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Protein Desulfurization: Sodium Tetraethylborate Makes It Fast

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Summary

Today, the development of powerful one-pot native chemical ligation/cysteine reductive desulfurization techniques is central to the production of proteins by chemical synthesis. In this issue of Chem, Xuechen Li and coworkers achieved this goal by combining sodium tetraethylborate and a phosphine as a post-ligation treatment.

The reductive desulfurization of alkyl thiols into alkanes is a useful chemical transformation, which has long been explored by chemists for a variety of analytical or synthetic applications (Fig. A).¹ For example, thiol desulfurization can be found as a key step in the total synthesis of natural compounds or in the elimination of some sulfur compounds from fuel feedstocks. Thiol reductive desulfurization was also early envisioned as a useful tool for structural elucidation of complex peptide molecules, as in the seminal work of Du Vigneaud on the peptide hormone oxytocin (Fig. B),² where Raney nickel was utilized to convert the two cysteine residues (Cys) into alanines (Ala). The fact that only one product was obtained during this transformation was considered as a strong indication that the Cys residues were connected through an intramolecular disulfide bond.

The interest in the reductive desulfurization of Cys into Ala for peptide and protein synthetic purposes turned to center stage following the introduction of the native chemical ligation by Kent and coworkers (NCL³, Fig. C). The NCL reaction enables the formation of a peptide bond to Cys by reaction of a C-terminal peptide thioester with an N-terminal Cys peptide. One hallmark of the NCL reaction is to proceed in water at neutral pH with high chemoselectivity, thereby permitting the assembly of proteins from unprotected peptide segments.⁴ It is the most popular method so far for accessing proteins by chemical synthesis. However, its dependency on the presence of Cys residues in the target protein limits its scope owing to the naturally low abundance of Cys in proteins (~1.5%). In this context, the post-assembly reductive desulfurization of Cys into Ala is considered as a powerful advance in the field since it extends the utility of NCL to the formation of much more frequent Xaa-Ala junctions (8.4%).⁵ Today, the combination of NCL with Cys desulfurization and especially those methods that enable both reactions to be done in one-pot are central to the field of chemical protein synthesis.⁶

Following the pioneering studies of Dawson and coworkers who applied Raney nickel or Pd/Al₂O₃, H₂ reductants for performing the desulfurization of Cys into Ala after NCL,⁵ alternative methods were developed to avoid the use of heterogeneous metal catalysts which often resulted in low yields. A key advance in the field was the report of Wan and Danishefsky in 2007. They showed that Cys can be desulfurized at the protein level by using a water-soluble phosphine such as *tris*(2-carboxyethyl)phosphine (TCEP) and a free radical initiator,⁷ a process that is reminiscent of the work of Hoffmann⁸ and Walling.⁹ The radical initiator decomposes into a carbon-centered free radical, which abstracts the Cys thiol hydrogen to produce a Cys

thiyl radical. The Cys thiyl radical combines with the phosphine to produce a phosphoranyl radical, the decomposition of which leads to an alanyl radical and a phosphine sulfide. Finally, the latter abstracts hydrogen from a hydrogen source, usually an exogenous thiol, to produce an alanine residue. Although complementary photo- or electrochemical desulfurization methods have been developed to trigger the in situ formation of the intermediate thiyl radical (Fig. D), the development of novel one-pot NCL/desulfurization techniques are desirable to enlarge the scope of existing methods.⁶ In particular, speeding up the Cys to Ala conversion and/or using non-toxic chemicals to effect this transformation in absence of side-reactions appear as timely challenges that mobilize the best research groups.

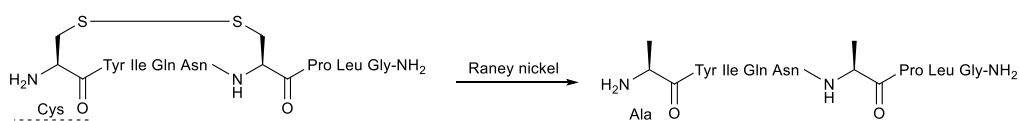
In this issue of Chem, Xuechen Li and coworkers report that fast desulfurization can be achieved by combining sodium tetraethylborate with TCEP,¹⁰ a commonly used reductant during NCL to avoid the formation of disulfides upon air oxidation. This discovery comes from an earlier observation from the authors regarding the use of superhydride (LiBEt_3H) for triggering the desulfurization of Cys in the presence of TCEP.¹¹ The dependency of the yield of desulfurization on the age of the superhydride batch led the authors to discover that a decomposition product of the superhydride, sodium tetraethylborate, was actually making the job (Fig. D). Adding sodium tetraethylborate to an NCL ligation mixture containing TCEP cleanly afforded the desulfurized product with outstanding rates, the reaction being completed in a few tens of seconds at room temperature. The authors established that sodium tetraethylborate was acting as a source of ethyl radicals by reacting with molecular oxygen. Complementary investigations also revealed that an efficient hydrogen donor source was present in solution, whose hydrogen donor capability was far superior to the classical thiols used for that purpose such as *tert*-butylmercaptan or glutathione (Fig. E). The authors propose that the hydrogen donor is the triethylborane-water complex shown in Fig. E, a hypothesis that is supported by literature data. Note that other boron species than sodium tetraethylborate such as BEt_3 or NaEt_3BH have been previously reported to perform desulfurization (Fig. D). However, these reactions were illustrated on simple substrates and required the presence of metals (Co^{2+} , Fe^{2+}) or light activation to work.

To sum up, Xuechen Li and coworkers show that sodium tetraethylborate is a powerful reagent for effecting the rapid desulfurization of Cys into Ala in one-pot following the assembly of the polypeptide using the native chemical ligation reaction. The extremely fast rates achieved with this reagent are unprecedented and its compatibility with NCL constitutes a strong asset. Sodium tetraethylborate provides the carbon-centered free radical needed to trigger the in situ formation of thiyl radicals as well as the hydrogen donor enabling the quenching of the alanyl radical.

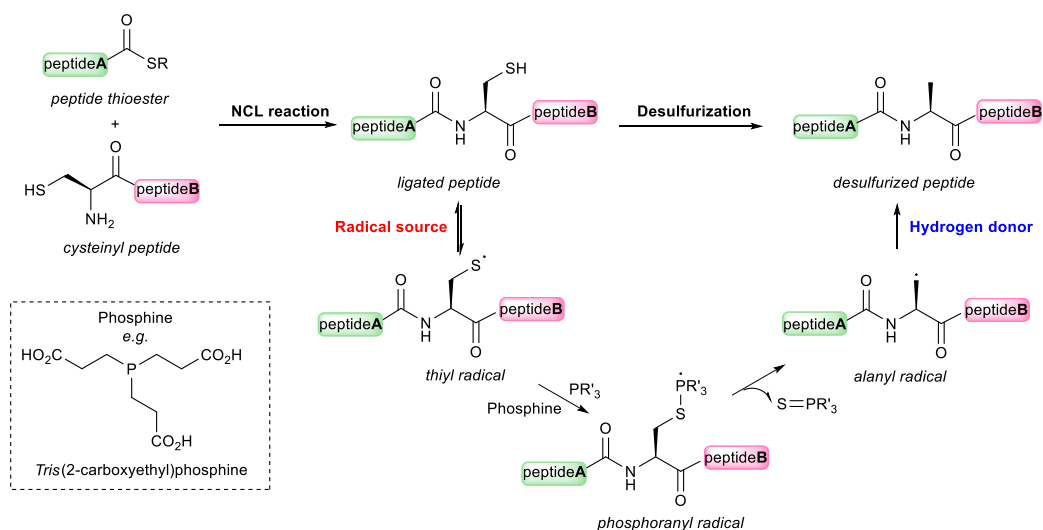
A Thiol desulfurization



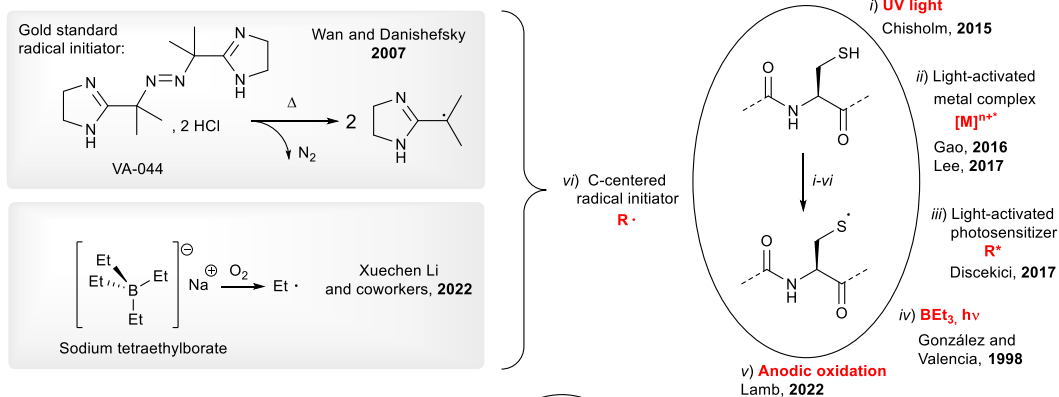
B The desulfurization of oxytocin



C Native chemical ligation/Desulfurization process



D Radical sources



E Hydrogen donors

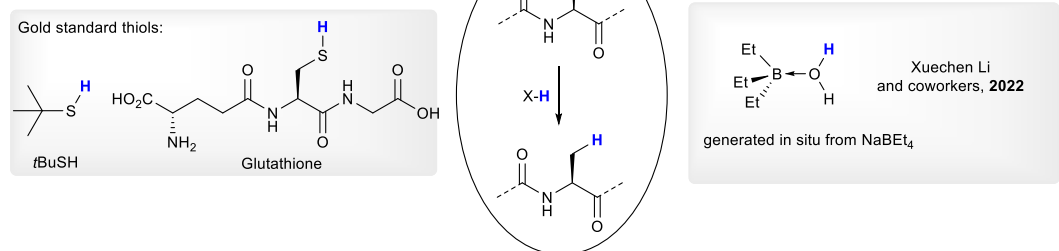


Figure. A) The reductive desulfurization of thiols into alkanes. B) In 1951, Du Vigneaud and coworkers used Raney nickel to desulfurize the peptide hormone oxytocin. C) The native chemical ligation/free-radical desulfurization strategy. D) Methods used so far for generating thiyl radicals in situ. E) Hydrogen donors used for quenching alanyl radical.

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