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Full Length Research Paper

Recent progress in the chemo-enzymatic peptide synthesis

Feifei Chen[1,2], Fangkai Zhang[1,3], Anming Wang[1]*, Haifeng Li[1], Qiuyan Wang[1], Zhaowu Zeng[1], Shuling Wang[1] and Tian Xie[1]

[1]Research Center for Biomedicine and Health, Hangzhou Normal University, Hangzhou 310012, People's Republic of China.

[2]College of Material, Chemistry and Chemical Engineering, Hangzhou Normal University, No. 222, Wenyi Road, Hangzhou 310012, People's Republic of China.

[3]College of Biological and Environmental Sciences, Hangzhou Normal University, Hangzhou 310012, People's Republic of China.

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Peptides are molecules of paramount importance in several fields, especially in health care and nutrition. They have many beneficial health effects, such as antimicrobial, antiviral, antitumor, neuroactive and immunoactive activity. Several technologies for their production are now available such as chemical synthesis, biosynthesis and chemo-enzymatic peptide synthesis. For combining the advantages of chemical and enzymatic synthesis methods, chemo-enzymatic peptide synthesis has been attracting the interest of researchers in the peptide synthesis. In this paper, new progress in this method was reported. Enzymes, solvent systems and possible mechanisms were presented. The main strategies of chemo-enzymatic synthesis of peptides and modification of enzymes by using different methods were also discussed.

Key words: Peptides, enzymes, solvent systems, chemo-enzymatic synthesis, genetic engineering, one-pot synthesis.

INTRODUCTION

Peptides are molecules of great importance in the pharmaceutical and food fields. They play important roles in the sensory appreciation of food toward four basic taste sensations (sweet, bitter, sour and salty) (Fuchise et al., 2010), they also can be used in multiple patholo-gies, including cardiovascular diseases, gastrointestinal dysfunction growth problem, immunity disease, oncology and so on (Stevenson, 2009; Dahiya et al., 2010) and present a broad antimicrobial activity (Lu et al., 2009) which represent important markets. The market for synthetic therapeutic peptides rose from €5.3 billion in 2003 to €8 billion in 2005. It has been estimated that it will reach €11.5 billion in 2013 (Pichereau and Allary, 2005).

Until now, several technologies for peptide production are available: (a) chemical synthesis; (b) enzymatic synthesis; (c) recombinant DNA technology. However, the application of recombinant DNA technology requires a long and expensive research and development phase (Noritomi et al., 2009). Moreover, it often remains impractical due to the low expression efficiencies obtained and difficulties encountered in product extrac-tion and recovery. For chemical synthesis, especially in solid- phase, the strategy can be considered now as the most mature technology for peptide preparation. Unfortunately, it has some problems such as racemiza-tion during peptide bond formation, the requirement of extensive protection of the side chain functionalities of amino acids, and the use of a large excess of coupling reagents and acyl donors. Enzymatic synthesis of peptides has drawn much attention because of enzyme stereospecificity, mild reaction conditions, minimum side-chain protection and avoidance of racemization.

The combination of chemical and enzymatic synthesis is probably the way to go as already suggested (Hou et al., 2005), since the good properties of each technology can be synergistically used in the context of one process

*Corresponding author. E-mail: anmwang@hotmail.com. Tel: +86-571-28861623. Fax: +86-571-28865630.

objective. Some drawbacks of chemical processes can be successfully overcome by enzyme biocatalysis (Guzmán et al., 2007). In this review, enzymes, solvent systems, possible mechanisms and the main strategies of chemo-enzymatic synthesis of peptides using different methods were discussed.

Enzymes

Enzymatic synthesis of peptides has a number of advantages, for instance, mild conditions of the reaction; the high regiospecificity of the enzyme allowing the use of minimally protected substrates; the reaction being stereospecific without racemization. Chymotrypsin, papain, alcalase, pronase, penicillin acylase and lipase are frequently used enzyme in peptide synthesis.

Chymotrypsin

Chymotrypsin is a potent catalyst in peptide synthesis and has strong specificity for aromatic amino acids (e.g., Tyr, Trp, and Phe). The yield of peptide synthesis is very high in an optimum environment when this enzyme was used. For example, the optimum condition for N- Ac-Phe-Gly-NH2 synthesis included an incubation time of 30.9 min, a reaction temperature of 35.8°C, an enzyme activity of 159.2 U and a pH of 8.98. The predicted and the actual (experimental) yields were 98.0 and 95.1%, respectively (Ju et al., 2009) In the present study, Narai-Kanayama et al. tried to synthesize Tyr-Tyr, an ACE inhibitory dipeptide simply constituted of Tyr only , using -chymotrypsin as a catalyst (Narai-Kanayama and Aso, 2009). Xing et al. also have successfully carried out the a-chymotrypsin catalyzed ZTyrGlyGlyOEt synthesis in 1-alkyl-3-methylimidazolium hexafluorophosphate and tetrafluoroborate ionic liquids. This is the first example to demonstrate that a-chymotrypsin can catalyze peptide bond formation in ionic liquids (Xing et al., 2007). For this enzyme, the cost of enzyme catalyst is a key problem in the enzymatic preparation of peptide. Reuse of the enzyme by immobilization and enhancement of its performance using genetic engineering technology would extend its use in the peptide production.

Papain

Papain is the most frequently used cysteine protease. It has been reported to catalyze the syntheses of the N-terminal tripeptide (CCK26-28), a fragment of CCK-8 (Meng et al., 2007). The step of CCK26-28 synthesis is shown in Figure 1. Papain-catalyzed dipeptide (Z-Ala-Ala-OH) synthesis from N-protected amino acid carbamoylmethyl esters and free amino acids in frozen aqueous solutions also had been studied (Salam et al., 2008).

Likewise, other papain-like peptidases could be applied as catalysts for similar reactions. The great biodiversity of South America (Hopkins, 2007; Peres et al., 2010) may be exploited as potentially useful source of biochemical catalysts for organic synthesis. As a matter of fact, asclepiadaceae, a cysteine protease, was purified from the latex of a local climbing milk weed Morrenia brachystephana Griseb (Domsalla and Melzig, 2008; Liggieri et al., 2009; Obregón et al., 2009), and proved to be a useful catalyst for peptide synthesis in an aqueous-organic biphasic system (Quiroga et al., 2008).

Alcalase

Alcalase, a cheap industrial serine protease from *Bacillus licheniformis* displays a relatively broad substrate tolerance and has been used in kinetically controlled enzymatic peptide synthesis. High yields are usually obtained due to the stability of the enzyme in nearly anhydrous (water contents <1 wt %) organic solvents, thereby minimizing product and/or ester hydrolysis (Nuijens et al., 2009). Hou et al. successfully synthesized Bz-Arg-Gly-NH2 (N-benzoylargininylglycinamide) [a precursor dipeptide of RGDS (Arg-Gly- Asp-Ser)] in acetonitrile/ 0.1 M Na2CO3/NaHCO3 buffer system (90:10, v/v) with a dipeptide yield of 82.9% (Hou et al., ,2006). Li et al. also reported the synthesis of a precursor dipeptide, Z-Asp- Val-NH2 of thymopentin (TP-5), catalysed by alcalase in acetonitrile=Na2CO3-NaHCO3 buffer system (9:1, V=V) (Li et al., 2008).

Pronase

During the past few decades enzymes are increasingly used to develop more efficient and cleaner chemical syntheses. Such biocatalytic conversions can involve one enzyme that carries out one specific reaction at a time, or multiple enzymes that carry out a series of conversions to yield a desired product (Lopez-Gallego and Schmidt-Dannert, 2010). In this work, a multiple enzyme, pronase is reported. Pronase, a mixture of proteases from *Streptomyces griseus*, is a commercially available (Boehringer Mannheim GmbH) mixture of several *Streptomyces griseus* proteolytic enzymes, endopeptidases and exopeptidases has been demonstrated that it can be employed successfully for the formation of dipeptides with yields up to 95%. It has also been employed successfully as catalyst for the enzyme assisted synthesis of a hexapeptide.

Penicillin acylase

Penicillin acylases are important enzymes in the

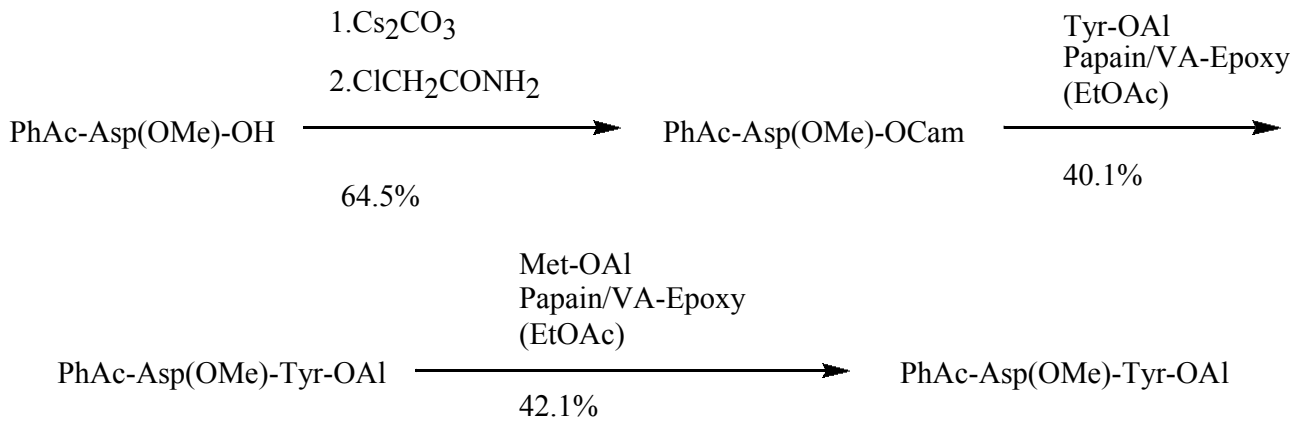

Figure 1. The steps of CCK26–28 synthesis.

pharmaceutical industry and their major industrial application is the production of 6- amino penicillanic acid (6-APA), catalyzing the hydrolysis of penicillins, they also have been applied in the peptide synthesis and the resolution of racemic mixtures of chiral compounds (Spence and Ramsden, 2007). Combining with proteases, penicillin acylase has been used in peptide synthesis as a selective protection and deprotection catalyst. For example PAC has been used as a biocatalyst in the synthesis of the sweetener aspartame, and further use has been in the preparation of D-phenyl dipeptides whose esters readily undergo ring closure to the corresponding diketopiperazines (Spence and Ramsden, 2007) . Such peptides are used as food additives and as synthons for fungicidal, antiviral and anti-allergenic compounds. In addition, PAC can hydrolyse phenylacetyl derivatives of a number of peptides and resolve enantiomers of some organic compounds.

Lipase

Lipases (triacylglycerol-hydrolases, EC 3.1.1.3) are serine hydrolases. They are generally stable and active in organic solvents and function without cofactors. These features have made lipases attractive in industrial use (Fan and Qian, 2010) such as in the peptide synthesis (Li and Kanerva, 2006; D'Arrigo et al., 2009). They work with a double displacement mechanism via an acyl-enzyme intermediate at the water-lipid interface, and an interfacial activation of the lipase is usually necessary. In addition to their physiological substrates, lipases have broad substrate specificity. In the peptide bond formation, they rarely have been shown to possess amidase activity as opposed to serine proteases (which act with the same mechanism) such as chymotrypsin and subtilisin which hydrolyse both amides and esters (D'Arrigo et al., 2009). The mechanism of lipase-catalysed dipeptide formation is shown in Figure 2. With this mechanism lipase (PPL) has been used to catalyze the formation of Bz-RGDS-NH2 in aqueous water- miscible organic cosolvent systems using Bz-Arg-OEt as the acyl donor and GDS-NH2 as the nucleophile (Huang et al., 2006).

Enzyme engineering

Usually, in spite of the impressive number of applications, enzymes do not exhibit satisfying performance or characteristics in terms of activity, stability and above all selectivity. Scientists have been exerting themselves to enhance these performances of enzymes by altering substrates (substrate engineering), modifying reaction system (medium engineering) or enzyme engineering. Enzyme engineering strategy mainly depends on chemical modifications of the protein, enzyme immobilization and modifications at the genetic (DNA) level.

Enzyme immobilization

The immobilization is the most used strategy to improve the operational stability of biocatalysts and provides the enzyme catalysis with some benefits including better control of reaction, flexibility of reactor design, and facilitated product recovery without much loss of catalyst. It will improve enzyme properties by immobilizing enzymes onto iron oxide magnetic nanoparticles (Kim et al., 2008) particularly with regard to enzyme stability and activity (Kim et al., 2006; Wang, 2006). In this work, the oligopeptides syntheses catalyzed by -Fe_2O_3/Fe_3O_4 magnetic nanoparticles (MNPs) with immobilized proteases were systematically studied. The results show that the -Fe_2O_3 MNPs were better for use as an immobilization matrix, rather than the Fe_3O_4 MNPs, owing to their smaller particle size and higher surface area. The use of nanobiocatalysts, with the combination of

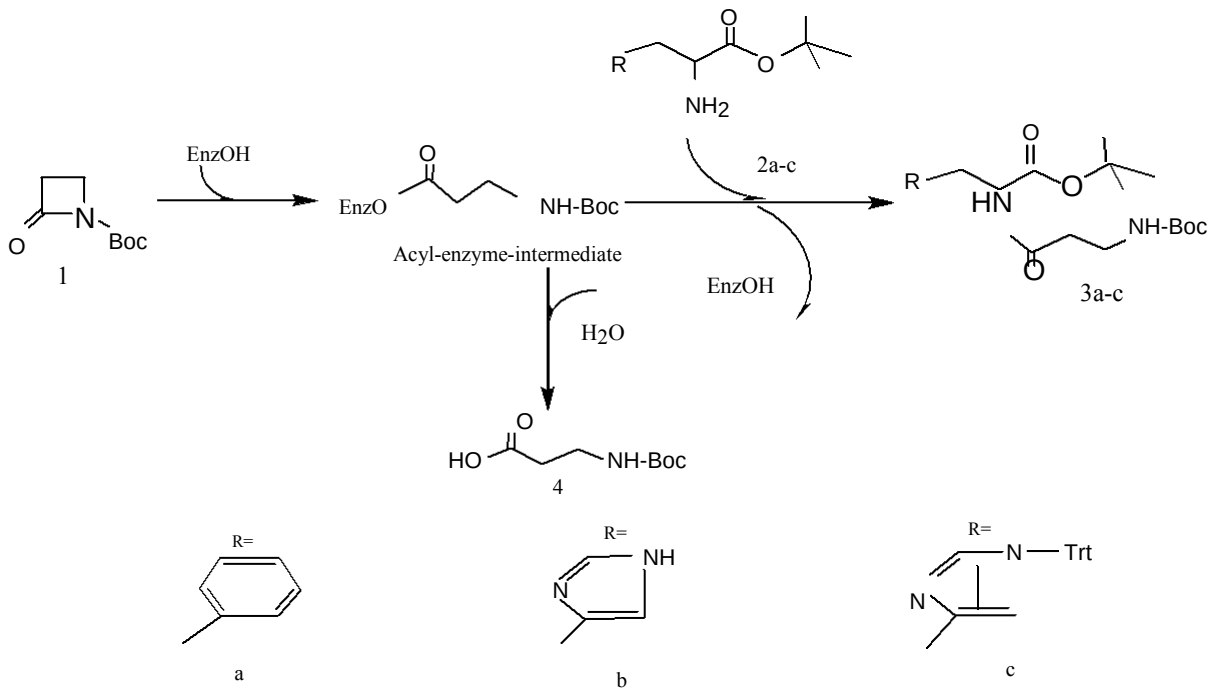

Figure 2. The mechanism of lipase-catalysed dipeptide formation.

nanotechnology and biotechnology, is considered as an exciting and rapidly emerging area (Xin et al., 2010).

Site-specific modifications

Besides the immobilization, specific chemical modifications of enzyme have also been used to enhance the enzyme performance in the enzymatic peptide synthesis. For example, chymotrypsin is unstable at pH 9; unfortunately, high pH is necessary for successful aminolysis of the acyl enzyme, since only the deprotonated amine is nucleophilic. Selective oxidation of methionine 192 to the methionine sulfoxide increases the stability of chymotrypsin toward basic conditions (West et al.,1990), raising the half -life at 25°C and pH 9.0 from 125 min to well over 4 h (at which point the study was terminated). Thus Met- 192-sulfoxide chymotrypsin is a more useful enzyme for peptide synthesis.

Genetic engineering

In improving the enzyme performance, genetic engineering has become an increasingly important strategy. A variety of techniques exist for introducing changes into the enzyme at the genetic level (Burton et al., 2002; Appella, 2010). During past years many research groups discovered mutations that improve enzyme properties such as selectivity, activity, alternate catalytic activity and thermal stability.

Random mutagenesis

The simplest method in genetic engineering is to use random mutagenesis followed by screening of the resultant mutants. Although it is one of the oldest techniques for DNA manipulation, random mutagenesis is still quite useful because it requires no prior knowledge about the enzyme's tertiary structure and no analysis of its structure. It has been used, for example, to screen for more solvent-stable and active mutants of *Subtilisin E* for peptide synthesis (Hult and Berglund, 2007; Chen et al., 2008).

Site-directed mutagenesis

Site-directed mutagen is, a technique that has become extremely simple with the advantage of overlap extension PCR (polymerase chain reaction), allows introduction of one or more defined changes at a time. With regard to peptide synthesis, site-directed mutagenesis has been useful for creating mutants with greater thermo-stability, higher solvent stability, altered substrate specificity, and an increased aminolysis to hydrolysis ratio. For example, starting from the trypsin variant D189S, which is already known for its low amidase activity, Ser189 and Ser190 were exchanged for Ala to further repress the inherent amidase activity of mutant trypsin D189S towards Arg/Lys and Tyr/Phe as well (Grünberg et al., 2001). However, this method requires that one have some idea of which residues are important in enzyme structure.

Solvent systems

According to conventional notion, enzymes are active only in water. However, this conventional notion has long been discarded, thanks to the numerous studies documenting enzyme activities in nonaqueous media, including pure organic solvents and supercritical fluids. Now solvent systems used for enzymatic catalysis may be categorized as: (1) aqueous; (2) organic solvents; (3) supercritical fluids; (4) ionic liquids (Cantone et al., 2007; Parvulescu and Hardacre, 2007; Giunta and Solinas, 2009).

Organic solvent

In contrast with aqueous solvents, there are numerous advantages in employing enzymes in organic solvents such as increased solubility of nonpolar substrates and shifting of thermodynamic equilibrium to favor synthesis over hydrolysis, reduction in undesirable side reactions (Gupta and Khare,2009; Doukyu and Ogino, 2010). Many hydrophobic small peptides have been synthesized in high yields using proteases under thermodynamic control or kinetic control in organic media as largely reported (Li et al., 2008; Bemquerer et al., 2009). However, because of the low solubility of hydrophilic amino acids in organic solvents, the synthesis of hydrophilic amino acid-containing peptides generally proceed in a rather low yield. A method available to overcome the difficulty is to use reverse micelle as reaction media. Reversed micelles, with their characteristic low water content (typically <5 M), provide favorable thermodynamic conditions for the enzymatic formation of peptide bonds (Dias et al., 2007). Organic solvents also can lead to the denaturation of enzymes and lower yield of the peptide product. To overcome this difficulty, water-organic cosolvents systems are used based on that enzymes require some water molecules to maintain structural flexibility and catalytic activity in organic solvents.

Supercritical carbon dioxide

Supercritical carbon dioxide (SC-CO_2) is often discussed as an alternative, environmentally benign reaction medium for chemical synthesis, due to its non-toxic and nonflammable nature, and its relatively low critical pressure and temperature (7.36 MPa, 31.0°C) which allows preservation of thermally unstable compounds. Furthermore, SC-CO2 is climate neutral and inexpensive because the CO2 used is a byproduct of industrial processes. Previous reviews have showed that supercritical CO_2 (SC-CO_2) has been used as a solvent for extractions (Zhang et al., 2010; Zermane et al., 2010), chromatography (Sui et al., 2010), stoichiometric and catalytic organic reactions (Ramsey et al., 2009).

Supercritical carbon dioxide has also been utilized as an environmentally benign solvent for enzymatic peptide synthesis. For example, surfactant-coated -chymotryp-sin (CT) has been used to synthesize dipeptides in SC-CO_2 (Mishima et al., 2003). Application of SC-CO_2 medium for performing industrially important enzyme-catalyzed processes is going to become important “green” and sustainable, environmentally friendly and economical synthetic protocols (Wimmer and Zarevúcka, 2010).

Ionic liquids

Ionic liquids are organic salts, which are liquids at ambient temperatures (Yang and Pan, 2005), and have attracted much attention in recent years for serving as good media for a variety of organic synthesis (Yang and Pan, 2005; de María, 2008; Sureshkumar and Lee, 2009; Gorke et al., 2010). It has been reported that different enzymes such as lipase (Rantwijk et al., 2006; Fan and Qian, 2010) protease (Nakashima et al., 2008; Noritomi et al., 2009; Malhotra et al., 2010), peroxidase, dehydrogenase, and glycosidase could maintain their activity when suspended in ionic liquids, which indicates that ionic liquids are very promising green alternatives to organic solvents for biotransformations. The enzyme catalyzed reactions, in particular, transesterification, with ionic liquids as solvent has been well investigated and the enzyme catalyzed peptide synthesis in ionic liquids has also been successfully carried out in recent years (Miao and Chan, 2005; Plaquevent et al., 2008). Typically, thermolysin catalyzed z-aspartame synthesis in BMIM•PF6 (Erbeldinger et al., 2008) and -chymotrypsin catalyzed a tripeptide, ZtyrGlyGlyOEt, synthesis in MOEMIM•PF6.

Mechanisms of synthesis

In general, the methods of enzyme-catalyzed peptide bond formation can be classified into two basic strategies according to the type of the carboxyl component used: thermodynamic and kinetic control (Kumar and Bhalla, 2005). In the thermodynamic approach, this component has a free carboxyl terminus, and the peptide bond formation occurs under thermodynamic control. In the kinetic approach the carboxyl component is employed in an activated form, mainly as an ester derivative, and the synthesis occurs under kinetic control (Meng et al., 2006).

Synthesis under thermodynamic control

The thermodynamically controlled synthesis of peptides (TCS) with proteases can be represented as shown in

Figure 3. Kion is the equilibrium constant of ionization and K_{con} is the equilibrium constant of conversion.

Figure 3. Proteases, as any catalyst, do not alter the equilibrium of the reaction but merely increases the rate to attain it. According to the principle of microscopic reversibility, both the formation and the hydrolysis of the peptide bond proceed by the same mechanism and through the same intermediate. From Figure 3, it is apparent that the equilibrium will be displaced to hydrolysis in an aqueous medium. The displacement of equilibrium towards peptide bond formation can be attained by manipulation of the equilibrium of ionization (that is, pH change) and the equilibrium of the reaction (that is, by product precipitation or by modification of medium composition) (Jakubke et al., 2003). The addition of organic cosolvents and the use of aqueous-organic biphasic systems are good strategies to displace the equilibrium towards synthesis. The presence of organic solvents will reduce the activity of water in the reaction medium, which will favour the equilibrium, but high concentrations of cosolvents are usually detrimental for enzyme activity (Jakubke et al., 2003). In biphasic systems, the partition of the peptide products from the aqueous phase that contains the enzyme to the organic phase drives the equilibrium towards synthesis, with the additional benefit that the product is no longer subjected to hydrolysis. However, reaction rates in biphasic systems are low because of limitation by substrate diffusion; besides, proteases tend to denature in the water-solvent interphase.

Synthesis under kinetic control

The kinetically controlled synthesis of peptides (KCS) with proteases can be represented by Figure 4 (Bordusa, 2002). As opposite to TCS, only serine or cysteine proteases can be used to perform KCS, because the enzyme acts in this case as a transferase and catalyzes the transference of an acyl group from the acyl donor to the amino acid nucleophile through the formation of an acyl-enzyme intermediate. Papain, thermolysin, trypsin and -chymotrypsin are the most used enzymes in KCS.

As shown in Figure 4, the intermediate [Ac-E] can be nucleophilically attacked by water and by the nucleophile (HN), which can be an amine, an alcohol or a thiol that will compete with water for the deacylation reaction. The success of the reaction of synthesis will depend on the kinetics of these nucleophilic reactions; this is why this strategy is termed “kinetically controlled”. It is desirable that the peptide product be removed from the reaction medium to avoid unwanted secondary hydrolysis. And as in TCS, the decrease of water activity, by using an organic cosolvent, favours synthesis in KCS by reducing the hydrolysis of the acylenzyme intermediate and the final product, but, again, the reaction medium can be harmful to the enzyme.

Strategies of chemo-enzymatic synthesis of peptides

In peptide synthesis, the combination of chemical and enzymatic synthesis is probably the way to go as already suggested (Hou et al., 2005), since the good properties of each technology can be synergistically used in the context of one process objective. In this method, some drawbacks of chemical processes can be successfully overcome by enzyme biocatalysis (Guzmán et al., 2007). Now, a lot of advanced strategies have presented such as membrane enhanced peptide synthesis, one-pot synthesis, peptide synthesis in eutectic-based media and frozen aqueous solutions.

Membrane enhanced peptide synthesis

Solid-phase peptide synthesis (SPPS) faces serious challenges including mass transfer, steric hindrance, and resin handling in peptide synthesis. Membrane separation coupled to solution phase synthesis can offer major advantages over SPPS by combining the advantages of ‘‘classical’’ solution phase synthesis with the ease of purification of the solid phase method.

Molecular separation in organic solvents via nanofiltration (Organic Solvent Nanofiltration-OSN) is an emerging technology (Vandezande et al., 2008), which would be an ideal separation method for in-cycle purification during peptide synthesis. Membrane Enhanced Peptide Synthesis (MEPS) is a new technology platform that advantageously combines OSN with solution phase peptide synthesis. As shown in Figure 5 (So et al., 2010), the cycle is repeated as many times as necessary, adding a further amino acid to each cycle, until the desired peptide sequence is obtained. Washing is carried out immediately after the coupling and deprotection steps using the reaction solvent and does not require any solvent exchanges. Membrane Enhanced Peptide Synthesis (MEPS) makes the process of peptide synthesis easier, saving many labour powers and material resources. It may be one way to be operated on

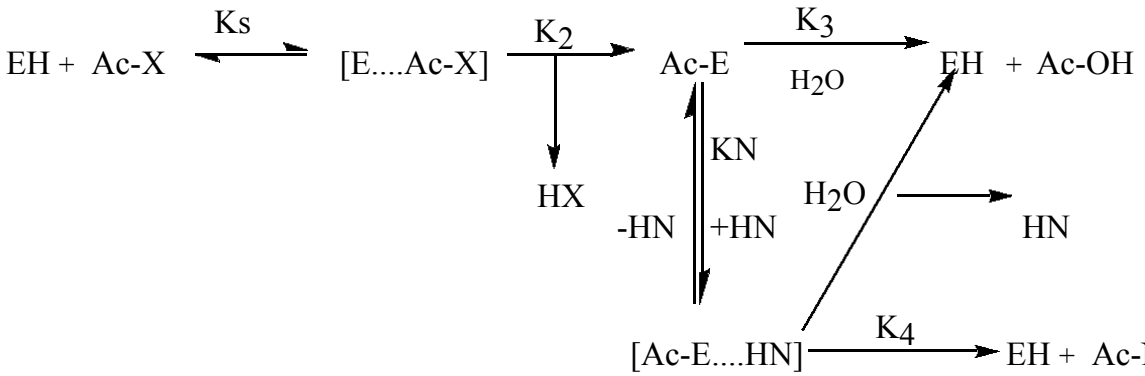

Figure 4. The kinetically controlled synthesis of peptides. Where EH is the free enzyme; Ac-X is the acyl donor substrate; [E...Ac -X] is the Michaelis-Menten acyl-enzyme complex; HX is the released group; Ac-E is the acylenzyme intermediate, HN is the acceptor substrate (nucleophile), Ac-N is the product of synthesis (peptide) and Ac-OH is the product of hydrolysis of the acyl donor.

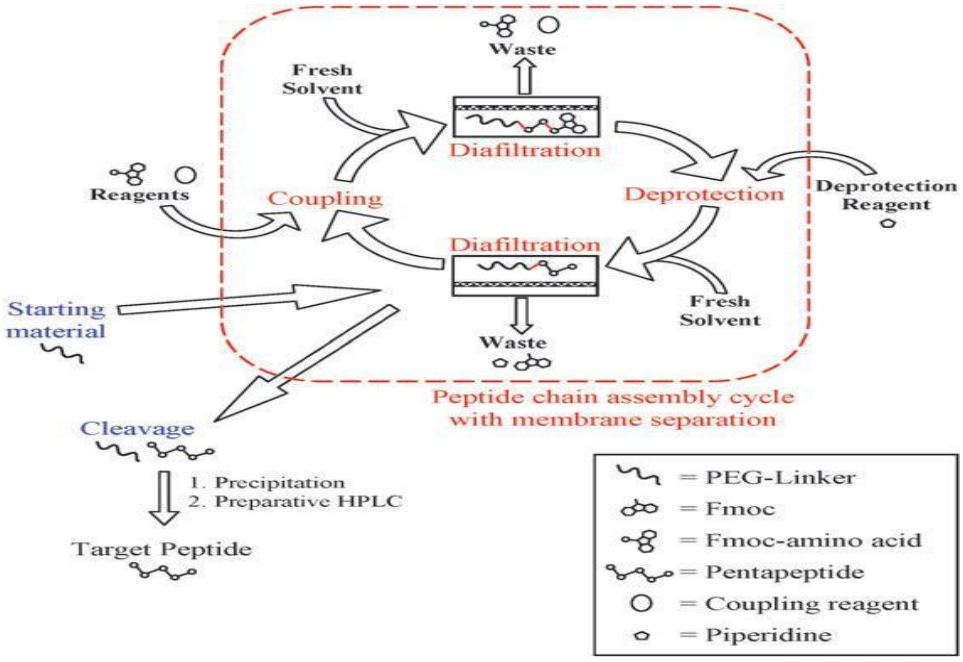

Figure 5. Schematic representation of membrane enhanced peptide synthesis (MEPS).

the large scale.

Peptide synthesis in eutectic-based media

Although numerous enzymatic synthesis have been devised for peptides, only one process, namely the thermolysin-catalysed synthesis of Aspartame is operating on the large scale (Gill and Valivety, 2002; Haddoub et al., 2009). In the context of “Green Process” development and high productivity biocatalysis, two novel approaches have recently been introduced, namely peptide synthesis in heterogeneous solid-liquid systems comprised of mixtures of substrates which undergo eutectic formation and solid-to-solid conversions in aqueous suspensions of substrates (Chaiwut et al., 2007; Basso et al., 2008). The former method relies upon the observation that amino acid and peptide derivatives commonly employed as substrates in peptide synthesis form low-melting point mixtures (completely liquid eutectic or liquid eutectic plus excess solid substrate) when combined together in the absence of bulk solvents. In these systems, the product concentrations and productivities are typically 5 -13-fold higher than those obtained in conventional solution systems (Gill and Valivety, 2002). Good reaction rates and high equilibrium

Table 1. a-Chymotrypsin-catalysed synthesis of peptide amides using Na-unprotected acyl donors and H-Leu-NH_2 as Nucleophile, at room temperature and in the frozen state -15°.

Acyl donor H-Xaa-Phe-OMe	Peptide product	Peptide yield (%)	
Xaa		**25°**	**-15°**
Gly	H-Gly-Phe-Leu-NH_2	56	84
Asp	H-Asp-Phe-Leu-NH_2	78	90
Leu	H-Leu-Phe-Leu-NH_2	56	91
Ala-Leu	H-Ala-Leu-Phe-Leu-NH_2	45	84

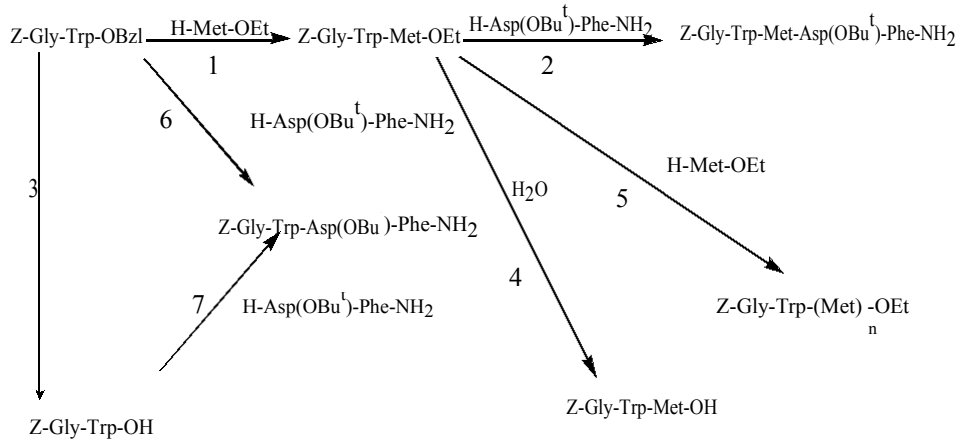

Figure 6. (a) Synthetic reactions 1 and 2; (b) hydrolysis of both acyl donors (reactions 3 and 4); (c) condensation of several methionine residues on Z-Gly-Trp-Met-OEt (reaction 5); (d) tetrapeptide synthesis by kinetic control over the dipeptide acyl donor (reaction 6) and by thermodynamic control over Z-Gly-Trp-OH (reaction 7).

yields can be achieved when most of the substrates and products remain in the solid form.

Frozen aqueous solutions

It has been shown in recent reports (Salam et al., 2006; 2008) that freezing the aqueous reaction mixture can prevent undesirable side reactions, the hydrolysis of the acyl enzyme, secondary hydrolysis of the formed peptide bond (Meng et al., 2006), and can drastically increase the yield of protease-catalyzed kinetically and thermodynamically controlled peptide synthesis reactions. -Chymotrypsin-catalysed peptide bond formation between N -unprotected dipeptide esters H-Xaa-Phe-OMe and even the tripeptide ester H-Ala-Leu-Phe-OMe as acyl donors and H-Leu-NH_2 as nucleophilic component was carried out at room temperature and at -15°, respectively. The obtained results are presented in Table 1 (Gerisch and Jakubke, 1997). As shown in Table 1, the yield of synthetical peptides are drastically increased, which indicates that freezing the aqueous reaction mixture is a great strategy in this area and is worth being developed in future.

One-pot synthesis

Obviously, the most interesting approaches are those of single-pot reactions, as well as the performance of consecutive reactions without intermediate separation (Clapés et al., 2007). Previous works (Meng et al., 2007; Joshi et al., 2008) showed the feasibility of the proposed enzymatic reactions using low water content media with adsorbed enzyme. As both reactions are catalyzed by the same enzyme, the proposed alternatives to link the reactions without intermediate purification are (i) simultaneous strategy: initial addition of the two nucleophiles and acyl donor on a compatible medium for both reactions; (ii) consecutive strategy: to perform the two reactions consecutively in the same reactor with a compatible medium, by adding the second nucleophile when the first reaction was already accomplished. For example, a single-pot enzymatic synthesis of Z-CCK5 (4-
8) is reported. The -chymotrypsin-catalyzed reactions involved in the synthesis of the target pentapeptide, starting from Z-Gly-Trp-OBzl, are presented in Figure 6 (Ruiz et al., 1997). The consecutive approach has been shown to be a suitable strategy. It can have a strong impact on process economics, since it will reduce the

number of operations required for the synthesis, will have less stringent requirement for equipment and reduce the energy input required and so on.

Conclusions

Peptides are molecules of great importance in the pharmaceutical and food fields. Chemo-enzymatic synthesis method has drawn much attention in the peptide production. Synthesis catalyzed by engineering enzymes in optimal conditions (temperature, substrates and solvent) will get a high yield and appropriate synthesis strategy will make the process more easy and more effective. As the progress in the enzyme immobilization technology and genetic engineering technology to enhance the enzyme properties in catalysis, enzyme would be used in peptide synthesis on a large scale. Enzymatic peptide bond formation can successfully and conveniently overcome some drawbacks of chemical processes. Since the good properties of each technology can be synergistically used in the context of one process objective, the combination of chemical and enzymatic synthesis is probably the way to go.

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