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Electrochemical studies of blocking properties of solid supported tethered lipid membranes on gold

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Abstract

The insulating properties of self-assembled thiolipid monolayers and tethered lipid bilayers on polycrystalline gold electrodes were studied by means of cyclic voltammetry (CV). These films were formed by two-step self-assembly processes. Electrochemical measurements of the heterogeneous electron transfer rate constant of different redox couples such as potassium ferrocyanide ($K_4[Fe(CN)_6]$) and dopamine (DP) were used to examine the molecular integrity and structural defects and pinholes within the monolayers. We demonstrate by means of cyclic voltammetry that the bilayer lipid membranes tethered to the gold surface are blocking, stable, yet retaining their dynamic properties and can be used as a model of the cell membrane.

Keywords: Cyclic voltammetry; t-BLMs; Potassium ferrocyanide; Dopamine; Membrane models

1. Introduction

Since the works of Allara and Nuzzo were first published [1–3], the self-assembled monolayers of alkanethiols and their derivatives on gold have been widely investigated [4–32]. Nowadays, it seems that such stable, well-organized films with well-defined structure and thickness can be applied, for example, in the studies of electron transfer [8–13], in the investigation of electrochemical behavior of different redox couples, in particular biological molecules covalently bound with the electrode surface [15–20], in the preparation of microelectrodes and microsensors [19–23,29–31] or in the modeling of the surface-supported biomimicking bilayer systems [24–32].

However, the structure and organization of the mono- and bilayer films are often very far away from ideal and the blocking properties are not satisfactory, therefore the use of such modified electrodes is to a certain extent limited. This is the main reason why it is very important to examine the properties of mono- and bilayers before further studies concerning applicability are performed [32]. This paper summarizes the results of our studies on blocking properties of two novel thiolipid monolayers as well as bilayers containing them, tethered to the polycrystalline gold electrode.

2. Experimental

2.1. Chemicals

All chemicals used during electrochemical experiments were of the highest purity commercially available: $LiClO_4$ (Aldrich, ACS grade), KCl (POCh, reagent grade), potassium ferrocyanide ($K_4[Fe(CN)_6]) \times 3H_2O$ (Sigma, 99%), 3-hydroxytyramine hydrochloride (DP) (Fluka, 99%). Aqueous solutions were prepared from water of high purity (Milli-Q). The gold used for the working electrode was a commercially available polycrystalline gold wire of 99.99% purity (0.5 mm in diameter). The two thiolipids dipalmitoylphosphatidylethanolaminomercaptopropionamide (DPPE-MPA) and cholesteryl 3-mercaptopropionate (Chs-MPA) were synthesized according to the previously described procedure [33,34].

2.2. Instrumentation

Electrochemical measurements were conducted with a PC-controlled, custom-built potentiostat/galvanostat (KSP Electronics, Poland), using a conventional small three-elec-

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trode cell. All potentials are referred to an Ag | AgCl | 1 M KCl_{aq} reference electrode. Potassium hexacyanoferrate(II), and dopamine solutions used for integrity tests on mono- and bilayers were thoroughly derailed with argon.

2.3. Electrode preparation

The electrodes were fabricated and modified by tethering of thiolipid monolayers to the surface of gold according to the procedure described previously [34]. Such modified electrodes were then examined for the presence of pinholes in the monolayer. These measurements were carried out in 0.1 M $LiClO_4$ aqueous supporting electrolyte containing one of the two electrochemical probes, hydrophilic $K_4[Fe(CN)_6]$ or amphiphilic dopamine. Most of the thiolipid-modified electrodes prepared that way exhibited good integrity and blocking behavior and were then used in the following experiments.

Then, a second layer was attached to a thiolipid-modified electrode by a subsequent transfer of a lipid layer from air/

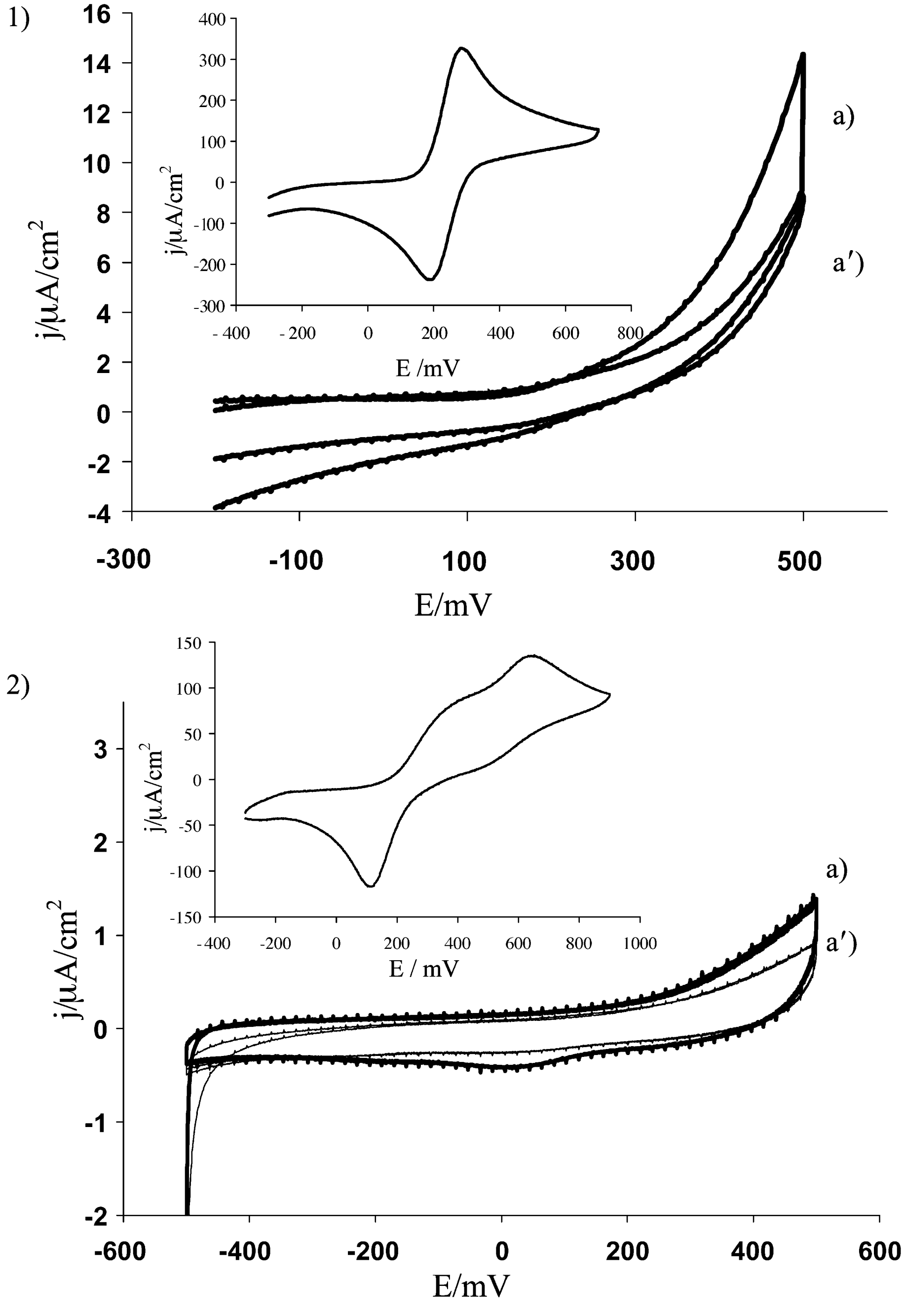

Fig. 1. Cyclic voltamograms of (1) 1 mM $K_4[Fe(CN)_6]$ and (2) 1 mM dopamine in 0.1 M $LiClO_4$ aqueous solution buffered with phosphate buffer pH 6.94 on Au electrode covered with self-assembled DPPE-MPA monolayer (a) and DPPE-MPA + DOPC bilayer (a′). Inlets show voltamograms for the same redox probes on bare gold.

water interface as in Refs. [33,34]. The driving force of this attachment is the hydrophobic effect that is connected with the reduction of free energy of thiolipid/water interaction by exposition of hydrophilic heads of phospholipid molecules into aqueous solution. Such parameters like the thickness of mono- and bilayers, the conformation of the thiolipids molecules on the surface and the flexibility and stability of the membranes have been described previously [33,34].

3. Results and discussion

The tests, the integrity and compactness (the absence of any pinholes and structural defects) of different self-assembled thiolipid monolayers: DPPE-MPA and Chs-MPA and two tethered t-BLMs containing these monolayers: DPPE-MPA + DOPC and Chs-MPA + DOPC were carried out by means of cyclic voltammetry (CV) [35]. For this purpose, we used kinetically facile hydrophilic redox probe—$K_4[Fe(CN)_6]$ ($E^0 = 237$ mV) as well as amphiphilic dopamine ($E_1{}^0 = 298$ mV, $E_2{}^0 = 498$ mV), (Fig. 1). As it is shown in Fig 1. The presence of the layers highly attenuated both the reduction and oxidation reactions of the redox couple. For a comparison, the inlets in Fig. 1 show the same voltammograms recorded on bare gold. From this figure, it is evident that the current density at the formal potential is negligible in comparison to a bare electrode. Moreover, one can notice almost perfect, exponential shape of anodic branch of voltammograms recorded on the monolayer and bilayer modified gold. The fact that the dependence of current density vs. potential is not sigmoidal in shape clearly demonstrates that the pinhole defects present in well-ordered mono- and, in particular, bilayers are electrochemically immeasurable. Therefore, we can draw a conclusion that the obtained mono- and bilayers are free of any considerable pinhole defects which means that the redox couples are unable to gain a direct contact with the electrode surface [36,37].

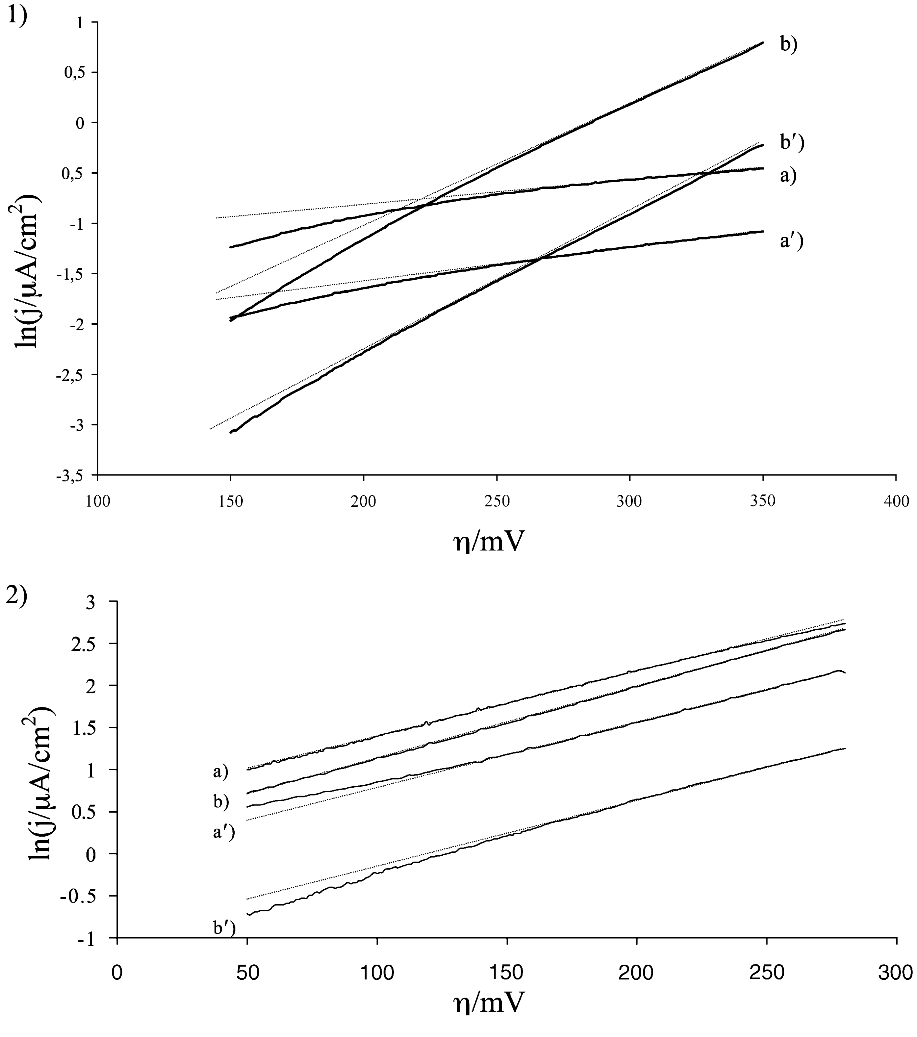

Fig. 2. Tafel plot of ln(j) vs. η for irreversible reaction of (1) 1 mM $K_4[Fe(CN)_6]$ and (2) 1 mM dopamine in 0.1 M $LiClO_4$ aqueous solution buffered with phosphate buffer pH 6.94 on Au electrode covered with self-assembled DPPE-MPA monolayer (a), DPPE-MPA + DOPC bilayer (a′), Chs-MPA monolayer (b) and Chs-MPA + DOPC bilayer (b′).

Table 1
Kinetic parameters for redox reactions of potassium ferrocyanide and dopamine on the gold electrode covered with DPPE-MPA, DPPE-MPA + DOPC and Chs-MPA and Chs-MPA + DOPC, respectively

Type of the redox probe	Layer type	k^s (m/s)	β
$K_4[Fe(CN)_6]$ (k^0 in 0.1 M KCl aq = 3.1×10^{-4} [11], k^0 in 0.1 M $LiClO_4$ aq = 4.38×10^{-5})	DPPE-MPA	8.42×10^{-7}	0.22
	DPPE-MPA + DOPC	7.65×10^{-7}	0.17
	Chs-MPA	5.55×10^{-7}	0.20
	Chs-MPA + DOPC	3.28×10^{-7}	0.22
Dopamine (k^0 in 0.1 M KCl aq = 5.78×10^{-5} [13], k^0 in 0.1 M $LiClO_4$ aq = 5.78×10^{-6})	DPPE-MPA	7.08×10^{-8}	0.33
	DPPE-MPA + DOPC	2.64×10^{-8}	0.35
	Chs-MPA	2.33×10^{-7}	0.10
	Chs-MPA + DOPC	9.87×10^{-8}	0.08

These results suggest that the electron tunneling through the layer is a general mechanism of the electron transfer for our studied systems. Therefore, we were able to use the Tafel approach for the kinetically controlled electrode processes [38–40]. The exemplary Tafel plots of the two redox couples for the mono- and bilayer, respectively, are presented in Fig. 2. Almost all of these plots are nearly linear for overpotential exceeding 200 mV [7]. Therefore, based on the Tafel equations, the transfer coefficients β for $K_4[Fe(CN)_6]$ and dopamine on both DPPE-MPA and Chs-MPA monolayers as well as DPPE-MPA + DOPC and Chs-MPA + DOPC bilayers were calculated from the slope of the linear part of the plot. Furthermore, we have estimated the values of the standard rate constants k_s of the reactions on the modified surface of the gold electrode. These kinetic parameters for both redox couples and all types of the layers that were used to modify the electrode surface are summarized in Table 1. Table 1 also presents the k_s values of the investigated redox couples on bare gold under the same experimental conditions. In order to evaluate these values, we used the voltammograms shown in the inlets of Fig. 1. In $LiClO_4$ aqueous supporting electrolyte, the kinetics of $K_4[Fe(CN)_6]$ is much slower as compared to KCl electrolyte [35]. The fact that the voltammogram of this couple behaves quasi-reversibly ($\Delta E_p = 110$ mV) enabled us to evaluate the standard rate constant k_s of this couple according to the procedure described by Nicholson [41]. The same procedure was used to evaluate the k_s value from the first pair of redox peaks for dopamine ($\Delta E_{p1} = 377$ mV and $\Delta E_{p2} = 257$ mV). Our experimental data for both redox probes on bare gold in 0.1 M $LiClO_4$ aqueous supporting electrolyte buffered with phosphate buffer at pH 6.94 are presented in Table 1. The obtained values are almost an order of magnitude smaller than those typically found in an aqueous solution of KCl as a supporting electrolyte.

Basing on the results presented in Table 1, it is reasonable to draw a conclusion that all mono- and bilayers affect the redox reaction mechanism differently for the different redox probes present in the solution. It is interesting that in the case of the hydrophilic probe $K_4[Fe(CN)_6]$, the standard rate constants are three orders of magnitude smaller as those on bare gold, while in the case of dopamine, the amphiphilic molecule, the standard rate constant decreases only two orders of magnitude. It is possible that the amphiphilic molecule of dopamine penetrates the layer more deeply (especially through the small defects, e.g. collapse sites, in the hydrophobic chains region that brings about the decrease of the real distance of the redox molecule to the electrode surface). In agreement with these results, our SERS results (not shown) of ordering the tethered DPPE-MPA [33] and Chs-MPA [34] monolayers show that both trans and gauche types of C–C bond orientation next to the sulphur atom contribute to the spectrum. This may be responsible for an imperfect organization of the hydrocarbon chains within the hydrophobic region of tethered monolayers. The addition of a second phospholipid layer, as it could be expected, increases the blocking properties of the thiolipid monolayer and the values of standard rate constants k_s are smaller than in the case of the monolayer (Table 1). It is also interesting that the obtained values of the standard rate constants k_s are comparable to the values obtained for the electrode modified with the shorter than thiolipids thiols such as for example $C_{18}SH$ [44,45]. This suggests that more liquid molecules of the thiolipid form quite blocking, well-ordered layers without significant pinholes what is in agreement with previous results on gold and mercury modified with thiols of different lengths [7,42–46]. These results confirm that the thiolipid mono- and bilayers have good blocking properties and they could be used in the study of a tunneling process through the incorporated species.

4. Conclusions

The blocking properties and integrity of the described mono- and bilayer systems containing Chs-MPA and DPPE-MPA monolayers tethered to the gold surface are satisfactory and even better than could be expected based on literature. Such mimetic systems due to their reproducibility, stability and simple way of preparation can be widely used as good models of biological lipid membranes in the future studies.

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References

[1] W.G. Golden, C.D. Snyder, B. Smith, Infrared reflection–absorption spectra of ordered and disordered arachidate monolayers on aluminum, J. Phys. Chem. 86 (1982) 4675–4678.

[2] D.L. Allara, R.G. Nuzzo, Adsorption of bifunctional organic disulfides on gold surfaces, J. Am. Chem. Soc. 105 (1983) 4481–4483.

[3] R.G. Nuzzo, F.A. Fusco, D.L. Allara, Spontaneously organized molecular assemblies: 3. Preparation and properties of solution adsorbed monolayers of organic disulfides on gold electrode, J. Am. Chem. Soc. 109 (1987) 2358–2368.

[4] H.O. Finklea, S. Avery, M. Lynch, Blocking oriented monolayers of alkyl mercaptans on gold electrodes, Langmuir 3 (1987) 404–413.

[5] M.D. Porter, T.B. Bright, G.L. Allara, C.E.D. Chidsey, Spontaneously organized monolayer assemblies: 4. Structural characterization of *n*-alcyl thiol monolayers on gold by optical ellipsometry, infrared spectroscopy and electrochemistry, J. Am. Chem. Soc. 109 (1987) 3559–3568.

[6] F. Sabatini, I. Rubinstein, R. Maoz, J. Sagiv, Organized self-assembling monolayers on electrode: Part I. Octadecyl derivatives on gold, J. Electroanal. Chem. 219 (1987) 365–371.

[7] H.O. Finklea, D.H. Hansew, Electron transfer kinetics in organized thiol monolayers with attached pentamine(pirydine)ruthenium redox couple, J. Am. Chem. Soc. 114 (1992) 3173–3182.

[8] R.J. Foster, L.R. Faulkner, Electrochemistry of spontaneously adsorbed monolayers. Equilibrium properties and fundamental electron transfer characteristic, J. Am. Chem. Soc. 116 (1994) 5444–5452.

[9] J.N. Richardson, G.K. Rowe, M.T. Carber, L.M. Tender, L.S. Curtin, S.K. Peck, R.W. Murray, Electron transfer kinetics of self-assembled ferrocene (C_12) alkanethiol monolayers of gold electrodes from 125 to 175 K, Electrochim. Acta 40 (1995) 1331–1338.

[10] K. Weber, S.E. Creager, Voltammetry of redox-active groups irreversibly adsorbed into electrode treatment using Marcus relation between rate and overpotential, Anal. Chem. 66 (1994) 3164–3172.

[11] L. Tender, M. Carter, R.W. Murray, Cyclic voltammetric analysis of ferrocene alkanethiol monolayer. Electrode kinetics based on Marcus theory, Anal. Chem. 66 (1994) 3173–3181.

[12] J.F. Smalley, S.W. Feldberg, C.E.D. Chidsey, M.R. Linford, M.D. Newton, Y. Liu, The kinetics of electron transfer through ferrocene-terminated alkanethiol monolayers on gold, J. Phys. Chem. 99 (1995) 13141–13149.

[13] H. Skulason, C.D. Frisbie, Self-assembled monolayers with charge-transfer functional groups: immobilization of the electron donor TMPD and electron acceptor TCNQ, Langmuir 14 (1998) 5834–5840.

[14] M.J. Honychurch, Effect of the interfacial potential distribution on the measurement of the rate constant for electron transfer between electrodes and redox adsorbates, Langmuir 14 (1998) 6291–6296.

[15] W.R. Fawcett, Discreteness-of-charge effects at an electrode covered with self-assembled monolayers containing a simple redox couple, J. Electroanal. Chem. 378 (1994) 117–124.

[16] A. Dalmia, C.C. Liu, R.F. Savinell, Electrochemical behavior of gold electrodes modified with self-assembled monolayers with an acidic end group for selective detection of dopamine, J. Electroanal. Chem. 430 (1997) 205–214.

[17] R. Naumann, A. Jonczyk, C. Hampel, H. Ringsdorf, W. Knoll, N. Bunjes, P. Graber, Coupling of proton translocation through ATPase incorporated into supported lipid bilayers to an electrochemical process, Bioeletrochemistry & Bioenergetics 42 (1997) 241–247.

[18] A.J. Guiomar, J.T. Guthrie, S.D. Evans, Use of mixed self-assembled monolayers in a study of the microenvironment on immobilized glucose oxidase, Langmuir 15 (1999) 1198–1207.

[19] S. Dong, J. Li, Self assembled monolayers of thiols on gold electrodes for bioelectrochemistry and biosensors, Bioelectrochem. Bioenerg. 42 (1997) 7–13.

[20] E. Sackmann, Supported membranes: scientific and practical applications, Science 271 (1996) 43–48.

[21] E. Katz, H. Heteg-Shabtai, B. Willner, I. Willner, A.F. Bückumer, Electrical contact of redox enzymes with electrodes: novel approaches for amperometric biosensors, Bioelectrochem. Bioenerg. 42 (1997) 95–104.

[22] M. Pontié, C. Gobin, T. Paupalé, F. Bedioui, Electrochemical nitric oxide microsensors: sensitivity and selectivity characterization, Anal. Chim. Acta 411 (2000) 175–185.

[23] P. Krysiński, Redox reactions on TCNQ-modified self-assembled monomolecular films, Adv. Mater. Opt. Electron. 8 (1998) 121–128.

[24] L.K. Tammu, M. McConnell, Supported phospholipid bilayers, Biophys. J. 47 (1985) 105–113.

[25] H.T. Tien, S.H. Wurster, A.L. Ottova, Electrochemistry of supported bilayer lipid membranes: background and techniques for biosensor development, Bioelectrochem. Bioenerg. 42 (1997) 77–94.

[26] M. Tominage, S. Kusawo, N. Nakshima, Tuning of lipid bilayer fluidity regulates mediated electron transfer reactions of glucose oxidase immobilized on lipid bilayer films on an electrode, Bioelectrochem. Bioenerg. 42 (1997) 59–62.

[27] F.T. Buaninsegini, R. Herrero, M.R. Moncelli, Alkanethiol monolayer and alkanethiol/phospholipid bilayers supported by mercury: an electrochemical characterization, J. Electroanal. Chem. 452 (1998) 33–42.

[28] K. Chiang, U.J. Krull, D.P. Nikolelis, Ellipsometric determination of the structure of surface-stabilized bilayer lipid membranes on silver metal, Anal. Chim. Acta 357 (1997) 73–77.

[29] D.P. Nikolelis, C.G. Siontorou, Globin modified bilayer lipid membranes (BLMs) biosensor for carbon dioxide detection, Bioelectrochem. Bioenerg. 42 (1997) 71–75.

[30] R. Naumann, E.K. Schmidt, A. Jonczyk, K. Fendler, B. Kadenbach, T. Liebermann, A. Offenhäusser, W. Knoll, The peptide-tethered lipid membrane as a biomimetic system to incorporate cytochrome *c* oxidase in a functionally active form, Biosens. Bioelectron. 14 (1999) 651–662.

[31] M. Šnejdarkova, M. Rehak, M. Babincova, D.F. Sargent, T. Hianik, Glucose minisensor based on self-assembled biotinylated phospholipid membrane on a solid support and its physical properties, Bioelectrochem. Bioenerg. 42 (1997) 35–42.

[32] P. Diao, M. Guo, D. Jiang, Z. Jia, X. Cui, D. Gu, R. Tong, B. Zhong, Fractional coverage of defects in self-assembled thiol monolayers on gold, J. Electroanal. Chem. 480 (2000) 59–63.

[33] P. Krysiński, A. Żebrowska, A. Michota, J. Bukowska, M. Rosa-Moncelli, Tethered mono- and bilayer lipid membranes on Au and Hg, Langmuir 17 (2001) 3852–3857.

[34] P. Krysiński, A. Żebrowska, B. Pałys, Z. Łotowski, Spectroscopic and electrochemical studies of bilayer lipid membranes tethered to the surface of gold, J. Electrochem. Soc., in press.

[35] H.O. Finklea, D.A. Snider, J. Fedyk, E. Sabatani, Y. Gafni, I. Rubinstein, Characterization of octadecanethiol-coated gold electrodes as microarray electrodes by cyclic voltammetry and impedance spectroscopy, Langmuir 9 (1993) 3660–3667.

[36] C. Amatore, J.M. Saveant, D. Tessier, Charge transfer at partially blocked surfaces: a model for the case of microscopic active and inactive sites, J. Electroanal. Chem. 147 (1983) 39–51.

[37] W.B. Caldwell, D.J. Compbell, K. Chen, B.R. Herr, C.A. Mirikin, A. Malik, M.K. Durbin, P. Dutta, K.G. Huang, A highly ordered self-

assembled monolayer film of an azobenzenealkanethiol on Au(111): electrochemical properties and structural characterization by synchroton in-plane X-ray diffraction, atomic force microscopy, and surface-enhanced raman spectroscopy, J. Am. Chem. Soc. 117 (1995) 6071–6082.

[38] J.A.V. Butler, Studies in heterogeneous equilibria: Part II. The kinetic interpretation of the Nernst theory of electromotive force, Trans. Faraday Soc. 19 (1924) 729–733.

[39] J.A.V. Butler, Studies in heterogeneous equilibria: Part III. A kinetic theory of reversible oxidation potentials at inert electrodes, Trans. Faraday Soc. 19 (1924) 734–739.

[40] A.J. Bard, L.R. Faulkner, Electrochemical Methods: Fundamentals and Application, Wiley, New York, 1980.

[41] R.S. Nicholson, Theory and application of cyclic voltammetry for measurement of electrode reaction kinetics, Anal. Chem. 37 (1965) 1351–1355.

[42] P. Krysinski, M.R. Moncelli, F. Tadini-Buoninsegini, A voltammetric study of monolayers and bilayers self-assembled on metal electrodes, Electrochim. Acta 45 (2000) 1885–1892.

[43] P. Krysinski, M. Brzostowska-Smolska, Capacitance characteristics of self-assembled monolayers on gold electrode, Bioelectrochem. Bioenerg. 44 (1998) 163–168.

[44] K. Slowinski, R.V. Chamberlain, C.J. Miller, M. Majda, Through-bond and chain-to-chain coupling. Two pathways in electron tunneling through liquid alkanethiol monolayers on mercury electrodes, J. Am. Chem. Soc. 119 (1997) 11910–11919.

[45] P. Diao, M. Guo, D. Jiang, Z. Jia, X. Cui, D. Gu, R. Tong, B. Zhong, Studies of structural disorder of self-assembled thiol monolayers on gold by cyclic voltammetry and ac impedance, J. Electroanal. Chem. 464 (1999) 61–67.

[46] Z. Gao, K.S. Siow, Permeability of mixed self-assembled monolayers self-assembled monolayers of poly(3-dodecylthiophene) and 3-mercaptopropionic acid on gold electrodes, Anal. Commun. 33 (1996) 223–225.