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Voltammetric behavior of selenocystine at modified gold substrates

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ABSTRACT

The voltammetric response of seleno-L-cystine has been studied at gold substrates under physiological conditions. The reactivity of diselenides is utilized to generate a modified electrode surface, which in turn allows for electroanalysis of solution-based selenium species. Stable and reproducible voltammetry is observed for selenocystine reduction at pH 7 on selenium-modified gold substrates ($E_{mid} = -486$ mV vs. Ag/AgCl) and is consistent with a diffusionally controlled process. Modification of the gold surface is readily achieved via electrochemical cycling in the presence of a diselenide source at conventional scan rates. These studies afford voltammetric characterization of the selenocystine/selenocysteine redox couple under physiologically relevant conditions and highlight the potential utility of selenium-modified substrates for electroanalysis of chalcogen-containing species.

1. Introduction

Selenium is a trace element in humans, found primarily in the form of the amino acids selenocysteine (SeCys) and selenomethionine (SeMet) [1–4]. Selenocysteine is present at the active site of several redox enzymes, such as thioredoxin reductases, and can react with its sulfur analog cysteine to form a selenylsulfide bond during the reduction of disulfides [5–15]. Much recent work has examined the diselenide bond that can form via intra- or intermolecular oxidation of two selenocysteine residues [16–23]. Native diselenides have been identified in proteins isolated from several aquatic organisms [17–19]. Though most human selenoproteins contain only a single selenocysteine residue, formation of diselenide bridges has been demonstrated under physiological conditions [3,17,24]. Rozovksy and coworkers recently reported that the mammalian membrane protein selenoprotein K forms an intermolecular diselenide bond in isolation [19]. If the diselenide formation proceeds in the cellular environment, it would mark the first example of a native Se–Se bond in a human protein system. Electrochemical characterization of diselenide bond formation and reduction under physiologically relevant conditions is needed to advance fundamental biochemical studies of selenium-containing amino acids and selenoproteins.

Electroanalysis of biological selenium species, however, is challenging because of unwanted oxidation and adsorption processes at metallic surfaces. The earliest reported electrochemical studies of the selenocysteine/selenocystine (SeCys/SeC) couple were measured on hanging mercury drop electrodes (HDME) and often in acidic conditions [25–27]. Polarographic studies revealed the formation of a mercuro-selenocystinate film [27]. In a more recent report, quasi-reversible voltammetric behavior was described for SeC reduction at HDME under physiological conditions [3]. While these measurements allowed the redox potential to be assessed and comparisons to the sulfur analog drawn, rigorous electrochemical evaluation of the selenocysteine/selenocystine couple under the reported conditions is limited by surface reactivity with the mercury substrate.

Over the past decade, the behavior of selenium-containing amino acids on gold and gold-modified electrodes has been explored [28–32]. Both selenomethionine and selenocysteine have been shown to adsorb onto gold substrates, the latter of which was proposed to form a self-assembled monolayer that supports diffusion controlled electron transfer to ferricyanide [28,30]. Bai and coworkers demonstrated that modification of glassy carbon substrates with a gold–selenium film afforded voltammetric detection of selenocystine that was not observable on the bare carbonaceous material [31,32]. These studies, however, are conducted in acidic media to promote solubility and film deposition, and thus do not reflect the native environment of the selenocysteine/selenocystine redox couple. Further, lengthy incubation times (>24 h) are required for film formation, limiting the applicability of the approach.

Here, we report voltammetric detection of the selenocystine/selenocysteine couple at modified gold substrates under mild experimental conditions and at relatively short timescales. The intrinsic reactivity of the dichalcogenide bond is harnessed to modify the metallic electrode surface, which in turn makes accessible electroanalysis of bulk selenium analyte. The described approach yields well-behaved

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and reproducible voltammetry for selenocystine reduction in a physiologically relevant environment.

2. Materials and methods

2.1. General

All chemical reagents used were of analytical grade. Potassium chloride, seleno-L-cystine, selenium dioxide, sodium phosphate dibasic, and sodium phosphate monobasic were purchased from Sigma Aldrich; gold(III) chloride was purchased from Acros Organics. Buffered solutions were prepared using distilled water and filtered prior to use. The pH was measured with an Accumet AB15 pH meter (Fisher Scientific). Seleno-L-cystine solutions were sonicated for 30 min and heated at 40 °C for 20 min to promote dissolution.

2.2. Electrochemical measurements

Electrochemical measurements were performed on μAutolab Type III and PGSTAT20 potentiostats (Eco Chemie, Metrohm) and in a Faraday cage. The electrochemical cell contained a platinum wire counter electrode, a Ag/AgCl reference electrode, and either a gold (d = 1.6 mm) or glassy carbon (d = 2.4 mm) disk working electrode. The electrodes were mechanically polished with 1.0 μm and 0.3 μm alumina slurries (Buehler). Solutions were purged with N_2 (g) prior to measurements.

2.3. Surface modification

A selenium-based film was formed on gold by electrochemically cycling the electrode in a seleno-L-cystine or selenium dioxide (SeO_2) solution or by incubating the electrode in a seleno-L-cystine solution at room temperature. A selenium-based film was formed on the glassy carbon electrodes following a reported protocol [32]. Briefly, a glassy carbon substrate was incubated in a mixture of SeO_2 (~80 mM/0.1 M KCl) and $AuCl_3$ (~10 mM/0.1 M KCl) under an applied potential of −0.85 V for 60 s.

3. Results and discussion

The electrochemical behavior of the selenocysteine/selenocystine redox couple was examined by cyclic voltammetry at physiological pH. Fig. 1 shows an overlay of the consecutive cycles recorded in a selenocystine/phosphate buffered saline (PBS, pH 7) solution. Initial voltammetric scans (n < 10) show essentially no redox activity on gold in the potential window under study (Fig. 1, inset). A well-defined voltammetric couple emerges with continuous cycling, however, and is assigned to the reduction and oxidation of selenocystine and selenocysteine, respectively, at a modified substrate. After 100 scans, a stable and reproducible signal is achieved (E_{mid} = −486 mV vs. Ag/AgCl) and the peak separation narrows from ΔE_p~350 mV (scan 15) to ΔE_p~65 mV (scan 100). This behavior is consistent with a two-step mechanism in which the diselenide is first cleaved to yield a selenium–gold adsorbate and then bulk selenocystine is reduced at the modified electrode. The formation of gold–selenium monolayers and films via Se–Se bond cleavage is well documented in the literature [33–43].

The voltammetric response achieved post-cycling is characteristic of a diffusionally controlled process. Fig. 2 illustrates the scan rate dependence observed for the SeC/SeCys couple once a steady signal is established. Peak currents scale linearly with the square root of scan rate for both the anodic and cathodic reactions over the range 0.02 to 1 Vs^{-1}. The peak separation (ΔE_p) varies with scan rate (Fig. 2, inset), indicative of irreversible behavior, while the midpoint potential remains essentially constant. Measured peak currents agree closely with those approximated for a two-electron reduction in the irreversible limit, using the diffusion coefficient of cystine as an estimate (Fig. 2, inset)

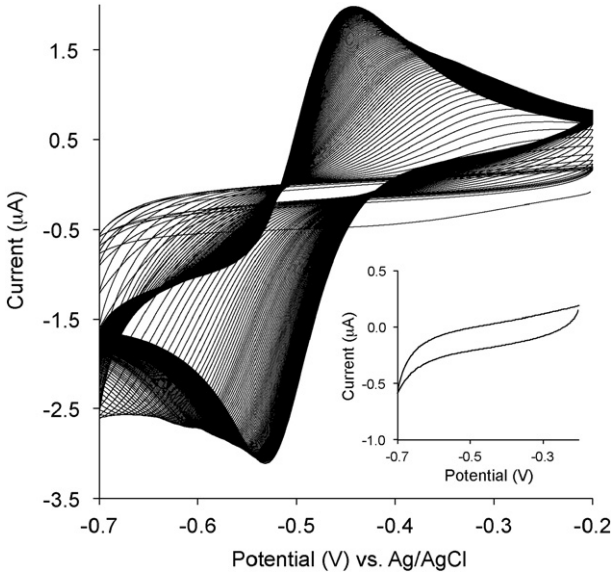

Fig. 1. Consecutive cyclic voltammograms of 0.4 mM seleno-L-cystine in 200 mM PBS (pH 7) at a gold electrode. 100 scans plotted. Scan rate: 0.1 Vs^{-1}. Inset: scan 3.

[44]. Ongoing electrochemical and spectroscopic studies will further assess the electrode kinetics and reduction mechanism.

Additional evidence for the diffusional nature of the observed voltammetry is provided by examining the behavior of the modified gold substrate in the absence and presence of bulk selenocystine. When an electrode that has been cycled in a SeC solution (Fig. 3, black trace) is placed into 'blank' phosphate buffer (no selenium analyte), all Faradaic activity is lost in the potential window of study (Fig. 3, gray line). Upon returning the electrode to a selenocystine solution, the redox couple reappears on the *first* scan (Fig. 3, inset) and is stabilized by the tenth (Fig. 3, dotted line). Moreover, the CV response is essentially unchanged after stirring the analyte solution under nitrogen, verifying that the redox activity does not result from selenocystine accumulation at the electrode/solution interface. These results confirm that the observed voltammetry is due to solution-based selenium analyte, not an

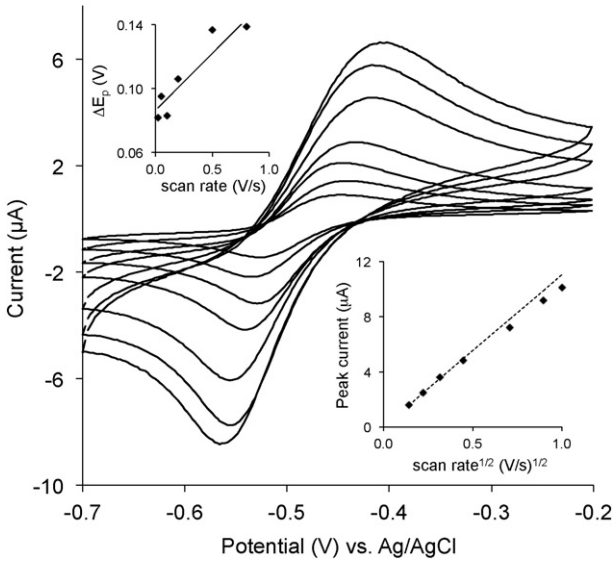

Fig. 2. Scan rate dependence (0.02–1 Vs^{-1}) of 0.4 mM seleno-L-cystine in 200 mM PBS (pH 7) at a modified gold electrode, post electrochemical cycling. Inset: (*left*) plot of peak separation (ΔE_p) as a function of scan rate; (*right*) plot of peak current as a function of the square root of scan rate (dashed line represents theoretical currents estimated assuming [SeC] = 0.4 mM, $D = 5.3 \times 10^{-6}$ cm^2/s, n = 2, α = 0.5).