Journal of Inclusion Phenomena and Macrocyclic Chemistry **49**: 187–191, 2004.

Measurement of Ibuprofen Binding to Mixed Monolayers Containing β-Cyclodextrin Active Sites

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(Received: 15 July 2003; in final form: 25 November 2003)

Key words: gold surface, ibuprofen, self-assembly, voltammetry

Abstract

A molecular imprinting method involving a three-step sequential self-assembly procedure was applied to prepare gold electrodes responsive towards ibuprofen. The electrode modified with the cyclodextrin derivative binds ferrocene to form an electroactive complex with the ferrocene oxidation current decreasing in the presence of ibuprofen in the solution. The competition of ferrocene and ibuprofen for the cyclodextrin cavities in the monolayer provided a means for the determination of the binding constants of ibuprofen with two derivatives of lipoylamide β-cyclodextrin of different hydrophobicity.

Introduction

Host/guest complexation studies with cyclodextrins are of importance for drug delivery systems [1, 2]. Cyclodextrins have been used as complexing agents for chromatographic and capillary electrophoretic separations of ibuprofen, a non-steroidal anti-inflammatory drug [3]. Drug-carrier interactions between ibuprofen and native and selected chemically modified β-cyclodextrins in solution and in the solid state have been also described [4, 5].

Self-assembled monolayers of mono-lipoyl-β-cyclodextrin derivatives with different chain lengths were first prepared by Fang and co-workers and used for the modification of electrodes [6]. Ferricyanide and *p*-aminophenol did not give any response on such modified gold electrodes while ferrocene carboxylic acid and hydroquinone entered the cavities of the cyclodextrin (CD) receptor and underwent redox processes easily detectable by the voltammetric method. From reductive desorption experiments of chemisorbed disulfides, the authors estimated the gold surface coverage with the lipoic-β-CD derivatives. The number of adsorbed molecules exceeded that corresponding to a monolayer which was explained assuming that the β-CD rings were partially folded in among the polyethylene chains. The same group described voltammetric sensors for organic compounds built of lipoylaminohexanoic-β-CD [7]. Molecules forming inclusion complexes with β-CDs were chosen as electroactive markers. When some electroinactive molecules were added to the solution, partial blocking of the β-CD sites was noticed and the peak currents of the electroactive probes decreased. On the basis of experimental data, calibration curves for seven non-electroactive guests were presented. Although these compounds can be self-assembled in monolayers on gold electrodes, no approach to use the monolayer for drug sensing has been made. The aim of this work is to establish the strength of binding of ibuprofen to monolayers of the lipoylamide β-CD and to find the optimal conditions for the determination of ibuprofen using the CD-modified electrodes.

Positional isomers of γ-CD derivatives with two lipoic ester groups per molecule were synthesized by Suzuki and co-workers [8]. Kitano and Taira presented the synthesis of 6-deoxy 6-lipoylamido α-CD with an average degree of substitution of 2.5 lipoic units in the CD molecules [9]. In our approach, two CD derivatives, mono(6-deoxy-6-lipoylamide)-per-2,3,6-*O*-acetyl-β-cyclodextrin and its per-*O*-Me analogue, were employed as electrode modifiers to induce the electrode response towards ibuprofen in the solution.

Experimental

Reagents

The synthesis of mono(6-deoxy-6-lipoylamide)-per-2,3,6-*O*-acetyl-β-cyclodextrin (**1a**) used for the modification of the gold electrode surface was described previously [10]. The per-*O*-Me analogue, mono(6-deoxy-6-lipoylamide)-per-2,3,6-*O*-methyl-β-cyclodextrin (**2**), was prepared in a similar way as described earlier [11]. Ibuprofen was purchased from SIGMA and used as received.

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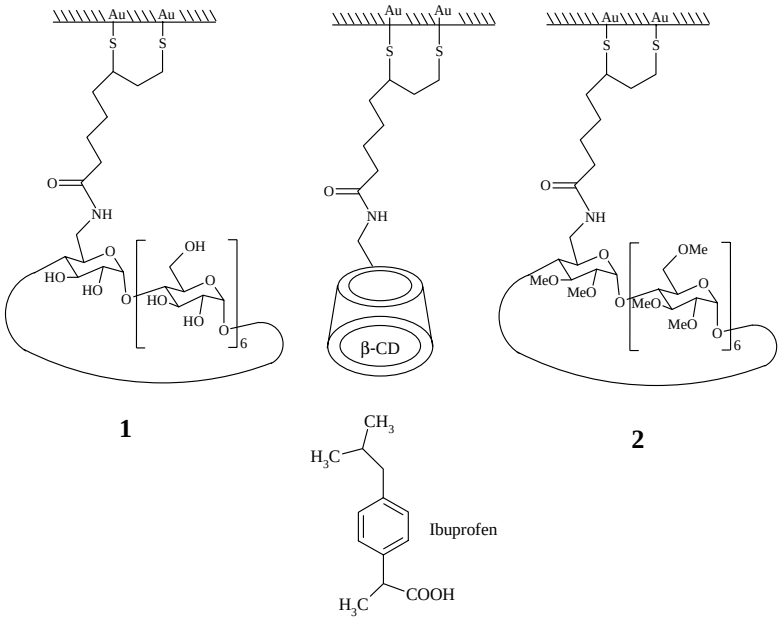

Scheme 1. Receptors used for the modification of the gold electrodes mono(6-deoxy-6-lipoylamide)-*β*-cyclodextrin (**1**) and mono(6-deoxy-6-lipoylamide-per-2,3,6-*O*-methyl)-*β*-cyclodextrin (**2**).

Preparation of electrodes

A stepwise self-assembly procedure was employed. First the mono(6-deoxy-6-lipoylamide)-per-2,3,6-*O*-acetyl-*β*-cyclodextrin (**1a**) monolayer was formed by immersing the gold electrode for ~16 h in 1 mM ethanol solution of **1a**. Next, the adsorbed layer was deacetylated by placing the electrode in alkaline solution for one hour. Then the electrode was transferred to 0.2 mM solution of ferrocene (Fc) in $EtOH/H_2O$ (50/50%). Inclusion of Fc into the cavities prevented inclusion of the alkanethiol molecules into the CD molecules in the following step [12, 13]. In the final step, an ethanol solution ~10 mM of octanethiol was added and the electrode was left in this solution for another two hours in order to block the surface not occupied by the *β*-CD receptors (the final concentration of octanethiol in the solution was ~1 mM). Before using the electrode substrates for the electrochemical experiments, they were carefully washed with ethanol and with Milli Q water.

For the Au electrode covered with mono(6-deoxy-6-lipoylamide)-per-2,3,6-*O*-methyl-*β*-cyclodextrin (**2**) no deacetylation step was needed and the same molecular imprinting method was employed.

Cyclic voltammetry

Cyclic voltammetry experiments were accomplished using the Autolab potentiostat (ECO Chemie, Netherlands) in a three electrode arrangement with the saturated Ag/AgCl separated from the bulk solution by double junction filled with 0.2 M Na_2SO_4 as the reference, a platinum wire as the counter, and gold disk electrode (2.85 mm^2 surface area) as the working electrode. Argon was used to deaerate the solution, and an argon blanket was maintained over the solution during the experiments.

Results

Two different *β*-CD derivatives **1** and **2** (Scheme l) were studied as electrode modifying agents responsible for the inclusion properties of the modified surface.

The gold electrodes imprinted as described in *Preparation of electrodes*. using mono(6-deoxy-6-lipoylamide)-per-2,3,6-*O*-acetyl-*β*-cyclodextrin (**1a**), ferrocene (Fc) and octanethiol, next deacetylated, washed, and transferred to a pure supporting electrolyte did not show the voltammetric signal of Fc which indicated effective removal of Fc to the solution (dotted line Figure 1). Typical CV curve obtained for (**1**) molecularly imprinted with Fc and octanethiol Au electrode is shown in Figure 1. The thiol 'sealing component' is needed to cover the parts of the electrode not occupied by the cyclodextrin (CD) molecules, while the Fc added in the imprinting step prevents unwanted inclusion of alkanethiols into the CD cavities. Using this approach, we obtain a well organized and stable monolayer on gold. Upon addition of Fc to this solution, a couple of surface peaks appeared corresponding to the Fc/Fc^+ system [10]. The peak height increased linearly with the Fc concentration in the solution proving that the electroactive guest binds to the surface (Figure 1).

At higher Fc concentrations (over $\sim 2.5 \times 10^{-5}$ M), the current attains a limiting value indicating that no more binding sites are available in the monolayer. Since the solubility of Fc in aqueous solution is 6×10^{-5} M

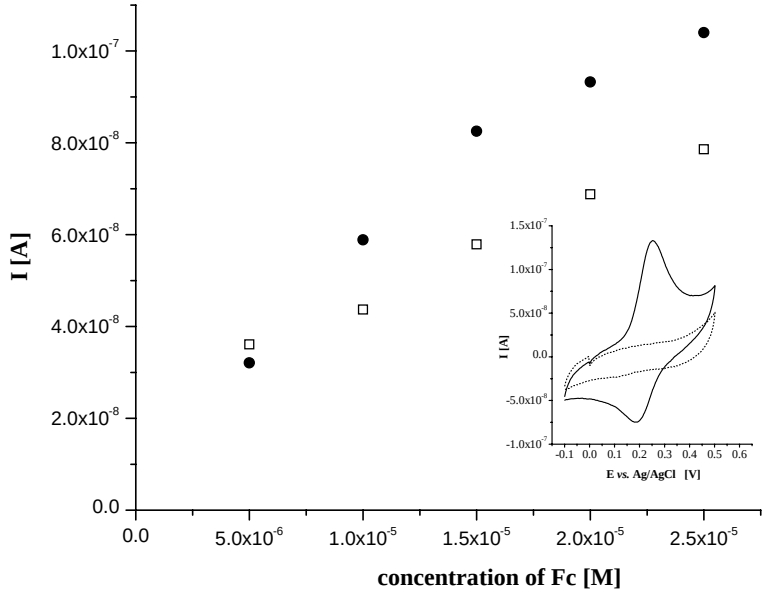

Figure 1. Plot of oxidation peak current *versus* Fc concentration measured using Au electrodes covered with monolayer containing receptor **1** (●), and receptor **2** (□), recorded in 0.2 M Na_2SO_4 solution. Inset shows voltammetric curves for electrode modified with **1**, recorded in pure supporting electrolite (*dotted line*) and in the solution containing 2.0×10^{-5} M Fc (*solid line*).

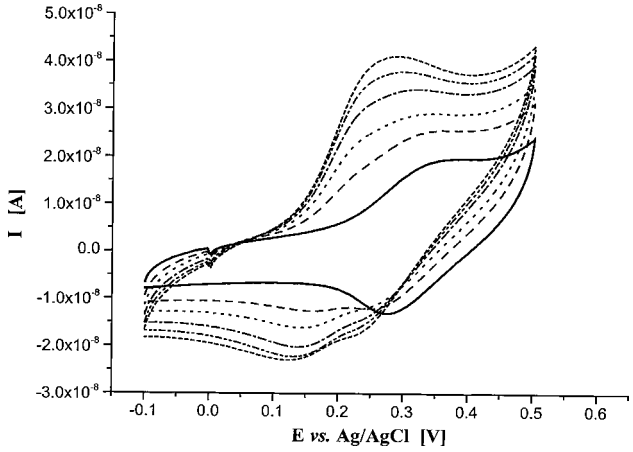

Figure 2. Voltammograms recorded in pure supporting electrolyte 0.2 M Na_2SO_4 (*solid line*) and in the solution containing also 0.5, 1.0, 1.5, 2.0, and 2.5×10^{-5} M Fc, recorded using Au electrode molecularly imprinted with receptor **2**. Scan rate 20 mV/s.

[12], this means that equilibrium concentration is attained earlier than the solubility limit.

Using the same procedure as for compound **1a**, monolayers containing compound **2** were also formed on the gold electrode. Interestingly, for the methylated CD, washing with water or ethanol did not remove the Fc molecules incorporated into the monolayer during the imprinting step. The anodic peak current was linearly dependent on the scan rate proving surface confinement of Fc. Upon addition of Fc stock solution to obtain concentrations higher than 5×10^{-6} M, a new peak can be recognized in the voltammogram (Figure 2). Square root peak current dependence on scan rate points to the diffusion control rather than surface immobilization of the species oxidized with the formation of this additional peak.

In case of receptor **2**, the peak current increased well over the concentration at which leveling of the current was observed for the CD receptor **1** (Figure 1), indicating much stronger binding. On the other hand, removal of Fc to a solution of pure supporting electrolyte (0.2 M Na_2SO_4) was extremely slow for the methylated receptor **2**. Even after leaving the electrode overnight in a stirred solution, some of the CD sites were still occupied (see non-zero intercept on the plot for receptor **2** in Figure 1). However, after adding Fc, no fast inclusion was observed either, which indicated that inclusion of alkanethiols present on the surface into the cavity may be more efficient than inclusion of Fc. Such a 'used' electrode placed in the imprinting solution did not return to its originally imprinted form. Figure 3 shows influence of the possible alkanethiol replacement on the shape of CV curves. The reduction peak current is decreased, but still present since a fraction of imprinted Fc remains in the cavities. This explains the non-zero value of intercept. This means that the thiol molecules were partially replaced into the cyclodextrin cavity. Similar behaviour was described by Choi and Park [13] for per(6-thio)-β-cyclodextrins as due to the dynamic and reversible alkanethiol adsorption process, more dynamic than that of the thiolated CD. Behaviour of this type was not observed for receptor **1**, probably due to less hydrophobic character of this receptor. The binding constant for receptor **2** was determined by fitting the experimental data to the following equation [14, 15]:

$$[\mathrm{Fc}]/I = 1/K_1 I_{\max} + [\mathrm{Fc}]/I_{\max}, \qquad (1)$$

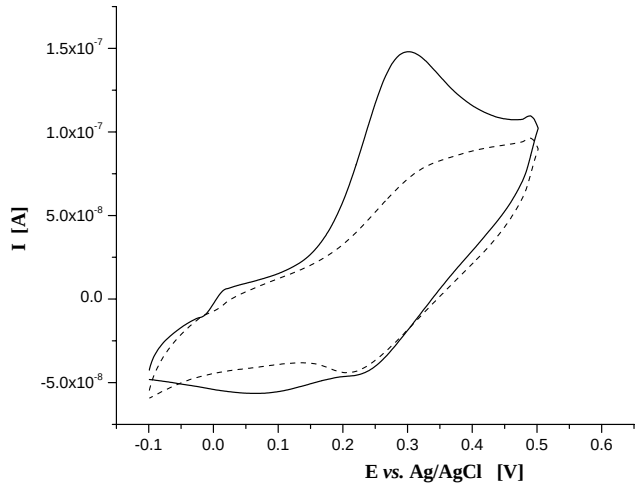

Figure 3. Cyclic voltammograms recorded in 0.2 M Na_2SO_4 solution containing 2.0×10^{-5} M Fc, using the electrode modified with receptor **2**, directly after imprinting (*solid line*) and after holding it overnight in pure 0.2 M Na_2SO_4 solution with stirring (*dashed line*). Scan rates 100 mV/s.

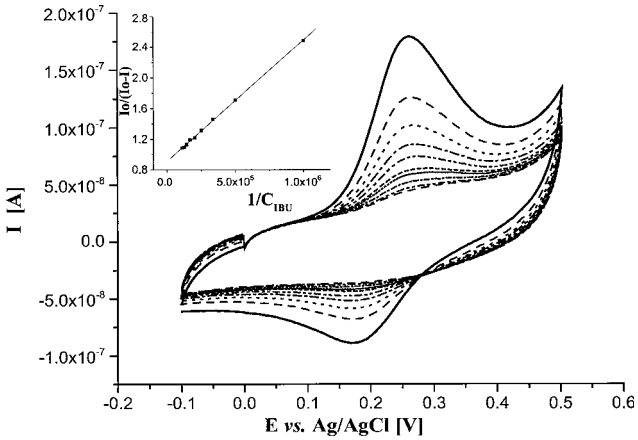

Figure 4. Voltammograms recorded using electrode modified with receptor **1** in 0.2 M Na_2SO_4 solution containing 1.5×10^{-5} M Fc before (*solid line*) and following addition of ibuprofen from 1 to 9×10^{-6} M (*dashed lines*). Scan rate 100 mV/s. Inset shows linear fit to $I_0/(I_0-I)$ *versus* $1/C_{IBU}$ plot.

where I is the anodic peak current for a given concentration of Fc, I_{max} is the maximum peak current when the current becomes constant and K_1 is the association constant of Fc with surface confined compound. The association constant for Fc and compound **2** in the monolayer was 1.33×10^5 M^{-1}, hence higher than with compound **1** (3.3×10^4 M^{-1} [10]) or with per(6-deoxy-6-thio)-β-CD (3.9×10^4 M^{-1}) reported by Rojas *et al*. [16].

Application of imprinted electrodes for ibuprofen sensing

*Monolayer with mono(6-deoxy-6-lipoylamide)-per-2,3,6-O-acetyl-β-cyclodextrin (**1**)*

The imprinted electrode was immersed in the supporting electrolyte solution and ethanolic solution of Fc was added to obtain 1.5×10^{-5} M concentration. The peak current in the absence of ibuprofen, I_0 was measured. Upon addition of small portions of stock solution of racemic ibuprofen (IBU) in ethanol the peak current decreased compared with the initial value I_0. The CV curves for increasing concentrations of IBU are presented in Figure 4.

The anodic peak current I of the oxidation of Fc is plotted as $I_0/(I_0-I)$ ratio *versus* $1/C_{IBU}$. The linear fit to this plot presented in the inset of Figure 4 is used for the calculation of ibuprofen binding constant (K_{IBU}) to the CD receptor.

Based on Equation [16]

$$(I_0 - I)/I_0 = K^*_{IBU} C_{IBU} / [(K^*_{IBU} C_{IBU}) + 1 + (K^*_{Fc} C_{Fc})]. \quad (2)$$

After simple transformation of Equation (2) we introduce B equal to

$$B = (1 + K_{Fc} C_{Fc})/K_{IBU}, \quad (3)$$

where B is the slope of the $I_0/(I_0-I)$ *versus* $1/C_{IBU}$ in Figure 4. Binding constant of ibuprofen to the monolayer of **1** is equal to 9.44×10^5 M^{-1}.

*Monolayer with mono(6-deoxy-6-lipoylamide)-per-2,3,6-O-methyl-β-cyclodextrin (**2**)*

The voltammetric curves for increasing ibuprofen concentration recorded using the electrode modified with receptor **2**, and the plot $I_0/(I_0-I)$ *versus* $1/C_{IBU}$ at 2×10^{-5} M Fc are shown in Figure 5.

The slope of the plot is three times larger than that for the electrode modified with compound **1** and the binding constant of ibuprofen was calculated as equal to 6.41×10^5 M^{-1}, hence smaller than for the receptor **1** with free hydroxyl groups. The decrease of current I_0-I as a function of increasing concentration of ibuprofen in the solution is shown in Figure 6 for both types of

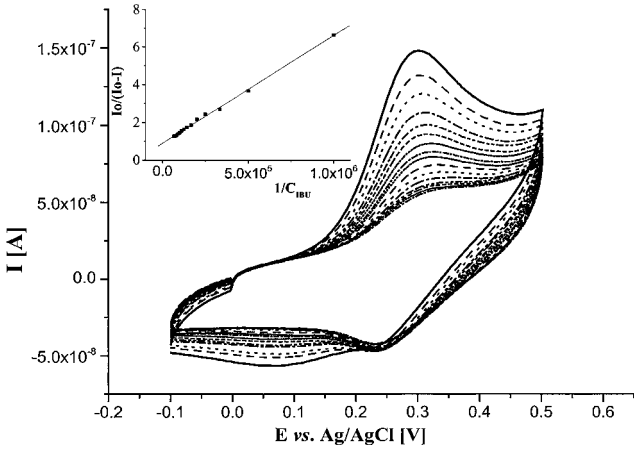

Figure 5. Voltammograms recorded using electrode modified with receptor **2**, in 0.2 M Na_2SO_4 solution containing 2×10^{-5} M Fc before (*solid line*) and following addition of ibuprofen from 0.1 to 1.5×10^{-5} M (*dashed lines*). Scan rate 100 mV/s. Inset shows linear fit to I_0/I_0-I *versus* $1/C_{IBU}$ plot.

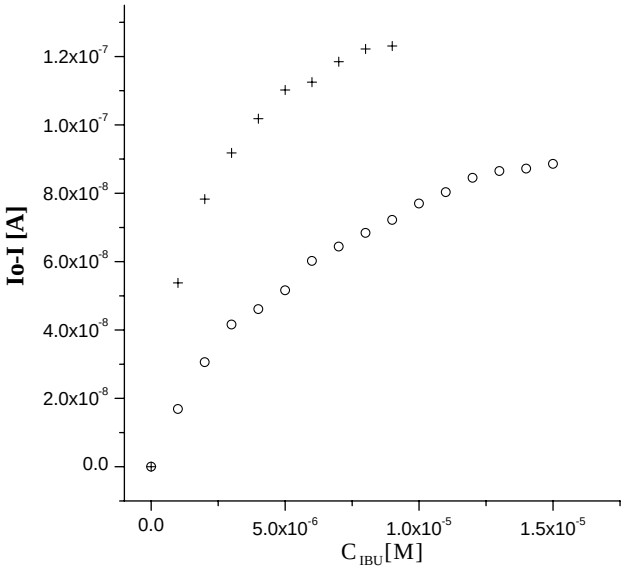

Figure 6. The decrease of current, I_0–I with increasing concentration of ibuprofen in the solution. Gold electrode modified with receptor **1** (+), and with receptor **2** (○).

modified electrodes. Monolayer containing receptor **1** shows higher sensitivity towards ibuprofen.

Conclusions

Electrodes modified with mixed monolayers containing either mono(6-deoxy-6-lipoylamide)-β-cyclodextrin or mono(6-deoxy-6-lipoylamide)-per-2,3,6-*O*-methyl-β-cyclodextrin were found applicable for the indirect determination of non-electroactive drugs. The approach described in this paper leads to a well organized and stable monolayer of ferrocene complex with cyclodextrin sensitive towards low concentrations of ibuprofen (as low as 5×10^{-7} M). The molecularly imprinted electrode is reusable and shows long-term stability ($\sim$2 months), hence may be considered e.g. for electrochemical detection of non-electroactive drugs following their chromatographic or electrophoretic separation.

Electrodes modified with mono(6-deoxy-6-lipoylamide)-β-cyclodextrin (compound **1**) and mono(6-deoxy-6-lipoylamide)-per-2,3,6-*O*-methyl-β-cyclodextrin (compound **2**) show slightly different behaviour towards ferrocene. The binding equilibria for the methylated receptor are established much slower. Since our method is based on the competition in binding of the electroactive marker – ferrocene and the drug – ibuprofen, the free hydroxyl receptor **1** is analytically favorable compared to methylated receptor **2**. Also the sensitivity towards the drug in the solution is lower for the electrode covered with the monolayer incorporating the receptor with free hydroxyl functionalities.

Acknowledgement

This work was supported by Warsaw University BW-1562/02/2002.

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