

Protein Assembly Using the Staudinger Ligation

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Introduction

New methods are facilitating the total chemical synthesis of proteins. In particular, the chemical ligation of synthetic peptides provides a convergent route to proteins. Currently, the most common ligation method is “native chemical ligation” [1]. In native chemical ligation, the thiolate of an *N*-terminal cysteine residue of one peptide attacks the *C*-terminal thioester of a second peptide. An amide linkage forms after S–N acyl transfer. “Expressed protein ligation” is an extension of native chemical ligation in which the *C*-terminal thioester is produced by recombinant DNA (rDNA) technology rather than chemical synthesis [2].

A limitation of native chemical ligation is its intrinsic reliance on having a cysteine residue at each ligation juncture. Cysteine is uncommon, comprising only 1.7% of all residues in proteins. Modern peptide synthesis is typically limited to peptides of ≤ 40 residues [3]. Hence, most proteins cannot be prepared by any method that requires peptides to be coupled only at cysteine residues.

The removal of the cysteine limitation by the development of a more general ligation reaction would greatly expand the scope and utility of total protein synthesis. We have developed such a reaction. Specifically, we have used the Staudinger reaction to unite two peptides, one with a *C*-terminal phosphinothioester and the other with an *N*-terminal azide [4–6]. A putative mechanism for this version of the ‘Staudinger ligation’ is shown in Figure 1. The reaction of a phosphinothioester with a peptide azide leads to the formation of the reactive iminophosphorane. Attack of the iminophosphorane nitrogen on the thioester leads to an amidophosphonium salt. Hydrolysis of the amidophosphonium salt produces the desired amide bond and a phosphine oxide. Significantly, no residual atoms remain in the amide product.

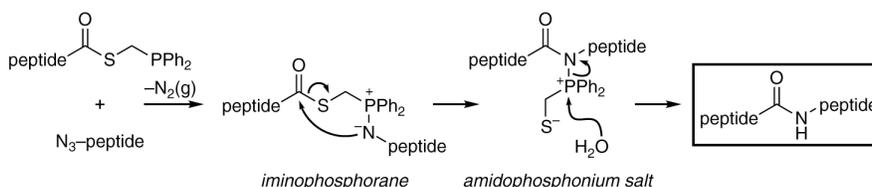


Fig. 1. Putative mechanism for the Staudinger ligation of peptides.

Results and Discussion

The Staudinger ligation of protected peptide fragments on a solid support is an orthogonal method to form an amide bond. Having demonstrated the efficacy of HSC₂PPh₂ in mediating the Staudinger ligation [4–6], we sought to exploit this new synthetic methodology in the assembly of a protein [7]. As a model system for semisynthesis, we chose ribonuclease A (RNase A). The 123 amide bonds in our semisynthetic RNase A were formed by using four distinct amide-bond forming methods. Fragment 1–109 was prepared as a *C*-terminal thioester by rDNA techniques [2]. The amide bonds in this segment were formed by *m*RNA translation by the

ribosome. Fragment 110–124 was prepared by combining two methods. The amide bonds between residues 110–111 and 113–124 were formed by standard solid-phase peptide synthesis utilizing HATU activation. The protected fragment 110–111 was elaborated as a phosphinothioester of HSCH₂PPh₂. The protected fragment 113–124 was elaborated as an *N*-terminal azide while immobilized to a solid support. The phosphinothioester of 110–111 was coupled to the 113–124 fragment on the solid support by using the Staudinger ligation. Finally, fragment 110–124 was liberated from the resin, deprotected, and coupled to fragment 1–109 via native chemical ligation to give the semisynthetic protein. The $k_{\text{cat}}/K_{\text{M}}$ value for catalysis by this semisynthetic RNase A was nearly identical to that of biosynthetic RNase A, as was its mass.

A promising application for the Staudinger ligation is in the total chemical synthesis of proteins. In our semisynthesis of RNase A we demonstrated the use of the Staudinger ligation to couple protected peptide fragments on a solid support. We envision expanding this methodology to assemble entire proteins on a solid support in an iterative and convergent manner (Figure 2). Our strategy is to divide a target protein into fragments of 20–30 residues. The *C*-terminal fragment is capped with an α -azido acid, and remains immobilized to the support. The remaining fragments are synthesized as protected peptides, and elaborated as *C*-terminal thioesters of HSCH₂PPh₂. The fragments are then coupled in turn to the growing polypeptide chain. After each Staudinger ligation, the nascent polypeptide is capped with an α -azido acid, preparatory for the next cycle. When the synthesis is complete, the polypeptide is deprotected and folded while still attached to the resin (to prevent aggregation), and then liberated from the support. Hence, protein assembly using the Staudinger ligation could facilitate access to the proteins encoded by the human genome.

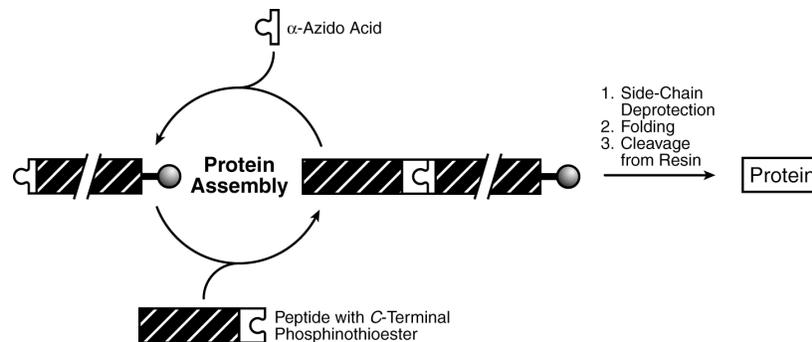


Fig. 2. Scheme for the total chemical synthesis of proteins using the Staudinger ligation.

Acknowledgments

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