

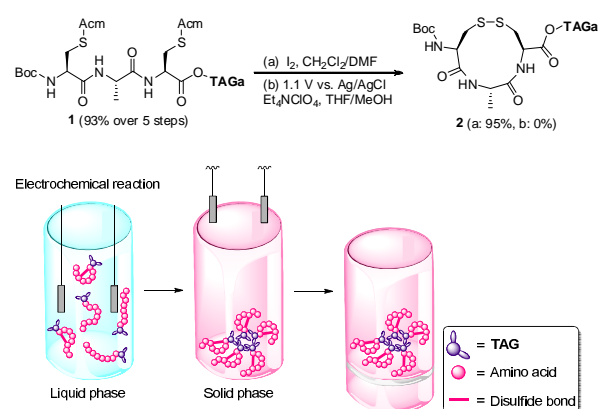
Soluble-Support-Assisted Electrochemical Reactions:  
Application to Anodic Disulfide Bond FormationShingo Kitada, Masahito Takahashi, Yusuke Yamaguchi,  
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Stemming from the pioneering work of Merrifield, the use of insoluble polymeric supports has comprised a substantial fraction of modern organic synthesis in both the academic and the industrial fields. The great advantages of such solid-phase techniques are rapid reaction work-up and product isolation in which reaction mixtures are filtered to recover the polymer-bound products, while excess reagents and impurities are rinsed away. Current chemical synthesis methods of peptides and nucleic acids, in particular, depend to a large extent on the advancement of such solid-phase techniques, enabling reliable production of the desired sequence in a programmed manner.

When it comes to electrochemical reactions, however, the use of such insoluble polymeric supports must be creative, because it is well known that electron transfer between two solid phases is severely limited, so that solid-phase-bound substrates are barely oxidized or reduced at the surface of the electrodes. Although several mediated systems have been devised, a more direct approach would be of considerable assistance in electrochemical reactions. By replacing insoluble polymeric supports with soluble variants, the familiar liquid-phase reaction conditions are reinstated, with unique product isolation methodologies including precipitation, crystallization, dialysis, centrifugation, and chromatography to ease reaction work-up and product isolation. In addition to macromolecules, several small hydrophobic molecules simply based on long alkyl chains have been proposed as soluble supports, particularly in the synthesis of oligosaccharides and peptides. In this context, we have developed soluble-support-assisted liquid-phase techniques using hydrophobic benzyl alcohols, leading to versatile preparation of bioactive peptides. Using this technique, excellent precipitation yields were realized through simple dilution of the reaction mixtures with poor solvent. Described herein is the application of soluble-support-assisted strategies to electrochemical reactions using oxidative disulfide bond formation as a model.

The present work began with the construction of electrolyte solutions applicable to hydrophobic-support-assisted techniques. We investigated numerous compositions of electrolyte solutions, using  $\text{Et}_4\text{NClO}_4$  as a less hydrophilic supporting electrolyte, and found that a 1:1 (v/v) mixture of THF and MeOH was effective for both the supporting electrolyte and the hydrophobic support. We then prepared a model peptide (**1**) for oxidative disulfide bond formation using a hydrophobic-tag-assisted method. In contrast to chemical oxidation by iodine, however, anodic oxidation of the peptide (**1**) gave no cyclized product (**2**) and the starting material was recovered quantitatively even after the application of a large excess of current. With this result in hand, we then tested other tetraethyl ammonium salts as supporting electrolytes.

Although  $\text{Et}_4\text{NI}$  was also found to be ineffective, anodic disulfide bond formation took place efficiently in the presence of  $\text{Et}_4\text{NBr}$  or  $\text{Et}_4\text{NBF}_4$ . Although the bromide anion is known to function as an electron transfer mediator, therefore the mediated mechanism can be proposed when  $\text{Et}_4\text{NBr}$  was used, this should also be proved by electrochemical analysis. For this reason, we carried out cyclic voltammetry measurements to illustrate clear-cut reaction pathway. However, when  $\text{Et}_4\text{NBr}$  was used as a supporting electrolyte (0.10 M), the oxidation current of bromide anion was observed dominantly at 0.73 V vs. Ag/AgCl and higher potentials because of its high concentration. On the other hand, the voltammogram of  $\text{Et}_4\text{NBr}$  as a substrate (1.0 mM) was recorded using  $\text{Et}_4\text{NBF}_4$  as a supporting electrolyte (0.10 M) to show reversible redox property, which changed significantly by the addition of the peptide (**1**) (1.0 mM). The oxidized species of the bromide anion generated through the electron transfer at the surface of the anode was reduced by the peptide (**1**) to regenerate the bromide anion, leading to the increased oxidative current and the decreased current. In addition to such mediated mechanism, direct electron transfer pathway might also be possible when  $\text{Et}_4\text{NBF}_4$  was used. It should also be noted that the reaction mixture was simply diluted with MeCN to induce precipitation of the product, which was recovered through filtration, while the supporting electrolyte and impurities were rinsed away.



In conclusion, we successfully applied a soluble-support-assisted technique to an electrochemical reaction to achieve anodic disulfide bond formation. The reaction was carried out in the liquid phase, which allowed electron transfer at the surface of the electrodes to occur the reaction. The support-bound product was recovered as a precipitate by simple dilution of the reaction mixture with a poor solvent. This strategy should find further application in selective disulfide bond formation, in combination with existing peptide chemistry.