Full Paper

Dopamine Oxidation at Per(6-deoxy-6-thio)-α-Cyclodextrin Monolayer Modified Gold Electrodes

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Abstract
The gold electrode is functionalized by sequential self-assembly of a monolayer of the title thiolated cyclodextrin with and without dopamine included in the cavities. The structure of α-cyclodextrin modified gold electrode is carefully characterized using STM and AFM. Surface complexation of dopamine is examined and its association constant is evaluated. Chemical reactions accompanying the electrode process of dopamine, which interfere in the electrochemical dopamine determination, are described and the conditions to avoid them are proposed. Dopamine incorporated in the α-cyclodextrin sites anchored to the electrode surface was found to provide electrochemical contact of the electrode with the solution-resident dopamine. Dopamine present in the α-cyclodextrin cavities has different properties compared to dopamine in the bulk buffer solution and can act as a mediator for the dopamine molecules diffusing to the electrode. This unique mediation effect leads to improvement of the sensitivity of dopamine determination using the α-cyclodextrin modified electrode and a procedure for the determination of dopamine in large excess of ascorbate is proposed.

Keywords: Dopamine, Voltammetry, Cyclodextrin, Modified electrode

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1. Introduction

The electrooxidation of 2-(3,4-dihydroxyphenyl)ethylamine (dopamine), an important neurotransmitter for the functioning of the central nervous system, is a complicated process in physiological pH, with a following chemical reaction [1–4]. Since monitoring dopamine concentration is crucial for the understanding of its role in the neuronal system, many methods to determine dopamine (DA) have been elaborated based on spectroscopy, chromatography, and electrochemistry [5–11].

The electrochemical determination causes problems connected with the presence of ascorbates (AA) usually in 100- to 1000-fold excess compared to the DA concentration. [9, 10, 12]. At the bare gold electrode surfaces both compounds are oxidized at the same potential (ca. 0.2 V), in the following voltammetric cycles the peak current decreases due to fouling of the electrode surface and additional chemical reactions interfere [8–14]. Several attempts to overcome all these problems have been made mainly by appropriate modification of the electrode surface i.e. charged Nafion [15–18], or other polymer films [19] usually on glassy carbon.

Dalmia and co-workers [20] used gold electrodes modified with monolayers of carboxylic or sulfonic acids to determine DA in the presence of AA. The selectivity depended on the charge of the terminal groups but also on the structural defects in the layer. The increase of the alkane chain length from C-2 to C-5 improved the selectivity at the expense of sensitivity.

In our recent papers, we presented a three step sequential self-assembly procedure applied to prepare gold electrodes functionalized with cyclodextrin (CD) and responsive towards ferrocene, methylene blue and ibuprofen [21–23]. Ibuprofen present in the solution lead to the decrease of ferrocene electrooxidation peak because the nonelectroactive ibuprofen molecule, due to larger affinity to β-cyclodextrin, substituted the electroactive ferrocene molecule in the macrocyclic cavity. We prepared also a CD modified electrode with methylene blue bound to the macrocyclic cavities. Methylene blue formed rather strong associate with the surface bound CD and was not easily substituted by species from the solution. On the other hand, it was useful as an electron-mediating unit to a solution resident enzyme-laccase catalyzing reduction of oxygen.

In the present paper, we present the concept for preparing stable dopamine responsive CD modified electrodes and explain the properties of dopamine included in the α-CD cavities.

Cyclodextrins are known to bind strong dopamine and its derivatives in solution with the dissociation constant of the DA-β-CD complex of 3.56×10^{-3} M^{-1} [24–25]. The natural precursor of dopamine, DOPA, forms a relatively strong inclusion complex with partially thiolated surface bound α-CD [26]. Molecular recognition of the guest molecules depends here on the size selectivity, thus α-CD should be the cyclodextrin forming the strongest inclusion complex with dopamine. Fragoso et al., however, employed β-cyclodextrins to complex dopamine using a mixed monolayer with 70% of the electrode covered with β-CD and rest blocked by thioctic acid. Such modification lead to repulsion of AA and attraction combined with binding of the positively charged DA to the molecular receptor. In a solution containing 1 mM DA, an increase of the voltammetric signal was observed for dopamine but in a solution containing dopamine and ascorbate, the voltammetric peak of AA decreased [27].

In the present paper we investigate the influence of the monolayer modification of the gold electrode with thiolated cyclodextrins on the oxidation process of dopamine. The electrode is modified with per(6-deoxy-6-thio)-α-cyclodextrin, its structure is characterized carefully using STM and AFM. Binding constant of dopamine to the electrode surface is evaluated using the Maeda procedure [26, 28] and a new procedure to determine dopamine in the presence of ascorbate is proposed.

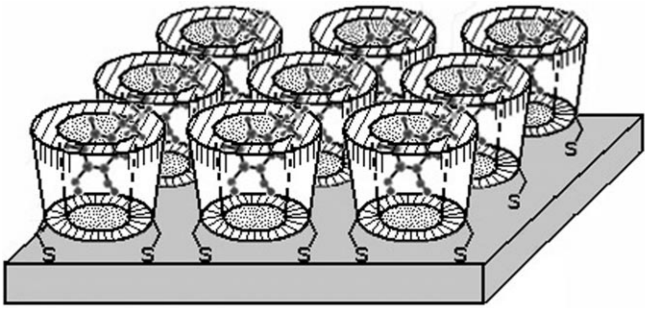
2. Experimental

2.1. Chemicals

Ethanol, perchloric acid and potassium hydroxide were purchased from POCh Gliwice. All other compounds used in this work were purchased from Sigma-Aldrich and Merck. Water was distilled and passed through a Milli-Q purification system. The final resistivity of water was 18.2 MΩcm^{-1}. The synthesis of per(6-deoxy-6-thio)-α-cyclodextrin was done according to the procedure of Stoddart and co-workers [29, 30].

2.2. Preparation of Electrodes

Monolayer modified Au(111) substrates were used as working electrodes. The substrates were 200 – 300 nm thick gold films evaporated onto borosilicate glass precoated with 1 – 4 nm underlayer of Cr (Arrandee). Before the deposition of monolayer, the substrates were cleaned by etching for 5 – 10 minutes in hot nitric acid, and then flame annealed until the sample glowed dark red [31]. Then the substrate was cooled down to room temperature. After annealing procedure, the substrates were transferred to a coating solution. Self-assembly was carried out from 1 mM α-CD in DMF solutions. After 16 h of being soaked, the substrates were washed with ethanol and water. The α-CD covered electrodes were immersed in 0.1 M DA solution in phosphate buffer for 1 h. The electrode was then rinsed thoroughly with water dried and used in the electrochemical or microscopy experiments. Scheme 1 presents idealized structure of the modified surface.



Scheme 1. Schematic representation of an Au electrode modified with per(6-deoxy-6-thio)-α-cyclodextrin and dopamine.

2.3. Electrochemistry

Electrochemical experiments were carried out in three-electrode cell with SCE as a reference electrode and platinum foil as a counter electrode. The supporting electrolyte was 0.1 M phosphate buffer or McIlvaine buffer. The measurements were performed using Autolab PGSTAT30. All electrochemical experiments were carried out at 25 °C.

2.4. Scanning Tunneling and Atomic Force Microscopy

STM and AFM measurements were performed using a Veeco Multi Mode Nanoscope IIIa (Digital Instruments, Santa Barbara, CA) equipped with a low current converter. The images were taken under ambient conditions with commercially available Pt-Ir tips. All AFM experiments are done using Veeco Silicon Probes TESP in the tapping mode with spring constant 20 – 100 N/m at 200 – 400 kHz.

3. Results and Discussion

3.1. Topography of the Modified Electrode

Figures 1 (A and B) show the AFM images of the surface of the electrode modified with per(6-deoxy-6-thio)-α-cyclodextrins. The monolayer is not very well organized and regions of higher and lower coverage can be recognized. Two-dimensional domains covered by CDs are seen and the tendency to form such structures is understandable since hydrogen bonding may connect neighboring molecules of α-CDs. The radius of the domain is about 20 nm and the height is ca. 1.2 nm. When the CDs are associated with dopamine it becomes ca. 2 nm (Fig. 1 C and D). This small increase of height indicates that the molecule of dopamine is protruding outside the α-CD molecule and only the flat aromatic ring of the neurotransmitter is accommodated inside the hydrophobic cavity.

Taking into account that the molecules are bound to the electrode surface through their thiol atoms, the height seen be the AFM is comparable to the height of a single molecule of α-CD (0.78 nm). This suggests that the domains appear as

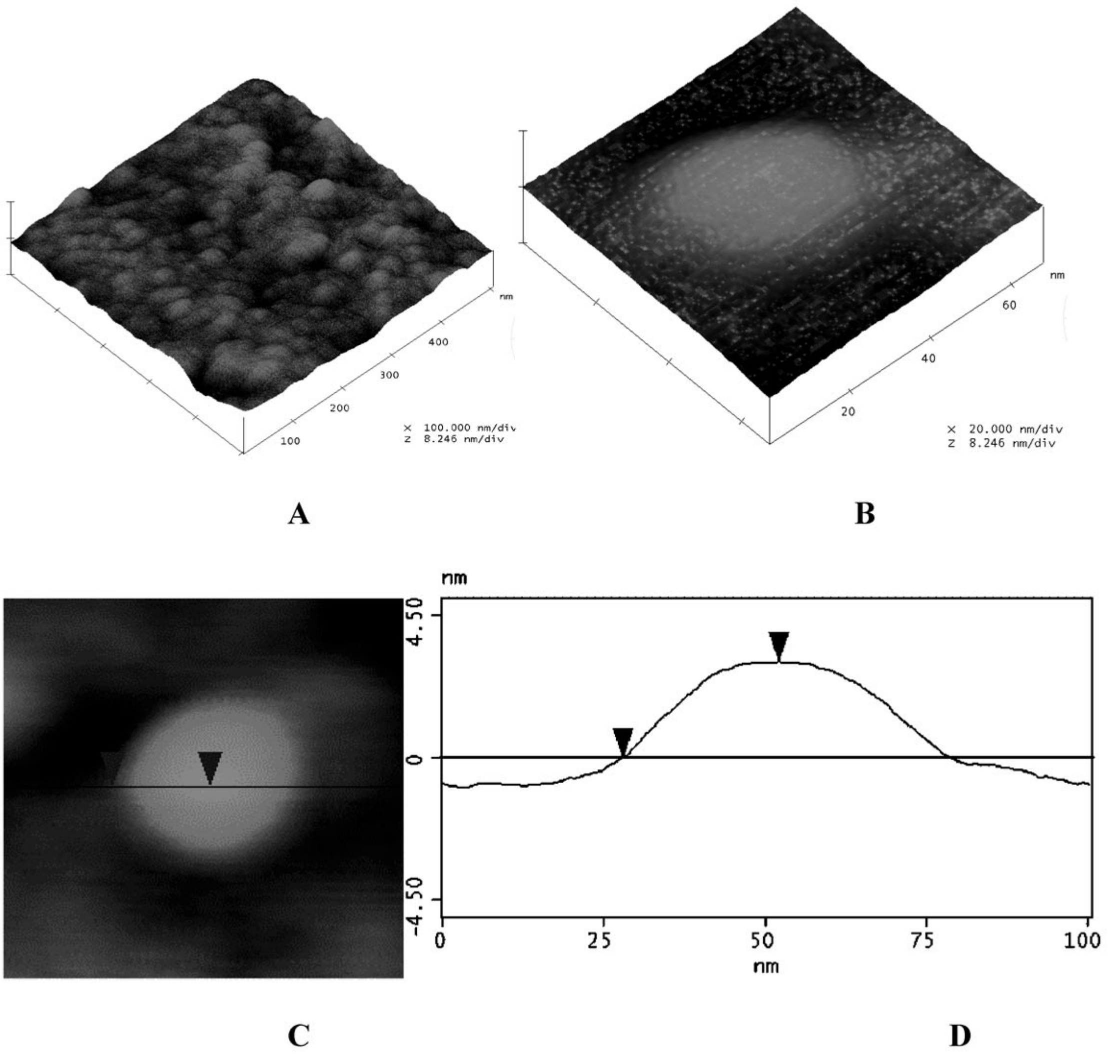


Fig. 1. A) AFM topography of the Au surface modified with α-CD. B) An α-CD domain formed on the surface of the Au electrode. C) AFM topography of the Au surface modified with α-CD and dopamine. D) A cross-section through an agglomerate of α-CD and dopamine.

two-dimensional structures on the gold surface and not three-dimensional aggregates.

Attempts to remove hydrogen bonding interactions by preparing a SAM using per(6-deoxy-6-thio-2,3-di-*O*-methyl)-α-cyclodextrin which is not a hydrogen bond donor (because of lack of free −OH groups) lead rather to aggregates formation than to the elimination of two-dimensional domains. Figures 2 (A and B) show STM images at higher resolution with ordering of the CDs inside the domains. The distance between centers of neighboring molecules is less than 2 nm, which is similar to the size of a single CD molecule (Fig. 2B).

3.2. Electrochemical Behavior of Dopamine Included in the α-CD Cavities

The electrooxidation of DA is complicated by following chemical reactions. There is a lot of confusion in the literature reports concerning these reaction. Therefore, detailed scheme showing the sequence of electrode (E) and chemical (C) steps is presented here as Scheme 2. The product of exchange of two electrons (first step E), dopaquinone (DOQ) as a Michael acceptor undergoes nucleophilic attack of amine in the side chain [2, 3]. The intramolecular proton transfer leads to a transient species, also an α,β-unsaturated ketone in equilibrium with the 1,4 dipole form. The latter under influence of water or other base, undergoes an elimination reaction leading to a stable aromatic species, the phenolate anion (step C). Upon protonation of this anion, dihydroxyindoline (DHI) is formed and oxidized further to the aminochrome (AC) (second step E). In the following reduction scan only part of the initial DA, still remaining in the DOQ form can be regenerated (Scheme 2, Reaction c_1) while the rest is reduced at a more negative potential (peak c_2) or lost due to these competing chemical reactions.

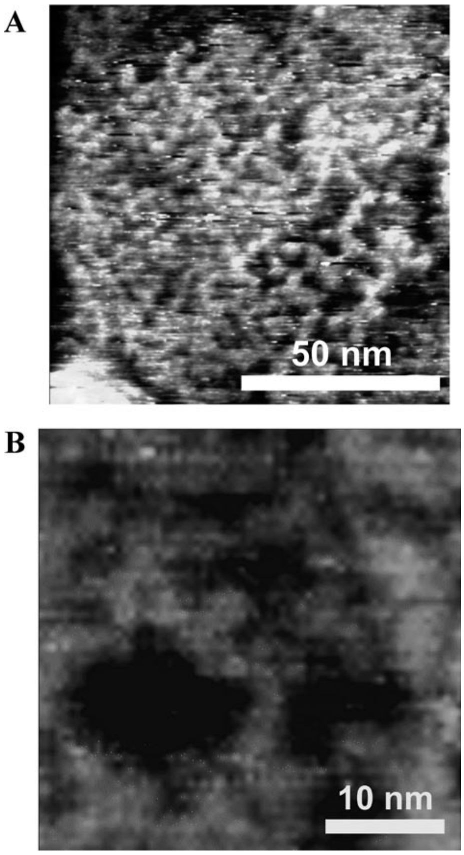


Fig. 2. STM topography of the Au surface modified with α-CD A) 100 nm, B) 40 nm.

The electrochemical behavior of an electrode covered by a self-assembled monolayer of α-CD molecules with dopamine included in their cavities can be easily understood based on the mechanism presented in Scheme 2. Two consecutive voltammetric curves are shown in Figure 3. In the first scan (Fig. 3, curve 1), DA is oxidized to DOQ (peak a_1) at $+0.147$ V, and reduced in part to DA (peak c_1). Part of DOQ undergoes cyclization reaction to form DHI (Scheme 2) which is immediately oxidized to AC. Upon scan reversal both c_1 and c_2 (reduction of AC, $E_p = -0.285$ V) can be easily recognized. In the following half-cycle, a_2 peak appears, corresponding to the oxidation of DHI to AC, while the DA oxidation peak, a_1 decreases at the expense of the AC/DHI peak couple. The contribution of chemical reaction is easily seen in the voltammograms recorded at low scan rates (Fig. 3).

The current of oxidation peak a_1 corresponding to the oxidation of dopamine included in the cyclodextrin, recorded in pure electrolyte solution, increases linearly with scan rate (Fig. 4. Inset). This proves surface immobilization of DA.

With increasing scan rate, the contribution of chemical reaction decreases and i_{pc1}/i_{pc2} ratio increases. Another way to decrease the contribution of chemical reactions is to change pH of the solution below pH 5.4 (Fig. 5). This means, however, that the physiological pH conditions are not maintained. The voltammograms obtained after ten cycles recorded in buffer solutions of pH 7.0 and 5.4 are compared in Figure 5. In both cases dopamine is included in the cavities of CD and no dopamine is present in the solution. The neurotransmitter is firmly held inside the cavities and the following cycles do not change the magnitude of the peaks. Under these conditions the DA@α-CD associate can be considered as a useful mediator for electrocatalytic and bioelectrocatalytic oxidations.

3.3. Evaluation of Dopamine Association Constant with Surface Immobilized Cyclodextrin

The association constant of dopamine to surface immobilized cyclodextrin was evaluated on the basis of $[DA]/I_{pa} - DA$ concentration dependence. The plot is linear to ca. 100 μM. Above this concentration, the values of current become constant. The $[DA]/I_p$ ratio vs. $[DA]$ is plotted. The association constant, K_{ass} is calculated using equation [22, 26, 28]:

$$[DA]/I_p = (1/K_{ass}I_{max}) + [DA]/I_{max} \tag{1}$$

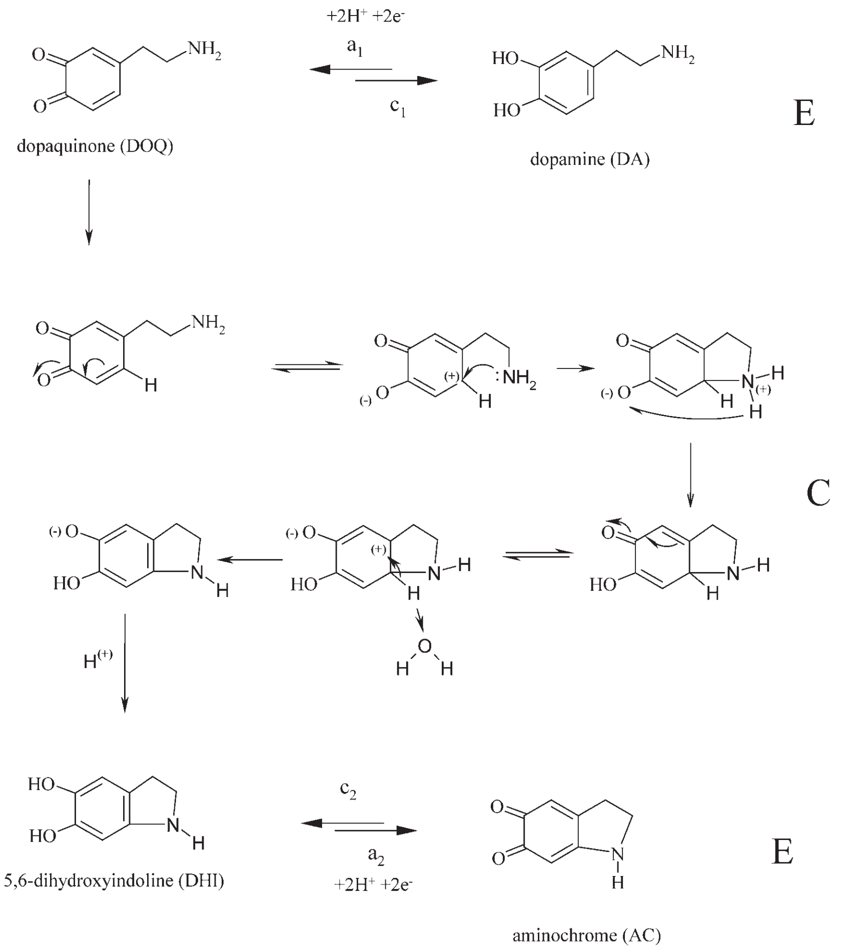
where I_p is the peak current for a given DA concentration and I_{max} is the limiting value of current at saturation of the surface. Polynomial regression is used to fit ($Y = A + B_1{*}X + B_2{*}X^2$) the data and K_{ass} is given by equation: $A = 1/K_{ass}I_{max}$.

A is found to be 0.18767 ($R^2 = 0.9896$, $SD = 0.1081$) and the K_{ass} is equal to 1.33×10^5 M^{-1}. This value is higher than the constants in the solution, and similar to the interfacial association constants calculated for β-CD with ferrocene and ibuprofen [22].

3.4. Electrooxidation of Bulk DA on the α-CD-Modified Electrode

The very interesting behavior of dopamine placed by the proposed preconditioning procedure inside the cyclodextrin cavities towards solution species is shown on Figure 6.

Curve 1 corresponds to the oxidation of 1 mM dopamine on a α-CD monolayer, curve 2 has been recorded for comparison on bare gold, while curve 3, on the electrode modified with the DA@α-CD complex. The peak a_1 in curve 3 (recorded using the electrode modified with the DA@α-CD complex) is higher than the a_1 peak recorded for the empty α-CDs on the electrode surface. Moreover, the corresponding cathodic peak, c_1 is smaller using the electrode modified by the complex instead of free ligand. This indicates a mediating effect of dopamine included in the receptor on the surface. In the latter case, due to electrostatics the potential at which DA oxidation appears, is more positive than on bare gold, since both dopamine in the cavities and in the solution are positively charged. DA in the inclusion compound seems therefore to have slightly different properties, than DA present in the bulk solution.



Scheme 2. Mechanism of dopamine oxidation.

Alfaro et al. mentioned earlier that dopamine immobilized on electrode surfaces may be used to mediate electrons to glucose oxidase [32].

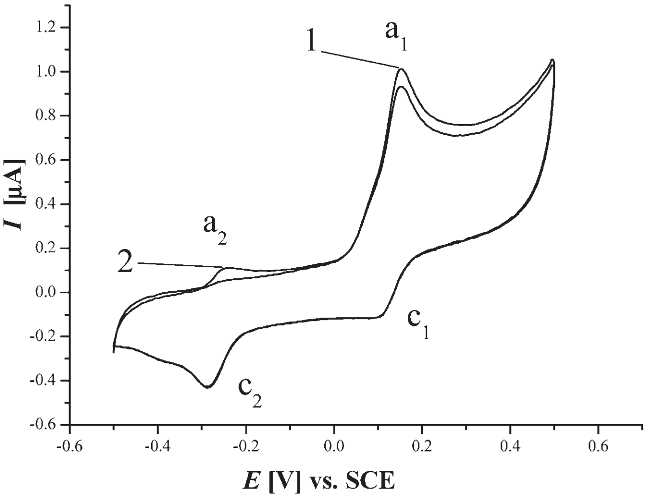


Fig. 3. Cyclic voltammetry curves recorded for an Au electrode modified by SAM film of DA@α-CD complex recorded in the pure supporting electrolyte solution (0.1 M phosphate buffer, pH 7.0) under Ar atmosphere: 1) first scan, 2) second scan. Scan rate 0.005 V/s.

With increasing concentration of dopamine in the solution the c_1 catalytic signal increases, thus it can be used for the determination of DA (Fig. 7). The peak current-concentration dependence has a nonzero intercept as expected since DA is present in the cavities of the cyclodextrin (Fig. 7, inset).

A small prepeak (a_1') at 0.05 V corresponds to the oxidation of dopamine on the uncovered gold surface and can be removed simply by filling the void places between CDs with cysteamine (CA). Following self-assembly of CD and inclusion of DA into the cavities, the electrode is immersed for 1 h into 1 mM CA solution in order to block the void places remaining on the electrode. CA is positively charged, binds to gold and repels electrostatically the positively charged dopamine from the uncovered Au areas.

3.5. Determination of Dopamine in the Presence of Excess of Ascorbic Acid in the Solution

The electrochemical determination of dopamine in the biological samples causes problems connected with the presence of ascorbate usually in large excess compared to the dopamine concentration. At unmodified electrode surfaces both compounds are oxidized at the same potential

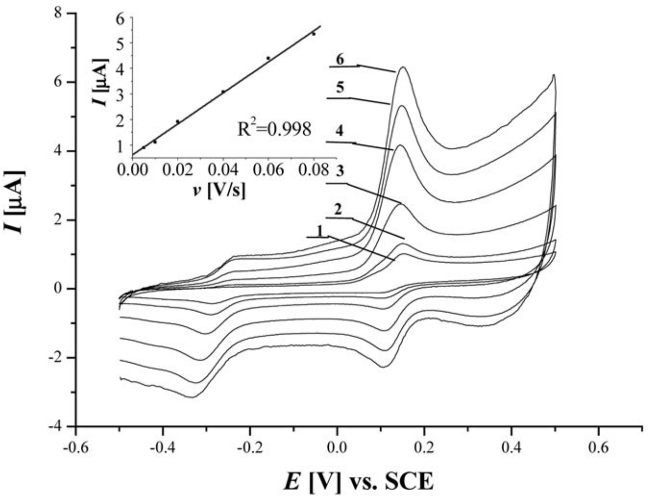


Fig. 4. Cyclic voltammograms for the Au electrode coated by a monolayer of DA@α-CD recorded 0.1 M phosphate buffer (pH 7.0) at scan rates: 1) 0.005, 2) 0.01, 3) 0.02, 4) 0.04, 5) 0.06, 6) 0.08 V/s.

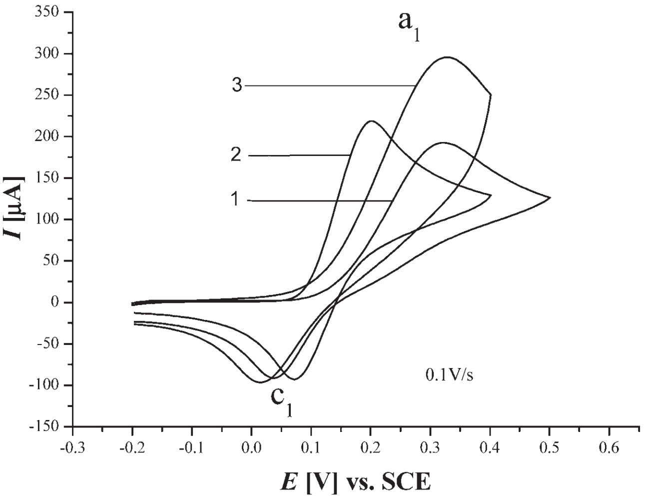


Fig. 6. Cyclic voltammograms for the Au electrodes: 1) coated by monolayer of α-CD, 2) uncovered gold electrode, 3) coated by a monolayer of DA@α-CD, scan rates 0.1 V/s for 10⁻3 M DA in phosphate buffer (pH 7.0).

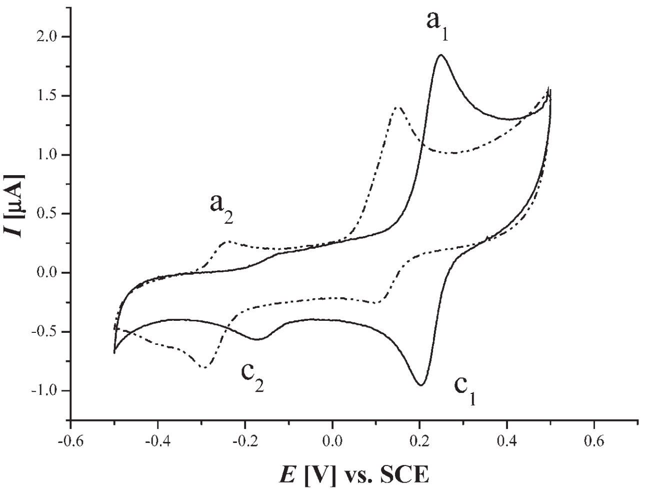


Fig. 5. Voltammograms recorded on gold electrode modified with a SAM film of DA@α-CD Tenth voltammetric scan in (solid line) phosphate buffer (pH 7.0), (dashed line) McIlvaine buffer (pH 5.4). Scan rate 0.01 V/s.

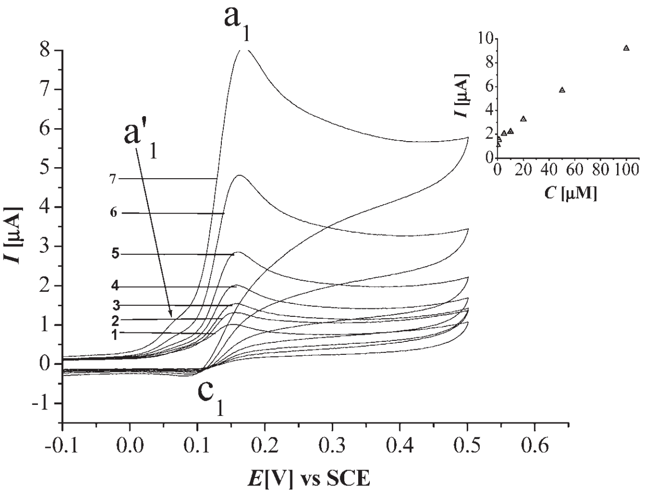
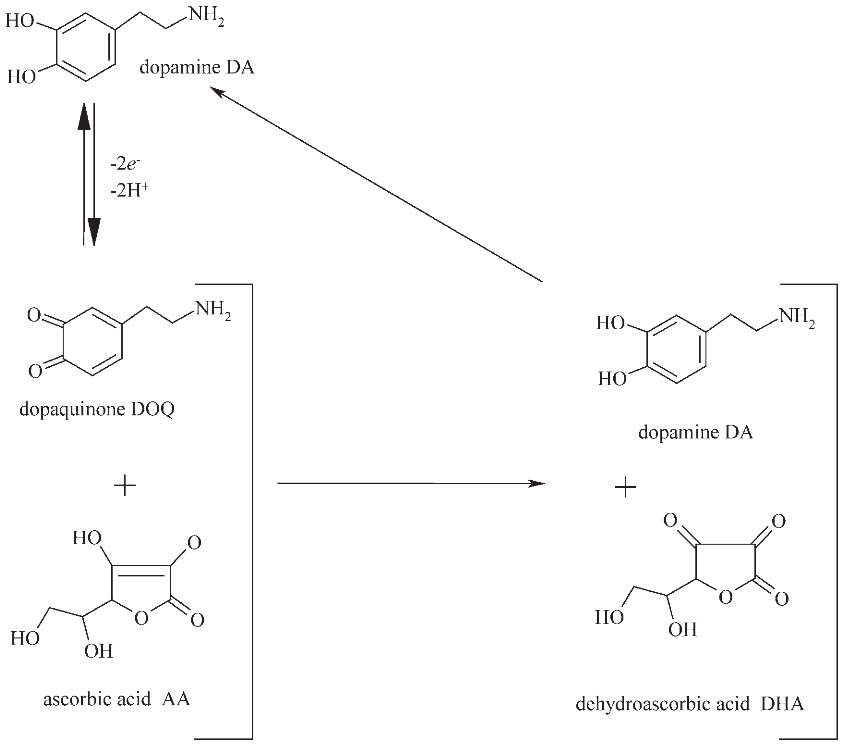


Fig. 7. Cyclic voltammetry curves for the Au electrode modified by SAM containing DA@α-CD for increasing concentrations of DA in phosphate buffer (pH 7.0): 1) 0, 2) 1, 3) 5, 4) 10, 5) 20, 6) 50, 7) 100 × 10⁻6 M. Scan rate 0.1 V/s.

(ca. 0.2 V), and in the following cycles the peak current decreases due to fouling of the electrode surface by the products of ascorbate oxidation. Additional complications connected with chemical reactions appear in the presence of AA, oxidized dopamine would catalyze the oxidation of ascorbate, which results in a single and broad peak. The mechanism of the latter has been explained by Dayton et al. and Doménech et al. [8, 13] and involves reaction of AA and DOQ with consecutive regeneration of initial DA according to Scheme 3.

Several attempts to overcome these additional problems have been made mainly by appropriate modification of the electrode surface [15–19, 33] usually on glassy carbon. The separation of DA and AA analytical signals was often based on electrostatic attraction of negatively charged AA (at physiological pH) to the positively charged films, which lead to a large negative shift of the voltammetric peak. Self-assembled monolayers of ω-mercaptocarboxylic acid were electrostatically attracting DA, thus its oxidation was observed at less positive potentials than in case of uncovered electrodes.

We propose to decrease the interfering role of AA in the determination of DA by oxidizing AA at more negative potential than that of DA. Under these conditions, the DOQ formed cannot react with AA since the latter is depleted in the diffusion layer. Figure 8 shows this procedure based on introduction of a preelectrolysis step before recording the voltammogram. Efficient decrease of the AA concentration in the diffusion layer has been achieved by preelectrolysis at potential +0.1 V. At 1000-fold excess of ascorbic acid, the



Scheme 3. Mechanism of simultaneous oxidation of DA and AA [8, 13].

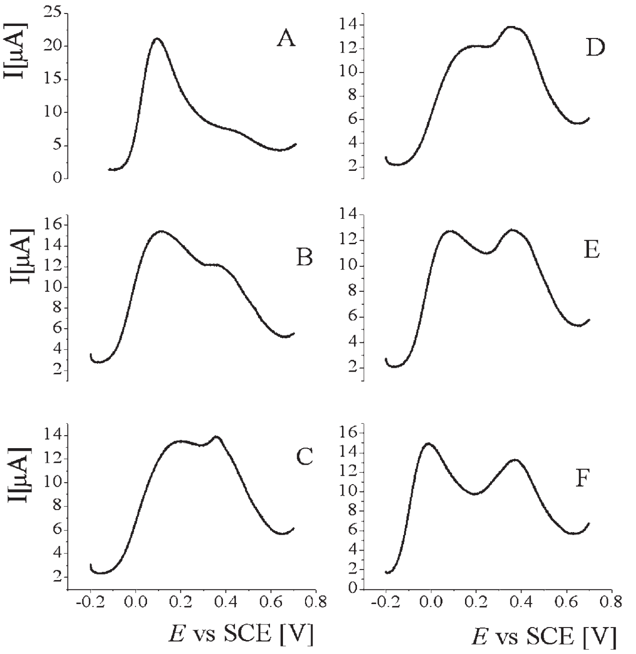


Fig. 8. Differential Pulse Voltammetry recorded for the Au electrode coated by a mixed monolayer of DA@α-CD and CA in solution of 10^{-6} M DA and 10^{-3} M AA in phosphate buffer (pH 7.0) for preelectrolysis time: A) 0, B) 1, C) 2, D) 3, E) 4, F) 5 min. $E_{\text{preelectrolysis}} = +0.1$ V, $\Delta E = 0.005$ V.

peak current of dopamine increases linearly with concentration and is separated from the AA oxidation by ca. 0.35 V.

4. Conclusions

We have applied per(6-deoxy-6-thio)-α-cyclodextrin in the sequential self-assembly procedure of the monolayer formation on gold electrode. The structure of the modified electrode surface is characterized carefully using STM and AFM microscopies. The electrochemical response of such electrode towards dopamine was found to be highly improved when dopamine was included in the cyclodextrin cavity. The association of dopamine with the monolayer CDs was strong and the binding constant was found to be 1.33×10^5 M^{-1}.

The molecule of dopamine included in the cavity of the α-cyclodextrin attached to the gold electrode surface acts as a mediator providing electrical contact between the electrode and the solution resident dopamine. The neurotransmitter immobilized in the α-CD has different properties than the one in the bulk buffer solution, and can act as mediator for the diffusing dopamine. This improved the sensitivity of the DA determination method and lead us to propose a new procedure to minimize the interference of ascorbic acid present at 1000-fold excess in the μ-molar solution of dopamine.

Introduction of positively charged cysteamine, moved ascorbate oxidation to more negative potential and allowed to oxidize it during the preelectrolysis step. In 1000-fold excess of ascorbic acid, the peak of dopamine increased linearly with concentration and was separated from the AA oxidation peak by ca. 0.35 V.

5. Acknowledgements

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6. References

[1] P. E. Phillips, G. D. Stuber, M. L. A. Helen, R. M. Wightman, R. M. Carelli, *Nature* **2003**, *422*, 614.

[2] M. D. Hawley, S. V. Tatawawadi, S. Piekarski, R. N. Adams, *J. Am. Chem. Soc.* **1967**, *89*, 447.

[3] A. W. Sternson, R. McCreery, B. Feinberg, R. N. Adams, *J. Electroanal. Chem.* **1973**, *46*, 313.

[4] R. M. Villanueva-Camañas, J. M. Sanchis-Mallols, J. R. Torres-Lapasió, G. Ramis-Ramos, *Analyst* **1995**, *120*, 1767.

[5] Z. E. Seckin, M. Volkan, *Anal. Chim. Acta* **2005**, *547*, 104.

[6] F. B. Salem, *Talanta* **1987**, *34*, 810.

[7] P. Nagaraja, K. C. Srinivasa Murthy, K. S. Rangappa, N. M. Made Gowda, *Talanta* **1998**, *46*, 3.

[8] M. Wightman, A. G. Ewing, M. Dayton, *Anal. Chem.* **1980**, *52*, 2392.

[9] C. R. Raj, T. Ohsaka, *J. Electroanal. Chem.* **2001**, *496*, 44.

[10] D. Sopchak, B. Miller, R. Kalish, Y. Avyigal, X. Shi, *Electroanalysis* **2002**, *14*, 473.

[11] R. D. O'Neill, *Analyst* **1994**, *119*, 167.

[12] P. Capella, B. G. Ghasemzadeh, K. Mitchell, R. N. Adams, *Electroanalysis* **1990**, *2*, 175.

[13] A. Doménech, H. Garcia, M. T. Doménech-Carbó, M. S. Galletero, *Anal. Chem.* **2002**, *74*, 562.

[14] J. Weng, J. Xue, J. Wang, J.-S. Ye, H. Cui, F.- S. Sheu, Q. Zhang, *Adv. Funct. Mater.* **2005**, *15*, 639.

[15] M. P. Brazell, R. J. Kasser, K. J. Renner, J. Feng, B. Moghddam, R. N. Adams, *J. Neurosci. Methods* **1987**, *22*, 167.

[16] F. Crespi, K. F. Martin, C. A. Marsden, *Neuroscience* **1988**, *27*, 885.

[17] J. Wang, P. Tuzhi, *Anal. Chem.* **1986**, *58*, 3257.

[18] E. W. Kristensen, W. G. Kuhr, R. M. Wightman, *Anal. Chem.* **1987**, *59*, 1752.

[19] Y. Zhang, G. Jin, Y. Wang, Z. Yang, *Sensors* **2003**, *3*, 443.

[20] A. Dalmia, C. C. Liu, R. F. Savinell, *J. Electroanal. Chem.* **1997**, *430*, 205.

[21] K. Chmurski, A. Temeriusz, R. Bilewicz, *Anal. Chem.* **2003**, *75*, 5687.

[22] K. Chmurski, R. Bilewicz, A. Temeriusz, *J. Incl. Phenom.* **2004**, *49*, 187.

[23] K. Chmurski, A. Koralewska, A. Temeriusz, R. Bilewicz, *Electroanalysis* **2004**, *16*, 1407.

[24] J. Szejtli, *Cyclodextrin Technology*, Kluwer Academic Press, Dordrecht **1988**.

[25] Z. N. Gao, X. L. Wen, H. L. Li, *Pol. J. Chem.* **2002**, *76*, 1001.

[26] T. Fukuda, Y. Maeda, H. Kitano, *Langmuir* **1999**, *15*, 1887.

[27] A. Fragoso, E. Almirall, R. Cao, L. Echegoyen, R. González-Jonte, *Chem. Commun.* **2004**, 2230.

[28] Y. Maeda, T. Fukuda, H. Yamamoto, H. Kitano, *Langmuir* **1997**, *13*, 4187.

[29] P. R. Ashton, R. Königer, J. F. Stoddart, *J. Org. Chem.* **1996**, *61*, 903.

[30] M. T. Rojas, R. Königer, J. F. Stoddart, A. E. Kaifer *J. Am. Chem. Soc.* **1995**, *117*, 336.

[31] A. S. Dakkouri, D. M. Kolb, in *Interfacial Electrochemistry* (Ed: A. Więckowski), Marcel Dekker, New York **1999**, pp. 151 – 173.

[32] I. M. Alfaro, E. I. Pizarro, L. Rodriguez, E. M. Valdes, *Bioelectrochem. Bioenerg.* **1995**, *38*, 307.

[33] P. R. Roy, T. Okajima, T. Ohsaka, *Bioelectrochem.* **2003**, *59*, 11.