ELECTROCHEMICAL BEHAVIOUR AT MACRO AND MICROELECTRODES OF CYTOCHROMES C AND C_3 FROM *D. VULGARIS*. A COMPARATIVE STUDY

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Abstract: A comparative study of heme proteins horse heart cytochrome c and cytochrome c_3 of *Desulfovibrio vulgaris* (strain Hildenborough) using different electrochemical techniques (linear scan and cyclic voltammetry, differential pulse and square wave voltammetry) with platinum and gold macro and microelectrodes is presented. The electrochemical response was evaluated analysing the reversibility of the electrode process and the formal potentials determined. Diffusion coefficients for both proteins were also calculated from the voltammetric data. The electrochemical behaviour at macro and microelectrodes was analysed and the advantages of microelectrodes pointed out.

Key words: Proteins electrochemistry, microelectrodes, cytochrome c, cytochrome c_3.

Introduction

The electrochemical behaviour of biologically important heme proteins is currently one of the most attractive research topics in bioelectrochemistry. Different electrochemical techniques may allow to have a better insight into the functional properties of the redox centers of the proteins. Electrochemistry at microelectrodes (critical dimension in the range 0.1 to 50 μm) may present some advantages over electrodes of conventional size. Namely enhanced mass transportation by non-linear diffusion allows the determination of kinetic parameters without the onset of capacitive currents. Moreover, due to their dimensions it is possible to work in very small volumes which is a very important feature when working with proteins. The aim of this work is to present a comparative study of the electrochemical behaviour of cytochrome c and cytochrome c_3 from *D. vulgaris* at macro and microelectrodes using different electrochemical techniques such as linear scan (LS) and cyclic voltammetry (CV), differential pulse (DP) and square wave (SW) voltammetry. Both proteins are heme type but while cytochrome c has one redox center (monohemic) cytochrome c_3 contains four hemes per molecule.

Experimental

The electrochemistry of horse heart cytochrome c (Sigma) and cytochrome c_3 from *D. vulgaris* (kindly supplied by Profs. I. Moura and J. Moura) was analysed at Pt and Au macro and microelectrodes. Solutions of both proteins with concentrations in the range 0.10 up to 0.33 mM were prepared in $NaNO_3$ 0.1 M and in phosphate buffer 10^{-2} M (pH = 7).

Voltammetric measurements were performed using a potentiostat/galvanostat AUTOLAB / PSTAT 10 with the low current module ECD from ECO-CHEMIE and the

data analysis processed by the General Purpose Electrochemical System (GPES 3.2) software package also from ECO-CHEMIE.

The potential was varied between +0.3 and -0.3V vs. SCE for cytochrome c and -0.1 and -0.8V vs. SCE for c_3. All measurements were done in deaerated solutions and at T = 20 ± 2 °C.

Results and Discussion

Cytochrome C: reduction at Pt and Au electrodes

In Fig. 1 CV, LS and SW voltammograms are shown for the reduction of cytochrome c at macro and microelectrodes. For all types of electrodes the charge transfer process at the metal electrodes only takes place at a reasonable rate in the presence of the promotor 4,4'-bipyridyl [3].

Due to the different time regime operating at both macro and microelectrodes a sigmoidal curve (B in Fig. 1) characterised by a steady state current, i_s, is obtained with microelectrodes for scan rates below 2 mV/s [1], contrarily to what happens with macroelectrodes where a peak shape curve is obtained (A in Fig. 1) In square wave voltammetry a bell shaped curve is obtained for the net current, no matter the electrode size (C and D in Fig. 1) [2].

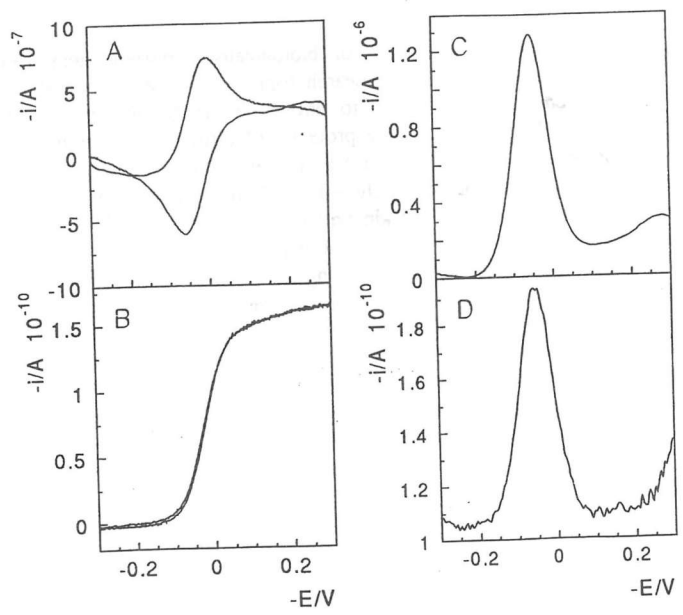

Fig. 1 - Voltammograms of cytochrome c 0.20 mM in $NaNO_3$ 0.1 M, pH = 7.0 in phosphate buffer 0.01 M and in the presence of 4,4'-bipyridyl 15 mM. a) Cyclic voltammogram at macro Pt electrode; b) Linear scan voltammogram at micro Pt electrode; c) Square wave voltammogram at macro Pt electrode; b) Square wave voltammogram at micro Pt electrode.

The analysis of the results obtained by CV showed a linear variation of the peak current (i_p) vs. the square root of the scan rate ($\sqrt{v}$) up to scan rates of 0.1 Vs^{-1} for both Pt and Au macroelectrodes. For the Pt electrode the ratio of the cathodic to anodic currents (i_p^c / i_p^a) was equal to one and the peak potential separation between the cathodic and anodic peaks (ΔE_p) was closed to 58 mV, and independent of scan rate. In the case of the Au electrode a ratio different from unit and an increase in peak separation (between 63 and 101 mV) was observed. So, one may conclude that the electrochemical reduction of cytochrome c although reversible at the platinum macroelectrode, occurs as a quasi-reversible process at the gold electrode.

As to the results obtained by LSV with the microelectrodes, analysis in terms of the Tomeš criterion ($E_{3/4}$ - $E_{1/4}$) and the slope of the logarithmic plots (E vs. $\log(i_s-i)/i$) lead to similar conclusions. In fact, for the reduction of cytochrome c at the Pt microelectrode a value of $E_{3/4}$ - $E_{1/4}$ = 56 ± 2 mV and a slope of 59 ± 2 mV were found in quite good agreement with the theoretical values for a totally reversible reaction (56.4 mV and 59.15 mV, respectively, for T = 25°C [4]). For the reduction at the Au microelectrode the values observed for the Tomeš criterion and the slope of log analysis were 70 ± 1 mV and 73 ± 2 mV, respectively.

Reproducible differential pulse voltammograms were also obtained for the reduction of cytochrome c at macro and microelectrodes of gold and platinum. However, the width of the peaks at half height, $W_{1/2}$, was larger for the gold electrodes (Table 1).

Table 1 - Values of $W_{1/2}$ (mV) of DPV for the reduction of cytochrome c.

Type of Electrode	Macro	Micro
Platinum	91	96
Gold	105	118

From Table 1 one may envisage that, although no theory has been developed for DPV with microelectrodes, $W_{1/2}$ values measured with these sensors may give us some insight into the degree of reversibility, as happens with macroelectrodes.

The results obtained by SWV analysed in terms of the variation of the net peak current with the square root of the frequency also suggested that while the electrochemical reduction of cytochrome c is reversible at the platinum macro and microelectrodes, a quasi-reversible process takes place at the gold electrodes.

For a nonreversible system kinetic data can be determined either from transient measurements (eg. CV at macroelectrodes) or from steady state measurements (eg. LSV at microelectrodes).

An estimate of the heterogeneous charge transfer rate constant, k_s, for the reduction of cytochrome c at the gold macroelectrode was then calculated from the CV results using Nicholson's method [5]. A value of $k_s = (1.3 \pm 0.3) \times 10^{-2}$ cm s^{-1} was obtained in quite good agreement with $k_s = 1.5 \times 10^{-2}$ cm s^{-1} determined from alternating current measurements [6], which indicates that the redox process is nearly reversible.

As to the results obtained by LSV with the gold microelectrode, according to the mass transfer parameter $m = \frac{D}{r} \sim 10^{-3}$ cm s^{-1}, where D is the diffusion coefficient of the electroactive specie ($\sim 10^{-6}$ cm^2 s^{-1}) and r is the electrode radius, values of $k_s > 10^{-3}$ cm s^{-1} are not accurately determined for the experimental conditions used. A smaller electrode should be necessary [7].

A very good agreement was found for the formal potential values, $E^{o'}$, estimated using either E_p or $E_{1/2}$ values from the several voltammetric techniques with macro and microelectrodes. Corrections due to a slower electrochemical reaction at gold electrodes were neglected since the redox processes are in the boundary between reversibility and quasi-reversibility [4]. Thus an average value of 265 ± 5 mV vs. NHE was calculated in excellent agreement with previously determined values of $E^{o'}$ for cytochrome c, $E^{o'} = 255 \pm 5$ mV vs. NHE [1].

The diffusion coefficient of cytochrome c was also determined from the results of CV, LSV and SWV (Table 2).

Table 2 – Diffusion coefficient D (cm^2 s^{-1}), of cytochrome c determined by CV, LSV and SWV.

Technique / Electrode	CV	LSV	SWV
Pt macro	1.0×10^{-6}	-	1.1×10^{-6}
Au macro	(0.7×10^{-6})	-	($0.8 - 2 \times 10^{-6}$)
Pt micro	-	1.2×10^{-6}	-
Au micro	-	1.4×10^{-6}	(2.0×10^{-6})

Once more it should be pointed out the good agreement among the values, even in the case where full reversibility was assumed in the calculations. However this is not a drawback as far as the results obtained by LSV with microelectrodes are concerned: steady state current is always achieved whose magnitude is directly proportional to D. For comparison a value of $D = 1.14 \times 10^{-6}$ cm^2 s^{-1} determined by non-electrochemical methods [8] is presented.

Cytochrome C_3: (from *D. vulgaris*) reduction at Au electrode

The use of microelectrodes in electrochemical studies of proteins was further analysed with the reduction of cytochrome c_3 at Au microelectrode. As happens with some macroelectrodes [9] cytochrome c_3 undergoes a direct electron-transfer at the gold microelectrode. This protein contains four heme groups that correspond to four similar (but non-equivalent) noninteracting redox sites. So, the voltammograms have a shape close to a one-electron transfer reaction but the current is proportional to the total number of electrons, n, being eventually smaller than the theoretical value according to n, unless the steady state is achieved [10].

From the analysis of the results obtained for cytochrome c_3 by CV and LSV following the procedure described for cytochrome c, we may conclude that the charge transfer of cytochrome c_3 at the gold macro and microelectrodes used is a quasi-reversible process. From the CV results at the Au macroelectrode and using Nicholson's method, a value of $k_s \geq 1.5 \times 10^{-3}$ cm s^{-1} was calculated from the increase in peak potential separation with the scan rate. Analysis of the steady state voltammograms at the Au microelectrode in terms of $E_{1/4} - E_{1/2}$ and $E_{1/2} - E_{3/4}$ allowed the determination of $k_s \geq 1.2 \times 10^{-3}$ cm s^{-1} in straightforward way [11].

From the good agreement between E_P values of the cyclic voltammograms and $E_{1/2}$ values of the linear scan voltammograms, a value of $E^{0'} \sim -292 \pm 5$ mV vs. NHE was estimated (neglecting the kinetic effect). One should point out that $E^{0'} = -281$ mV vs. NHE was obtained from the voltammetric data due to the reversible reduction of this protein at mercury electrodes [9].

From the steady state currents achieved in linear scan with the gold microelectrode, a value of $D = 0.9 \times 10^{-6}$ cm^2 s^{-1} was easily determined. Taking into account the molecular weight of both cytochrome c and c_3 (12384 and ~13000, respectively) a similar, or eventually slightly smaller value for the diffusion coefficient of c_3 was expected when comparing with cytochrome c (in the same medium).

Conclusions

From the above results it is apparent that microelectrodes can be useful tools in electrochemical studies of proteins. Namely, the steady state currents readily attained due to the very small dimensions of the electrodes make the measurement of electrochemical kinetics and transport parameters straightforward procedures. Due to their size electrochemistry in µl of solution is also feasible, which is an important aspect when dealing with proteins.

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ELECTROOXIDATION OF LIGNIN IN ALKALINE MEDIUM

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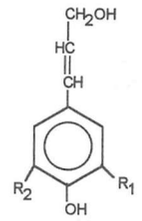
Abstract: The electrochemical behavior of lignin, in alkaline medium, was studied by cyclic voltammetry and compared with that of some lignin model compounds e. g. guaiacol, eugenol, vanillic alcohol and vanillin, in order to elucidate the process involved in electrode reactions. Galvanostatic electrolyses of lignin were carried out using different electrode materials in order to investigate the influence of electrode material on the product distribution.

Introduction:

According to the widely accepted concept, lignin may be defined as an amorphouse polyphenolic material arising from an enzyme mediated dehydrogenative polymerization of three phenylpropanoid monomers, coniferyl (1), sinapyl (2), and p-coumaryl (3) alcohols [1].



(1) $R_1 = OCH_3$; $R_2 = H$

(2) $R_1 = R_2 = OCH_3$

(3) $R_1 = R_2 = H$

The estimated annual production of lignin as a by product of paper making industry is about 30 millions tons. 95% of this amount are burned or dumped as polluting effluent and only 5% are converted into useful chemicals [2]. Electrochemistry of lignin has ben the subject of numerous studies including the depolymerization, the preparation of new polymers for special applications and the conversion into useful compounds [3].

The aim of this work is to evaluate the performance of the electrochemical way in low molecular weight carbonyl compounds production from the oxidative degradation of lignin.

Experimental :

Electrochemical equipment

The preliminary studies were carried out by cyclic voltammetry. The voltamograms were recorded using a PPRI model waveform generator (HI-TEK) a DT2101 model potentiostat (HI-TEK) and a PM 8043 model XY recorder (Philips). A thermostated three-electrode electrochemical cell was used. A saturated calomel electrode (SCE) served as a reference electrode but all the potentials are given in the