Modified electrodes with new phenothiazine derivatives for
electrocatalytic oxidation of NADH

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Received 10 November 1999; received in revised form 23 February 2000

Abstract

Phenothiazine derivatives containing two linearly condensed phenothiazine moieties, 16H,18H-dibenzo[c,l]-7,9-dithia-16,18-diazapentacene (I), dibenzo[c,l]-16,18-diacetyl-7,9-dithia-16,18-diazapentacene (II) dibenzo[c,l]-16,18-dibenzyol-7,9-dithia-16,18-diazapentacene (III), and 16H,18H-dibenzo[c,l]-7,9-dithia-16,18-diazapentacene-7,7,9,9-bis-dioxide (IV), strongly adsorb on spectrographic graphite resulting in modified electrodes with electrocatalytic activity for NADH oxidation. From cyclic voltammetry measurements, performed in aqueous buffer solutions at different potential scan rates and pH values, the rate constants of the heterogeneous electron transfer and the transfer coefficients were estimated. The linear dependence between the peak current and the potential scan rate, corroborated with the slope of the formal standard potential versus pH linear regression, pointed out to a quasi-reversible, surface confined redox process involving 1e−/1H+. The electrocatalytical efficiency, evaluated from cyclic voltammetry, and the second order electrocatalytical rate constant (k1), calculated from rotating disk electrode experiments, revealed the same sequence of the activity decrease: III > II > IV > I. Compound III-modified electrodes were characterized by the highest k1 (1.8 × 103 M−1 s−1, at pH 7.0) as well as by the highest stability, expressed by the lowest rate of the surface coverage depleting under continuous potential cycling. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: NADH electrocatalytic oxidation; Phenothiazine derivatives; Modified electrodes; Amperometric sensor

1. Introduction

The electrochemical oxidation of reduced β-nicotinamide adenine dinucleotide (NADH) has been investigated extensively, since this reaction is of considerable interest for NADH recycling used in many active research domains as: construction of dehydrogenase based amperometric biosensors [1–5], fine chemicals industry using NAD+ -dependent biocatalysts [6,7], conversion of chemical energy into electrical energy via biofuel cell [8] etc.

Since the direct electro-oxidation of NADH on conventional electrode materials requires high overpotentials [9–11] many efforts were directed towards discovering and characterizing new efficient electrocatalysts (see the recently published comprehensive reviews [1–5]). Among the most frequently investigated mediating schemes are those based on the direct adsorption of electron mediators onto electrode surface to obtain modified electrodes with electrocatalytic activity for
NADH oxidation ([12], and references cited therein). Taking advantage of their remarkable strong adsorption on graphite surface associated with high electrocatalytic efficiency, organic dyes, i.e. phenazines, phenoxazines and phenothiazines derivatives were extensively used as electrocatalysts for NADH oxidation [13–15]. However, the organic dye-modified electrodes have as a major drawback a low chemical stability, especially at high pH values [1].

Recently, a strategy to improve the operational stability of NADH sensors was demonstrated by using poly (methylene blue)-modified electrodes, obtained by methylene blue electropolymerization [16]. Nevertheless, a significant decrease of electrocatalytic activity of the modified electrodes was still noticed during long-term stability experiments.

Starting from the idea that an increase of the intramolecular conjugation of the phenothiazine derivatives will increase their electrochemical stability, in this paper, a new strategy to obtain modified electrodes for NADH oxidation was proposed. Mainly, this strategy is based on using phenothiazine derivatives having two linearly condensed phenothiazine moieties [17,18]. In order to check this new strategy, four newly synthetized phenothiazine derivatives (compounds I–IV from Scheme 1) based on the 16$^H$,18$^H$-dibenzo[c,l]-7,9-dithia-16,18-diazapentacene structure, were used to obtain (I–IV)-modified graphite electrodes by simple adsorption. Cyclic voltammetry (CV) measurements, performed at different potential scan rates and pH values, in conjunction with rotating disk electrode experiments, were used to investigate the electrochemical as well as the electrocatalytical behavior of the modified electrodes. Based on the conformational structure and the energetical characteristics of the investigated compounds, an explanation of the sequence of the decreasing activity towards NADH oxidation, revealed by the quantitative estimation of the electrocatalytical activity, was attempted.

![Scheme 1. Structures of the dibenzodiaza-thiapentacene derivatives.](image)

2. Experimental

2.1. Materials

The phenothiazine derivatives, 16$^H$,18$^H$-dibenzo[c,l]-7,9-dithia-16,18-diazapentacene (I), dibenzo[c,l]-16,18-diacetyl-7,9-dithia-16,18-diazapentacene (II) dibenzo[c,l]-16,18-dibenzoyl-7,9-dithia-16,18-diazapentacene (III) and 16$^H$,18$^H$-dibenzo[c,l]-7,9-dithia-16,18-diazapentacene-7,7,9,9-bis-dioxide (IV), were synthesized according to previously published procedures [17,18].

$\beta$-Nicotinamide adenine dinucleotide, reduced form (NADH) was purchased from Sigma (St. Louis, MO, USA) as disodium salt. Phosphate buffer solutions were prepared using $\text{Na}_2\text{HPO}_4\cdot2\text{H}_2\text{O}$ and $\text{NaH}_2\text{PO}_4\cdot\text{H}_2\text{O}$ from Merck (Darmstadt, Germany. All other reagents were of analytical grade and used as received.

2.2. Electrode preparation

A spectrographic graphite rod (Ringsdorff-Werke, GmbH, Bonn-Bad Godesberg, Germany), of $\approx$ 3 mm diameter, was wet polished on fine (grit 400 and 600) emery paper (Buehler, Lake Bluff, Ill., USA). Then, a graphite piece of suitable length was carefully washed with deionized water, dried, and finally press-fitted into a PTFE holder in order to obtain a graphite electrode having, in contact with the solution, a flat circular surface of $\approx$ 0.071 cm$^2$.

The modified graphite electrodes were obtained by spreading onto the electrode surface 1 mm$^3$ of 2 mM phenothiazine derivative solution in dimethylformamide, and leaving them for 20 min at room temperature to evaporate the solvent. Before immersion in the test solution the modified electrodes were carefully washed with deionized water, dried, and finally press-fitted into a PTFE holder in order to obtain a graphite electrode having, in contact with the solution, a flat circular surface of $\approx$ 0.071 cm$^2$.

The modified graphite electrodes were obtained by spreading onto the electrode surface 1 mm$^3$ of 2 mM phenothiazine derivative solution in dimethylformamide, and leaving them for 20 min at room temperature to evaporate the solvent. Before immersion in the test solution the modified electrodes were carefully washed with deionized water. For each electrode, the surface coverage ($I$, mol cm$^{-2}$) was estimated from the under peak areas, recorded during the CV measurements at low scan rate ($v < 10$ mV s$^{-1}$), and considering as 1 the surface redox valency [19]. All presented results are the average of at least 3 identically prepared electrodes, if not otherwise mentioned.

2.3. Electrochemical measurements

CV measurements were carried out in a conventional three-electrode electrochemical cell. A saturated calomel electrode (SCE) and a coiled Pt wire served as reference and counter electrode, respectively. The cell was connected to a computer-controlled voltammetric analyzer (Autolab-PGSTAT10, Eco Chemie, Utrecht, Netherlands).

Steady state amperometric measurements at different rotating speeds of the working electrode were performed using a modulated speed rotator (model AMS-
Using the same strategy, four new phenothiazine derivatives containing two linearly condensed phenothiazine moieties were synthesized and characterized [17,18,26]. X-ray diffractometry and mechanical molecular calculations showed that single crystals of compound I, grown from N,N-dimethylformamide, contain 1.5 solvent molecules per donor molecule, which means that one solvent molecule is shared by two 16H,18H-dibenzo[c,l]-7,9-dithia-16,18-diazapentacene molecule (brute formula C\textsubscript{26}H\textsubscript{16}N\textsubscript{2}S\textsubscript{2} 1.5C\textsubscript{3}H\textsubscript{7}NO). This half of a solvent molecule is situated on a second order symmetry axis and is characterized by an important disorder, especially regarding the positions of the carbonyl carbon atom and one of the methyl group carbon atom.

As expected, the pentacene core of compound I is slightly bent. We measured a folding angle of 157.5° between the central benzene ring and one terminal naphthalene unit, whereas the other similar subunit shows a folding angle of 168.2°. The observed conformation is the one with the two terminal naphthyl groups folded in the same direction regarding the central benzene unit (see ORTEP diagram). The preference for the ‘cis’ stereochemistry of the compound I, thus accounting for the stability of the resulting modified electrodes, may be explained by the crystalline field interactions. Calculated energies for the frontier orbitals of the two possible conformers of 16H,18H-dibenzo[c,l]-7,9-dithia-16,18-diazapentacene [27] are very close. On the other hand, mechanical molecular calculations, performed in Sybyl-Tripos force field with default parameterization, showed that the difference between the energy values for a planar conformation of one phenothiazine subunit and a folded conformation (corresponding to an average folding angle of 165°) is only 6.4 kcal mol\textsuperscript{-1}.

Taking advantage of the strong adsorption of compounds I–IV on graphite, due to the extended aromatic character and to their almost planar conformation, the electrochemical behavior of I–IV-modified graphite electrodes was investigated by CV in different experimental conditions.

The cyclic voltammograms recorded for the compounds I–IV, adsorbed on the surface of a graphite electrode (Fig. 1A) present one wave with a formal
standard potential placed at 260–290 mV versus SCE at pH 7.0. The voltammetric behavior of the investigated compounds, characterized by the electrochemical parameters summarized in Table 1, corresponds to a quasi-reversible, monoelectronic process ($\Delta E_p < 50$ mV and $I_{pa}/I_{pc} \approx 1$). The discrepancies between the theoretical peak width at half peak height ($E_{FHWM} = 90.6$ mV) and the experimental values (Table 1) prove the existence of repulsive interactions between the adsorbed redox species (cation radicals generated in the anodic process) and attractive ones (neutral molecules or dimers formed during the cathodic process) [28,29]. Additionally, as expected for lateral interaction [30], it was noticed that peak parameters were affected by the surface coverage. Furthermore, as predicted for surface confined redox active species [31], the cyclic voltammograms recorded for a wide range of potential scan rates (10–1280 mV s$^{-1}$) showed a linear dependence of the peak currents ($I_p$) on the electrode potential scan rate ($v$) (Table 2). The deviations from the linearity observed at scan rates larger than 1280 mV s$^{-1}$ (Fig. 1B), could be attributed to the effect of the uncompensated ohmic drop [32]. The electron number, estimated from the $I_p$ versus $v$ dependence [33], was found (within ±10%) close to 1, in accordance with the predicted value for the cation radical formation.

Using the treatment proposed by Laviron [34], from the variation of the peak potentials with the potential scan rate (Fig. 2), the heterogeneous electron transfer rate constant ($k_s$ s$^{-1}$) for the compounds (I–IV) redox process was estimated (Table 3). As can be seen, for all compounds the transfer coefficient ($a$) is close to 0.5, while for compound III, the $k_s$ value is significantly higher than for the others. This peculiar behavior should be attributed to the presence of the two benzoyl substituents, known to exert a beneficial influence on the mediator adsorption on graphite surface [35,36] and expected to increase the mediator electrochemical stability due to their interactions with the intramolecular conjugated system.

As expected for the redox behavior of an N-unsubstituted phenothiazine moiety-containing compound [20] the formal redox potentials, $E^0$, (estimated as the average of cathodic and anodic peak potentials) for compounds I and IV were pH-dependent (Table 4). The slope of the regression line corresponding to the $E^0$—pH dependence, obtained from the experimental data in a wide pH range (2–11), proved that in the redox process is involved an equal number of electrons and protons. Consequently, the voltammetric response, corresponding to the formation of the cation radical, is based on a $1e^-/1H^+$ transfer. Surprisingly, a similar dependence was observed for compounds II and III (Table 4) suggesting a partial hydrolysis of these compounds.

The sequence of $E^0$ values for compounds I–IV does not fit that corresponding to the energies for the HOMO orbitals (I: 7.09; II: 7.72; III: 7.57; IV: 9.00 eV), calculated with the HYPER CHEM program. A possible explanation could be the influence exerted by the strong adsorption upon the reactivity of these compounds.

Based on the electrochemical behavior of the I–IV modified graphite electrodes, it was concluded that compound III presents the most convenient redox re-
Table 1
Electrochemical parameters of the voltammetric response of I–IV phenothiazine derivatives, adsorbed on spectral graphite

<table>
<thead>
<tr>
<th>Compound</th>
<th>ΔE_{peak} (mV)</th>
<th>E_{FWHM} (mV)</th>
<th>I_{oa}/I_{pc}</th>
<th>Surface coverage (10^{10} \text{ mol cm}^{-2})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Anodic</td>
<td>Cathodic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>39</td>
<td>102</td>
<td>80</td>
<td>1.06</td>
</tr>
<tr>
<td>II</td>
<td>49</td>
<td>124</td>
<td>80</td>
<td>1.03</td>
</tr>
<tr>
<td>III</td>
<td>38</td>
<td>107</td>
<td>85</td>
<td>1.05</td>
</tr>
<tr>
<td>IV</td>
<td>35</td>
<td>84</td>
<td>66</td>
<td>1.01</td>
</tr>
</tbody>
</table>

* Experimental conditions: as in Fig. 1.

Table 2
Linear regression parameters for the dependence of the peak current on the potential scan rate (0.01–1.28 V s^{-1}), observed for I–IV phenothiazine-modified graphite electrodes

<table>
<thead>
<tr>
<th>Compound</th>
<th>Slope (10^6 AV s^{-1})</th>
<th>R/no. exp. of points</th>
<th>Surface coverage (10^{10} \text{ mol cm}^{-2})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Oxidation</td>
<td>Reduction</td>
<td>Oxidation</td>
</tr>
<tr>
<td>I</td>
<td>38.4 ± 1.6</td>
<td>-46.7 ± 1.4</td>
<td>0.9889/11</td>
</tr>
<tr>
<td>II</td>
<td>25.0 ± 0.8</td>
<td>-29.4 ± 0.9</td>
<td>0.9903/12</td>
</tr>
<tr>
<td>III</td>
<td>8.4 ± 0.2</td>
<td>-8.7 ± 0.4</td>
<td>0.9958/12</td>
</tr>
<tr>
<td>IV</td>
<td>39 ± 1.5</td>
<td>-47.5 ± 0.7</td>
<td>0.9895/11</td>
</tr>
</tbody>
</table>

* Experimental conditions: as in Fig. 1.

The electro-oxidation of NADH has been achieved at many phenothiazine-modified electrodes at much lower overpotentials than at bare electrodes [1,4], but, generally, this approach suffers because of the low stability of mediators, especially at high pH values.

Starting from the favorable electrochemical behavior of the compounds I–IV adsorbed on graphite, their electrocatalytic activity was tested by CV. The electrocatalytic efficiency, estimated as the ratio (I_{cat})_{NADH}/(I_{cat}) at +320 mV versus SCE, was found decreasing in the following sequence: III (141.7) > II (53.6) > IV (37.4) > I (24.3).

For a better quantitative evaluation of the electrocatalytic behavior, kinetic measurements of NADH electro-oxidation at I–IV-modified graphite electrodes were performed at constant NADH concentration and pH 7, using the RDE technique. In all cases, in order to have a global electrode process controlled by the chemical reaction between NADH and the mediator, the applied
### Table 3
Kinetic parameters for the heterogeneous electron transfer at I–IV phenothiazine-modified graphite electrodes

<table>
<thead>
<tr>
<th>Compound</th>
<th>$k_s$ (s$^{-1}$)</th>
<th>$\alpha$</th>
<th>$R$/no. of exp. points</th>
<th>Surface coverage ($10^{10}$ mol cm$^{-2}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Oxidation</td>
<td>Reduction</td>
</tr>
<tr>
<td>I</td>
<td>30.1</td>
<td>0.61</td>
<td>0.9950/6</td>
<td>0.9888/6</td>
</tr>
<tr>
<td>II</td>
<td>21.8</td>
<td>0.45</td>
<td>0.9924/8</td>
<td>0.9885/6</td>
</tr>
<tr>
<td>III</td>
<td>135</td>
<td>0.43</td>
<td>0.9803/7</td>
<td>0.9602/7</td>
</tr>
<tr>
<td>IV</td>
<td>34</td>
<td>0.64</td>
<td>0.9971/8</td>
<td>0.9758/6</td>
</tr>
</tbody>
</table>

*Experimental conditions: starting potential, 0 mV versus SCE; supporting electrolyte, 0.1 M phosphate buffer, pH 7.0.

**potential was taken more positive with 120 mV [37] than the actual value of the formal standard potential of the phenothiazine-mediators. For all investigated systems, a well behaved mixed control (diffusional-kinetic) behavior, was observed for the $I - \omega^{1/2}$ dependence (2 mM NADH; pH 7; rotation speeds between 10 and 3615 rpm).

The so-called ‘Koutecky–Levich’s’ interpretation [1], in fact a $1/I_{cat}$ versus $\omega^{-1/2}$ plot at constant NADH concentration (Fig. 3), allowed to calculate the second order rate constant ($k_1$, M$^{-1}$ s$^{-1}$) for the NADH electrocatalytic oxidation. The calculated rate constants revealed the same sequence for the decrease of the catalytic activity as CV measurements: III (1800) $> II$ (1080) $> IV$ (670) $> I$ (320), pointing out to compound III as the most active mediator among the investigated compounds. The high electrocatalytic efficiency of compounds II and III must be attributed to the beneficial effect of electron density depletion on the reaction centers (N atoms), induced by the acetyl and benzoyl substituents, respectively [27]. The $k_1$ value measured for compound III-modified electrode at pH 7 was found smaller than the highest value ($3 \times 10^4$ M$^{-1}$ s$^{-1}$) ever-reported [1] for a dye mediator, Meldola Blue, a phenoxazyne compound.

**Table 4
Linear regression parameters corresponding to the pH dependence of the formal standard potential for I–IV phenothiazine-modified graphite electrodes

<table>
<thead>
<tr>
<th>Compound</th>
<th>Parameters of linear regression</th>
<th>$R$/no. of exp. points</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intercept (V) Slope (V/pH)</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>0.671 ± 0.01 0.054 ± 0.002</td>
<td>0.9970/10</td>
</tr>
<tr>
<td>II</td>
<td>0.69 ± 0.02   0.055 ± 0.002</td>
<td>0.9954/8</td>
</tr>
<tr>
<td>III</td>
<td>0.693 ± 0.02  0.061 ± 0.003</td>
<td>0.9940/8</td>
</tr>
<tr>
<td>IV</td>
<td>0.685 ± 0.01  0.059 ± 0.001</td>
<td>0.9987/9</td>
</tr>
</tbody>
</table>

*Experimental conditions: starting potential, 0 mV versus SCE; scan rate, 50 mV s$^{-1}$; supporting electrolyte, 0.1 M phosphate buffer; pH range, 2–11; surface coverage, $\approx 3.5 \times 10^{-10}$ mol cm$^{-2}$.

Batch amperometric measurements at constant applied potential (+ 300 mV versus SCE), performed in a magnetic stirred solution at pH 7, proved that compound III-modified electrode works well as an NADH amperometric sensor. The following analytical parameters describe its performances: linear range, from 2 μM to 1 mM (the correlation coefficient was 0.999 for 36 experimental data); sensitivity, 99 mA/(M cm$^{-2}$); detection limit, $\cong 0.5$ μM ($S/N$ 3); response time, $\cong 1$ min. The slope of the log $I$–log[NADH] dependence, examined for the concentration range corresponding to the amperometric response, was close to unity (0.93 ± 0.02) confirming once more that the NADH sensor works under kinetic control.
4. Conclusion

A comparative study on the electrochemical behavior and electrocatalytical activity for NADH oxidation, involving four new synthetized phenothiazine derivatives containing two linearly condensed phenothiazine moieties, confirmed the strategy, based on the enlargement of the intramolecular conjugation system of the mediator in order to increase its electrochemical stability.

In this context it was established that a nearly planar conformation and the benzoyl substituents at N-atoms determined both a stronger adsorption on graphite surface and a higher catalytic activity for NADH electro-oxidation. The resulting modified electrodes exhibited remarkable stability even at high pH values (11), but they showed a moderate electrocatalytic activity. Researches for further improvement of the mediator efficiency are in progress.

Acknowledgements

Financial support from the Romanian Academy of Sciences (Grant 55/1999) is gratefully acknowledged.

References