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Ion-channel mimetic sensor incorporating an anion-binding cyclopeptide designed for sulfate determination in dilute aqueous solutions

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ABSTRACT

A sensor designed for anion detection in water based on an ion-channel mimetic system is described. This sensor consists of a gold electrode modified with an anion-binding cyclopeptide **CP** that mediates the recognition of sulfate. The number of **CP** molecules covalently attached to the gold electrode via Au$-$S bonds was controlled by using 6-mercapto-1-hexanol (**MCH**) as "diluent" molecule. The optimal molar ratio **CP**/**MCH** (10^{-6} M/10^{-6} M) in the solution used for electrode modification was determined experimentally. The presence of **CP** molecules as well as their orientation on the gold surface was studied by using voltammetry techniques, contact angle measurements, and atomic force microscopy. The electrolyte composition as well as the appropriate pH suitable for anion recognition was also determined. Anion detection was explored by using cyclic voltammetry (CV) and Osteryoung Square wave voltammetry (OSWV). The developed sensor was shown to allow detection of sulfate in highly dilute aqueous media with a 0.25 pM detection limit. Chloride, phosphate and acetate anions generated substantially lower responses.

1. Introduction

A large number of artificial anion receptors are nowadays available [1–3]. Many of these receptors bind their respective substrates only in organic solutions, however [4–13]. Solvent molecules and also the presence of competing salts typically substantially impair the anion affinity of many of these receptors in water [3]. Therefore, the design of receptors, which can overcome these problems and bind anions in water media with high affinities is a very challenging task [14–21]. Interestingly, several peptide or cyclopeptide-derived receptors have been shown to recognize sulfate with relatively high selectivity in organic solvents and also in more competitive aqueous solvent mixtures [18–32]. An example is the cyclic hexapeptide comprising an alternating sequence of L-proline and 6-aminopicolinic acid subunits that binds halide and sulfate anions by sandwiching them between two cyclopeptide rings [22]. Bis(cyclopeptides) containing two cyclopeptide rings covalently linked via appropriate linkers or analogues of the monotopic cyclopeptide containing hydroxyproline instead of proline subunits form 1:1 complexes with halides and sulfate anions in water [29–32], but the incorporation of such hosts in analytical tools is still rare [25].

In our group, electrochemical sensors based on self-assembled monolayers (SAM) on gold electrode surfaces were developed for the selective detection of various analytes [33–35]. One of our approaches involves the use of an ion-channel mimetic sensor, which has first been reported by Umezawa and co-workers in 1987 [36]. In this system, the binding of ions present in the surrounding aqueous medium to receptor molecules attached to the electrode surface changes the charge of the analytically active layer. As a consequence, the accessibility of a redox marker present in the sample solutions, which depends on electrostatic forces, is improved or impaired [36–38]. This mode of action, adapted for the ion channel sensor described here, is shown in Fig. 1.

Examples of anion sensors for halides and phosphate based on self-assembled monolayers have already been reported [39–42], but to the best of our knowledge, systems that respond to the presence of sulfate ions have not been described yet. From the viewpoints of medical diagnosis and environmental analysis the development of methods for the sensitive and selective detection of anions in water is, however, highly relevant.

In this paper, we present a new electrochemical sensor, which can fulfil this need. This sensor contains the cyclopeptide receptor molecule **CP** (Fig. 1) together with 6-mercapto-1-hexanol (**MCH**) attached to the gold electrode surface through Au$-$S covalent bonds. The recognition processes between **CP** and anions were explored by using cyclic voltammetry and Osteryoung square wave voltammetry (OSWV) in the presence of $[Fe(CN)_6]^{3-/4-}$ as a redox marker. Based on the reported

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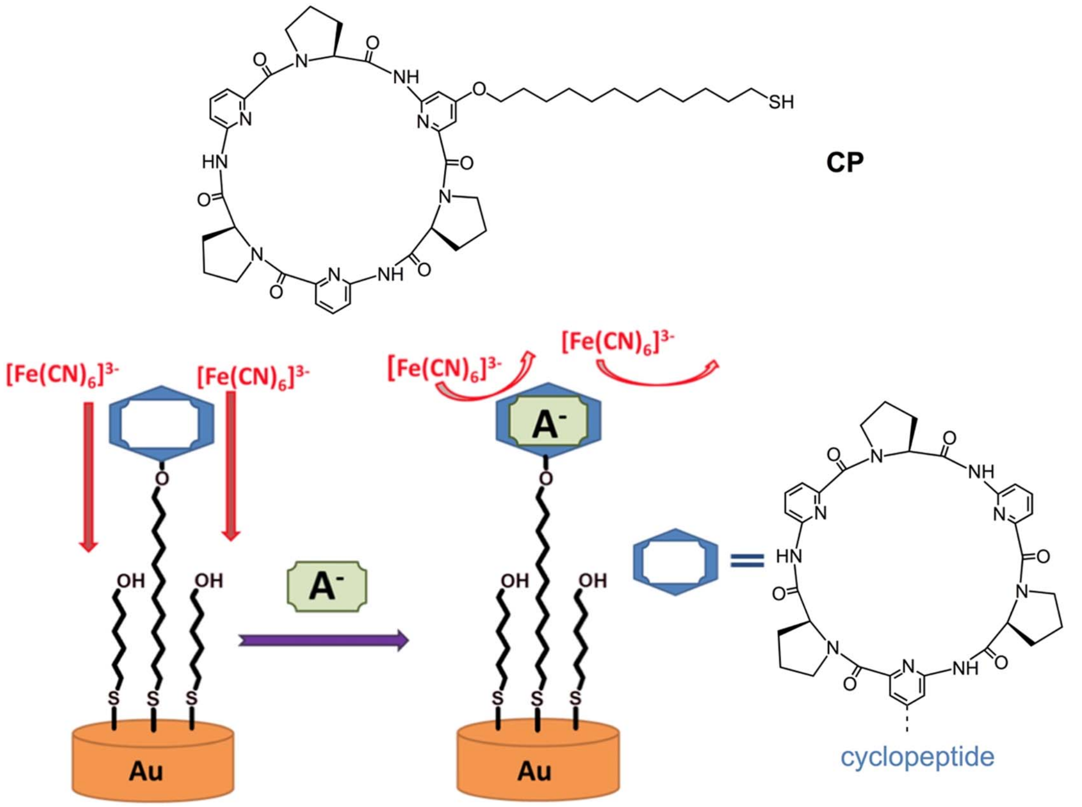

Fig. 1. Schematic assembly of the investigated ion-channel mimetic sensor with **CP** as a receptor.

anion selectivity of **CP**, SO_4^{2-} was chosen as the target anion. As reference anions, $H_2PO_4^-$, Cl^- and CH_3COO^- that should bind less strongly to **CP** were selected to assess the selectivity of the sensor [22].

2. Experimental section

2.1. Materials and chemicals

6-Mercapto-1-hexanol (**MCH**), dichloromethane, cyclohexane, sodium nitrate, sodium phosphate dibasic, sodium chloride, sodium acetate, potassium hexacyanoferrate(III) and potassium hexacyanoferrate(II) were obtained from Sigma-Aldrich (Poznań, Poland). Methanol, boric acid, potassium hydroxide and sulfuric acid were purchased from Avantor Performance Materials Poland S.A. (Gliwice, Poland). All aqueous solutions were prepared with deionized water (resistivity of 18.2 M Ω cm) obtained with a Milli-Q reagent grade water system (Millipore, Bedford, MA). All experiments were carried out at room temperature. The synthesis of **CP** is described in the Supporting information. This compound was used as a mixture of the free thiol and the respective disulfide.

2.2. Gold electrode modification

Gold disk electrodes with a diameter of 2 mm (Bioanalytical Systems (BAS), West Lafayette, IN, USA) were used for the experiments. They were initially cleaned mechanically by polishing with alumina slurries (Alpha and Gamma Micropolish, Buehler; Lake Bluff, IL, USA) with particle sizes of 0.3 and 0.05 μm on a micro cloth pad (BAS) for 5 min each. Afterwards, they were carefully rinsed with Milli-Q water. The polished electrodes were further cleaned electrochemically by cyclic voltammetry. At first, they were dipped in 0.5 M KOH solution, swept with a potential between −400 mV and −1200 mV against the Ag/AgCl reference electrode (with 3 M KCl inner solution) and the platinum wire counter electrode with a scan rate of 100 mV s^{-1}. Initially, they were submitted to 3 cycles, then to 10 cycles, and finally to 3 more cycles, performed under the same conditions.

Next, the electrodes were cleaned in 0.5 M H_2SO_4. Cyclic voltammograms (CVs) in the potential window between −300 mV and +1500 mV were recorded for 3 cycles, then to 10 cycles and finally to 3 more cycles. All of these cycles were performed under the same conditions. Before modification, the surfaces of electrodes were refreshed in a 0.5 M KOH solution for 10 cycles. Then, after washing with Milli-Q water, methanol and dichloromethane/methanol, 1:1 (v/v), the electrodes were immersed in dichloromethane/methanol, 1:1 (v/v) containing **CP** and **MCH** in concentrations of 10^{-6} M each. After washing with the dichloromethane/methanol mixture, methanol, Milli-Q water, and buffer, the electrodes were conditioned in buffer overnight.

2.3. Electrochemical measurements

All electrochemical measurements were performed with a potentiostat–galvanostat AutoLab (Eco Chemie, Utrecht, The Netherlands) with a conventional three electrode configuration. The following electrode configuration was applied: a gold (BAS) working electrode, an Ag/AgCl reference electrode and a Pt counter electrode. CV was performed with potentials from +0.6 V to −0.2 V with a scan rate of 0.1 V/s. OSWV was registered in a potential window from +0.6 V to −0.1 V with a step potential of 0.001 V, a square-wave frequency of 25 Hz, and an amplitude of 0.05 V.

2.4. Contact angle measurements

Au(111) films with a thickness of 100 nm, deposited on mica covered with Ti (10 nm) (generous gift from Interuniversity Microelectronics Centre, Leuven, Belgium) were washed with Milli-Q water, methanol, and cyclohexane. Next, the films were cleaned in a UV/ozone chamber for 25 min. Subsequently, gold-mica plates were annealed with a hydrogen flame. The films were modified in the same