condensation of more peptide bonds than desired as well as unwanted peptide bond hydrolysis), and the cost of enzymes. Of these deficiencies, the first two likely can be addressed by further testing of current methods. The third problem could be overcome most efficiently by employing immobilized instead of soluble enzymes. That proteases can be immobilized without loss of function is well established (*86–88*). And, several recent studies have sought to explore such species for peptide synthesis (*33–35*). Further evaluation and use of immobilized enzymes would seem a particularly important need for the development of enzymatic peptide synthesis beyond its present level.

The advent of current enzymatic approaches in peptide synthesis comes at the same time as that of recombinant DNA techniques. As with the former, the latter provides a "biological" alternative to chemical synthesis for making polypeptides of controlled sequence (*89–92*). Recombinant methods can allow this through protein biosynthesis coded by specific genetic sequences, either synthetic or native, that are incorporated into high-producer clones. Though such a process likely will be inappropriate for small peptides, or larger ones containing amino acids not coded by normal DNA triplets, large polypeptides, proteins, and many of their analogs are reasonable targets for future work. It is likely that both enzymatic and recombinant DNA methods will be useful for particular polypeptide synthesis objectives. The degree to which each takes its place along with or instead of chemical methods remains to be determined by further evaluation.

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