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Soluble-support-assisted Electrochemical Reactions: Application to Anodic Disulfide Bond Formation

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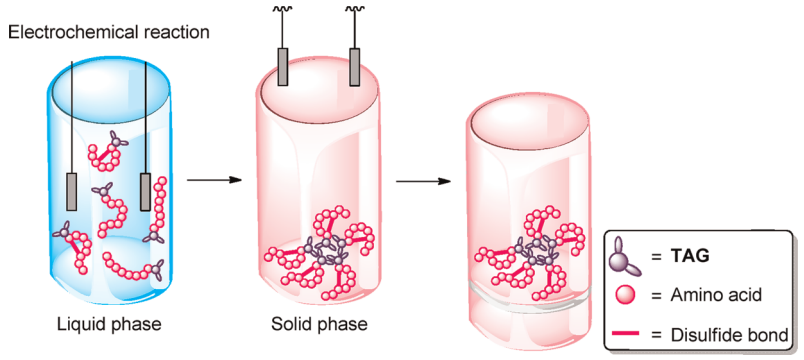
ABSTRACT



A soluble-support-assisted technique was successfully applied to electrochemical reactions, leading to anodic disulfide bond formation. The support-bound peptide was soluble in electrolyte solution, allowing electron transfer at the surface of the electrodes. After completion of the reaction, the support-bound product was recovered as a precipitate by simple dilution of the reaction mixture with poor solvent.

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.[1] The great advantages of such solid-phase techniques are rapid reaction workup 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,[2] 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,[3] 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,

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and chromatography to ease reaction workup and product isolation.[4]

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.[5] In this context, we have developed soluble-support-assisted liquid-phase techniques using hydrophobic benzyl alcohols,[6] leading to versatile preparation of bioactive peptides.[7] 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 (Figure 1). We investigated numerous compositions of electrolyte solutions, using Et_4NClO_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 (Scheme S2, Supporting Information). 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 (Scheme 1).

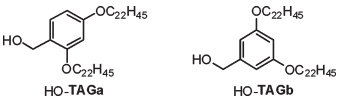
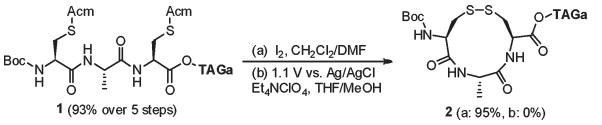


Figure 1. Structures of the hydrophobic tags (HO-**TAGa**, HO-**TAGb**) used in this work.

Scheme 1. Oxidative Disulfide Bond Formation using Iodine or Anode as Oxidant



With this result in hand, we then tested other tetraethyl ammonium salts as supporting electrolytes (Table 1). Although Et_4NI was also found to be ineffective, anodic disulfide bond formation took place efficiently in the presence of Et_4NBr or Et_4NBF_4. Although the bromide anion is known to function as an electron transfer mediator, the mediated mechanism can be proposed when Et_4NBr is used (Scheme 2), this should also be proved by electrochemical analysis. For this reason, we carried out cyclic voltammetry measurements to illustrate a clear-cut reaction pathway (Figure 2). However, when Et_4NBr 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 (Figure S1, Supporting Information). On the other hand, the voltammogram of Et_4NBr as a substrate (1.0 mM) was recorded using Et_4NBF_4 as a supporting electrolyte (0.10 M) to show reversible redox property (black line in Figure 2), which changed significantly by the addition of the peptide (**1**) (1.0 mM) (red line in Figure 2). 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 reductive current. In addition to such mediated mechanism, a direct electron transfer pathway might also be possible when Et_4NBF_4 was used. It should also be noted that the reaction mixture was simply diluted with

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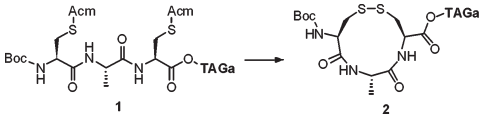
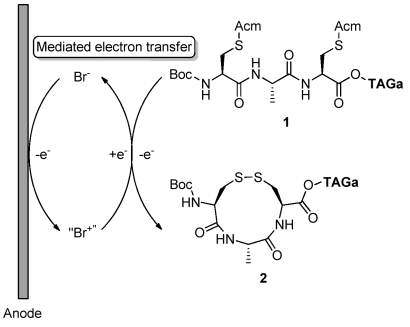
MeCN to induce precipitation of the product, which was recovered through filtration, while the supporting electrolyte and impurities were rinsed away (Scheme 3).

Table 1. Anodic Disulfide Bond Formation by Peptide **1**



solvent	supporting electrolyte	yield (%)
THF/MeOH	Et_4NClO_4	0
THF/MeOH	Et_4NI	0
THF/MeOH	Et_4NBr	88
THF/MeOH	Et_4NBF_4	98

Scheme 2. Proposed Reaction Mechanism of the Anodic Disulfide Bond Formation



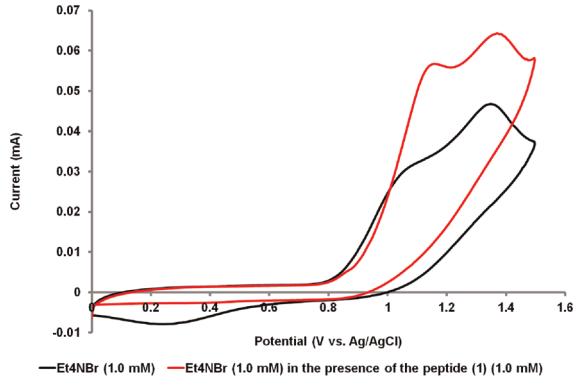
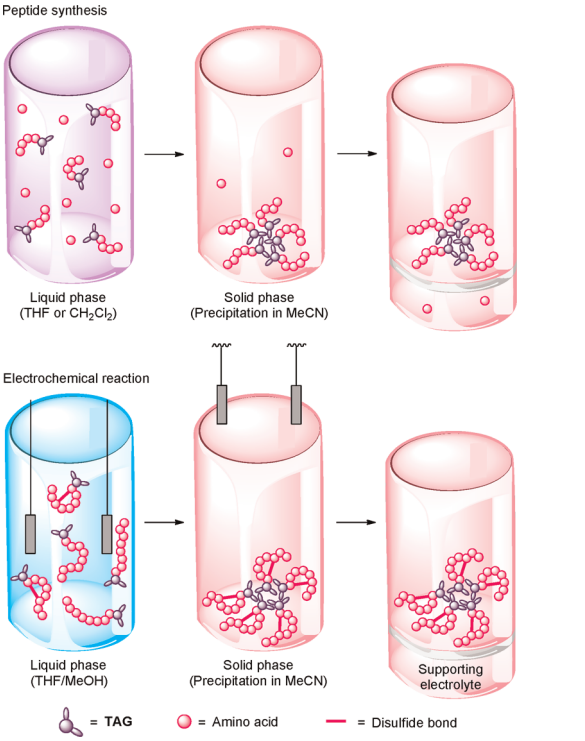


Figure 2. Cyclic voltammogram of the Et_4NBr in the absence and presence of the peptide (**1**) using Et_4NBF_4 as a supporting electrolyte.

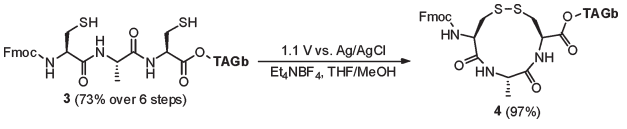
With the aim of investigating the scope of the anodic disulfide bond formation, we also prepared the side-chain-deprotected variant (**3**) using the hydrophobic-tag-assisted method (Scheme S3, Supporting Information). In this case,

Scheme 3. Overall Experimental Procedure for Soluble-support-assisted Anodic Disulfide Bond Formation



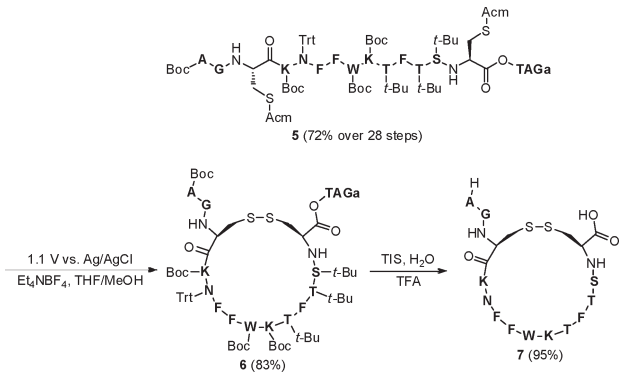
acidic deprotection was required to remove the trityl groups. Thus, the acid-resistant tag HO-**TAGb** and the Fmoc group were employed instead of HO-**TAGa** and Boc. The anodic disulfide bond formation of the peptide (**3**) was also attempted in the same electrochemical condition, resulting in the formation of the desired cyclic peptide (**4**) in excellent yield, which largely similar to that of the peptide (**1**) (Scheme 4).

Scheme 4. Anodic Disulfide Bond Formation in the Peptide (**3**)



Encouraged by these results, we then applied the soluble-support-assisted anodic disulfide bond formation method to bioactive peptide synthesis using a growth-hormone-inhibiting peptide, somatostatin (**7**), as a model (Scheme 5). The corresponding sequence (**5**) was elaborated based on the hydrophobic-tag-assisted method, followed by anodic disulfide bond formation to afford the desired cyclic peptide (**6**) in high yield. Finally, all protective groups were removed under acidic conditions to give somatostatin (**7**) in excellent yield.

Scheme 5. Synthesis of Somatostatin (**7**) by Anodic Disulfide Bond Formation



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 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.

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Supporting Information Available. Additional schemes, figure, and experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.