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Digest Paper

Development of thiol-independent peptide ligations for protein chemical synthesis

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ABSTRACT

Development of chemoselective peptide ligation methods and strategies has enabled complex proteins to be chemically synthesized. The key components in these effective ligations include tolerating the side chain functionalities present in the protein sequence and using unprotected peptides for reaction. Moreover, generation of the natural peptidic linkages after ligation is most desired. The most used ligations are the cysteine-mediated native chemical ligation (NCL) and its variants. Over the past decade, some thiol-independent peptide ligation methods have emerged to offer alternative tools for protein chemical synthesis.

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Introduction

In 1953, the first synthesis of a biologically active polypeptide hormone was reported. Du Vigneaud successfully synthesized oxytocin (a nonapeptide hormone) by means of solution phase peptide synthesis using protected amino acids as building blocks and the strategy of deprotection and coupling cycle.[1] The synthetic oxytocin was chemically and biologically identical to the natural product. This pioneering work of du Vigneaud started to bridge the biology and chemistry of active peptides. Later, bovine insulin, a heterodimeric peptide, was chemically synthesized in 1964 also via sequential solution phase synthesis.[2] Nevertheless, solution phase peptide synthesis methods are laborious and tedious, as purification after each step is needed. A major breakthrough in the synthetic peptide chemistry was the development of solid phase peptide synthesis (SPPS) by Merrifield in 1963, in which the growing peptide chain was attached to a solid support.[3] Multiple times of simple filtration to wash out excess reagents after each round of coupling is adequate for the purification step. Generally speaking, SPPS is suitable for synthesizing peptides of size up to 50 amino acids, as the trace of byproduct from incomplete coupling accumulates a lot over the long linear sequential synthesis and produces significant various truncated peptide products, resulting in the difficulty in purification of the final product. The properties of truncated peptides can be very similar to the target peptide, making it extremely difficult to isolate the desired final peptide in high purity from the reaction mixture by reverse-phase HPLC, if not impossible. In addition, the long peptide sequence tends to aggregate on the resin, causing the elongation site to become less accessible to the coupling.

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An effective strategy to solve the problem of difficulty in synthesizing long peptides via SPPS is to use convergent synthesis methods, which condense two short peptide segments obtained from SPPS together in solution or on a solid support. With this strategy, even the condensation reaction is not complete, or one of the reacting peptide segments is used in excess, the starting materials can be readily separated from the coupled product, as they differ a lot in size. Traditionally, people use dehydration reagents to activate the C-terminus of one peptide, with which to react with the N-terminus of the other peptide fragment. Using this strategy, side chain functional groups (e.g., amine, hydroxyl, carboxylic acid) of the peptide have to be fully protected to avoid the side reaction. Most of protecting groups used in peptides are very hydrophobic, which often causes peptide aggregation and poor solubility of the peptide in any organic solvent. Furthermore, the process of C-terminus activation can lead to serious epimerization via formation of 5(4H)-oxa-zolone at the non-Gly/Pro residue.[4] Thus, the development of method using unprotected peptides and not involving in situ C-terminus activation has been a long-seeking goal in synthetic protein chemistry. It is challenging to develop a chemical reaction having enzymatic reaction characteristics. Of all the ligation methods developed over the past decades[5–8], native chemical ligation (NCL) by Kent[8] became the most effective and widely used method for protein synthesis nowadays. NCL uses an unprotected peptide with a C-terminal thioester and another unprotected peptide with N-terminal cysteine for a chemoselective ligation.[8] The high chemoselectivity of NCL tolerating all side chain functionalities within the peptide sequence lies in the superior and unique nucleophilicity of the cysteine thiol group.

However, the abundance of cysteine residue in proteins as the lowest among the 20 amino acid residues has promoted people to seek alternative methods to realize chemoselective and natural peptide linkage-producing peptide ligation.[9] As far, the NCL-desulfurization methods, using β- or γ-thiol containing unnatural amino acids as cysteine surrogates[10], have been developed and well adopted for delivering synthetic native proteins. Alternatively, our research group and others have been searching for thiol-independent peptide ligation methods. In this Digest, we will only discuss the thiol-independent methods which have been used to deliver synthetic proteins.

KAHA ligation

The α-ketoacid-hydroxylamine (KAHA) ligation was reported by the Bode group.[11] Originally, this method involves the chemoselective condensation between a C-terminal α-ketoacid and an N-terminal *N*-hydroxylamine to generate an amide product (Fig. 1).[11] Later, they have used 5-oxaproline (Opr) to replace the *N*-hydroxylamine at the N-terminus, which has been shown to be more suitable for ligating large peptide fragments.[12] However, a non-proteinogenic homoserine (Hse) is generated at the ligation site after the reaction. The ligation pathway was recently revised to form a homoserine depsipeptide first, which could rearrange to the amide in basic buffers.[13]

Bode and co-workers have applied Opr-KAHA to synthesize Hse-containing analogues of 63-mer prolaryotic-ubiquitin-like protein (Pup) and 66-mer probable cold shock protein A (cspA).[14]

Ser/Thr ligation

In our efforts, we have recently developed a Ser/Thr ligation (STL), which uses N-terminal Ser or Thr to mediate a chemoselective peptide ligation.[15] STL involves an unprotected peptide with a C-terminal salicylaldehyde (SAL) ester reacting with another unprotected peptide with an N-terminal serine/threonine residue to generate an *N,O* benzylidene acetal linked product, which upon acid treatment is converted to a natural peptide linkage, realizing a chemoselective peptide ligation (Fig. 2). Along the mechanistic pathway, the first step is thought to be the capture of the N-terminal serine/threonine by the C-terminal aldehyde group through imine formation followed by cyclization from the hydroxyl group of the N-terminal Ser or Thr to form an oxazolidine.[16] Both the

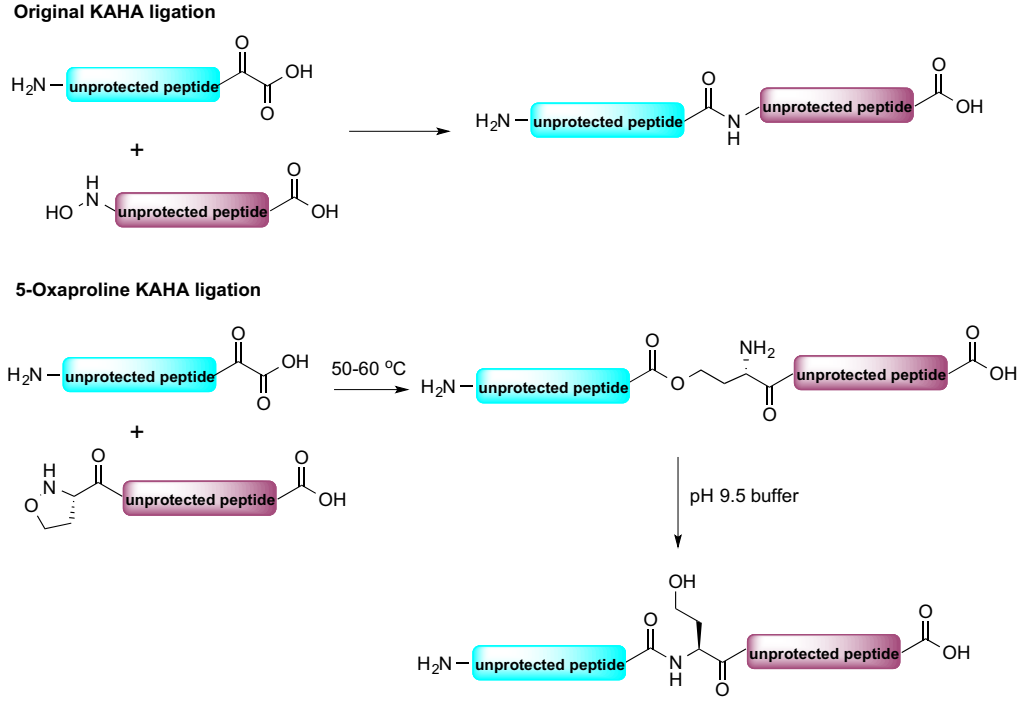

Figure 1. KAHA ligations.