Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Chemical Biology

Recent extensions to native chemical ligation for the chemical synthesis of peptides and proteins

Lara R Malins and Richard J Payne

Native chemical ligation continues to play a pivotal role in the synthesis of increasingly complex peptide and protein targets twenty years after its initial report. This opinion article will highlight a number of recent, powerful extensions of the technology that have expanded the scope of the reaction, accelerated ligation rates, enabled chemoselective post-ligation modifications, and streamlined the ligation of multiple peptide fragments. These advances have facilitated the synthesis of a number of impressive protein targets to date and hold great promise for the continued application of native chemical ligation for the detailed study of protein structure and function.

Addresses
School of Chemistry, The University of Sydney, Sydney, NSW 2006, Australia

Corresponding author: Payne, Richard J (richard.payne@sydney.edu.au)

Current Opinion in Chemical Biology 2014, **22**:70–78

This review comes from a themed issue on **Synthetic biomolecules**

Edited by **Paul F Alewood** and **Stephen BH Kent**

For a complete overview see the Issue and the Editorial

Available online 3rd October 2014

http://dx.doi.org/10.1016/j.cbpa.2014.09.021

Introduction

Proteins exhibit a diverse array of structure and function. The immense variety of functional roles played by these macromolecules results from the precise make-up of the polypeptide amino acid sequence, the consequent three-dimensional structure of the folded protein molecule, and finally, the potential for adornment with post-translational modifications. Thus, as a result of the multi-layered structural complexities of these important molecules, the total chemical synthesis of homogeneous, functional proteins represents a challenging facet of modern organic synthesis.

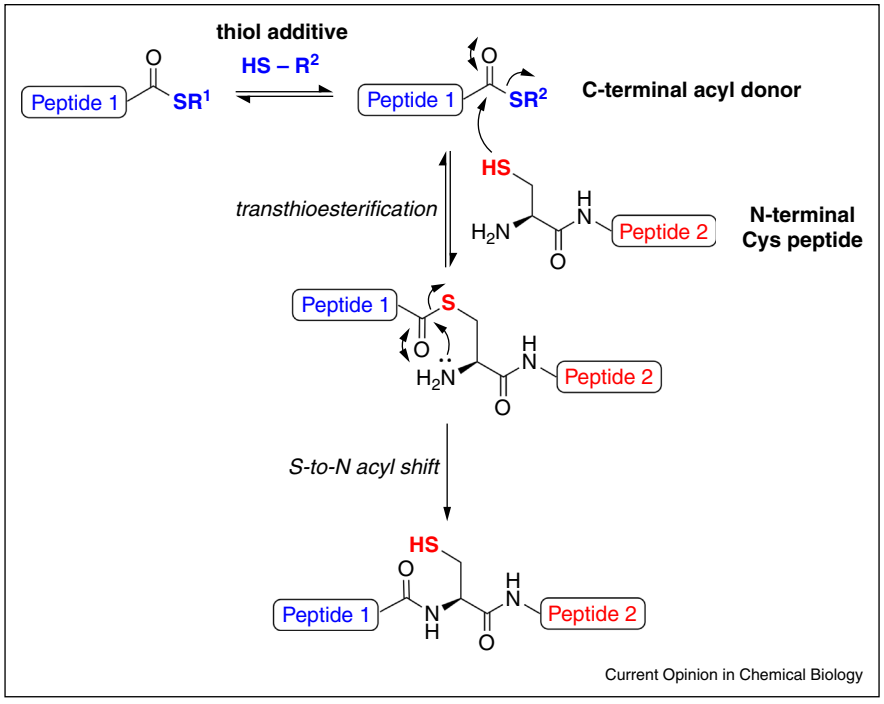
One of the most influential advancements in the chemical synthesis of peptides and proteins has been the development of chemoselective ligation protocols, in particular the discovery of native chemical ligation [1]. This reaction enables the condensation of two unprotected peptide fragments, in aqueous media and under mild reaction conditions, to generate a native amide linkage in an efficient and high-yielding manner. First reported in 1994 by Kent and coworkers, this methodology involves the reaction of a peptide containing a C-terminal thioester with a peptide bearing an N-terminal cysteine (Cys) residue (Figure 1). Mechanistically, the reaction proceeds *via* an initial transthioesterification between the thiol side-chain of Cys and the C-terminal acyl donor to generate an intermediate thioester-linked adduct, which rearranges through an intramolecular S-to-N acyl shift *via* a 5-membered ring intermediate to generate a native peptide bond. Since its inception, a number of advances to the initial ligation methodology have aimed to increase the scope of the reaction (particularly to address the requirement for an N-terminal Cys residue), accelerate the rate of ligation, and to facilitate iterative ligations and chemoselective post-ligation amino acid modifications. These advancements can be broadly distilled into three main research areas: 1) the development of new N-terminal Cys surrogates; 2) the development of new C-terminal acyl donors; and 3) the inclusion of various exogenous thiol additives to modulate the reactivity of the C-terminal thioester moiety [2] (see Figure 1). Over the years, there have been several comprehensive reviews of native chemical ligation and the application of the technology in the chemical synthesis of peptides and proteins [3–9]. In addition, Dirksen and Dawson provided an insightful perspective on the expanding scope of ligation strategies, including native chemical ligation, in 2008 [10]. On the 20th anniversary of the seminal report of the reaction, this opinion article serves to update the reader by highlighting a number of recent, powerful extensions to the native chemical ligation manifold in each of the three areas listed above as well as provide a commentary on current limitations and future challenges.

Development of new N-terminal Cys surrogates

Ligation-desulfurization chemistry

Native chemical ligation traditionally relies on the presence of an appropriately placed Cys residue in a peptide or protein sequence in order to effectively disconnect the target. However, the relatively low abundance of Cys (1.1%) in naturally occurring proteins has prompted intense efforts to develop N-terminal Cys surrogates that can extend the applicability of the reaction, *via* a similar pathway to native chemical ligation, but that can be subsequently removed or manipulated to generate other proteinogenic amino acids at the ligation junction. In the early 2000s, there was a flourish of activity in the study of removable N-linked and side-chain ligation auxiliaries in the ligation-based assembly of peptides and proteins [10].

Figure 1



Mechanism of the native chemical ligation reaction.

Challenges in expanding the scope and increasing the yields of these auxiliary-promoted ligations, however, have prompted the exploration of alternative approaches. These efforts have recently converged on the use of ligation-desulfurization chemistry [11,12], a concept first demonstrated by Yan and Dawson through reductive desulfurization of Cys following the ligation event to generate a native Ala [13••]. The development of a mild and metal-free radical desulfurization protocol employing the water-soluble radical initiator VA-044 in the presence of tris(2-carboxyethyl)phosphine (TCEP) and *t*-BuSH [14••] or reduced glutathione [15] has further fueled the adoption of post-ligation desulfurization methodologies.

The concept of employing unnatural amino acid derivatives bearing suitably positioned thiol auxiliaries [13••] in ligation-desulfurization chemistry has recently come to fruition (Figure 2I) and represents a crucial extension to the native chemical ligation methodology. Beginning with the preparation of β-thiol phenylalanine (Phe) [16,17] and the application of this Phe derivative in peptide ligation followed by a post-ligation reductive desulfurization with nickel boride [17], a number of additional thiol-derived amino acids have been added to the ligation-desulfurization toolbox. These additions include access to post-desulfurization Xaa-Yaa ligation junctions where Yaa can be valine (Val) [15,18], lysine (Lys) [19–21], threonine (Thr) [22], leucine (Leu) [23,24], proline (Pro) [25,26], glutamine (Gln) [27], arginine (Arg) [28], aspartic acid (Asp) [29•,30], glutamic acid (Glu) [31] or tryptophan (Trp) [32] using suitable thiol-derived amino acid building blocks (Figure 2Ia-c). Importantly, thiolated amino acids tend to exhibit increased rates of reactivity and improved reaction scope relative to N-linked and side-chain appended auxiliaries, owing in part to the decreased steric bulk at the ligation junction (relative to N-linked auxiliaries) and the ability to proceed primarily through 5-membered (for β-thiol derivatives, Figure 2Ia) or 6-membered (for γ-thiol derivatives, Figure 2Ib) ring intermediates in the S-to-N acyl transfer step. Ligation-desulfurization chemistry using these thiol-derived building blocks has also been successfully employed in the synthesis of a number of complex peptide and protein targets, including the construction of human parathyroid hormone [33•], a mucin 1 (MUC1) glycopeptide oligomer [28] and to facilitate side-chain ubiquitination of α-synuclein [34].

On-going challenges in the development of ligation-desulfurization methodologies include the development of more practical routes to thiol-derived amino acid building blocks. With the exception of penicillamine [15] and γ-thioproline building blocks [25], which are commercially available, and the late-stage installation of a Trp thiol auxiliary onto unprotected peptides [32], the