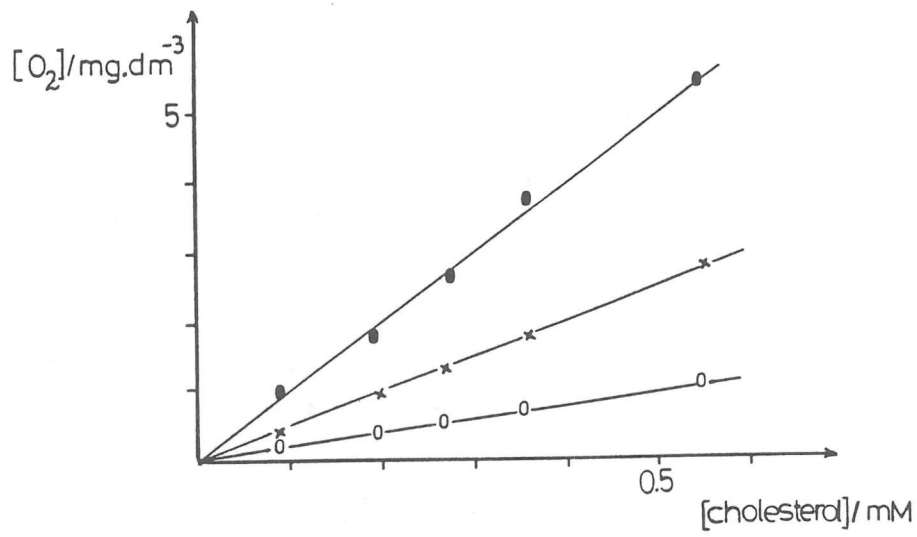

Fig. 2 Biosensor response on: ● day 1, X day 10 and 0 day 30.

The response time depends on the membrane thickness and using the Teflon membrane the steady-state value was attained very rapidly, as shown in Fig. 1. A linear response was obtained for a range of concentrations of cholesterol of 90μM → 0.55mM, Fig. 2, and the detection limit determined was 50μM. Fig. 2 shows that the activity of the biosensor drops after 30 days of use and it was found that the lifetime of this biosensor was 35 days.

In conclusion, the standard oxygen electrode with the modified Teflon membrane becomes a multifunctional sensor that quantifies oxygen, hydrogen peroxide and cholesterol.

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CURRENT/POTENTIAL STUDIES ON TETRAHEME CYTOCHROMES c_3.

SIMULATION OF THE ELECTROCHEMICAL BEHAVIOR OF MULTIREDOX SYSTEMS

C. Moreno[1], A. Campos[2], M. Teixeira[1,2], J. LeGall[3], M.I. Montenegro[4], I. Moura[1,2], C. van Dijk[5] and J.J.G. Moura[1,2]

[1] Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2925 Monte da Caparica, Portugal.
[2] Centro de Tecnologia Química e Biológica, Apartado 127, 2780 Oeiras, Portugal.
[3] Depart. of Biochemistry, University of Georgia, Athens, Ga 30602, USA.
[4] Centro de Química Pura e Aplicada, Universidade do Minho, Largo do Paço, 4700 Braga, Portugal.
[5] Depart. of Biochemistry and Food Processing, ATO, P.O. Box 107, 6700 AA, Wageningen, The Netherlands.

The direct (unmediated) electrochemical response of the tetrahemic cytochrome $\underline{c}_3$, isolated from the sulfate reducer Desulfovibrio baculatus, was evaluated using different electrode systems (graphite (edge cut), gold and semiconductors (indium oxide)) and different electrochemical methods (cyclic voltammetry and differential pulse voltammetry).

Tetrahemic cytochromes $\underline{c}_3$ are found in sulfate-reducing bacteria. They represent an ideal situation for studying a multielectronic transfer system with four redox centres in a fixed geometry. Each heme, in this class of cytochromes, is covalently bound to the polipeptide chain by two thioether linkages involving cysteinyl residues and the fifth and sixth heme-iron ligands are histidinyl residues. The four hemes are localized in non-equivalent protein environments and have a negative and different mid-point redox potential.

Figure 1 shows the direct voltammetric response observed for D.baculatus cytochrome $\underline{c}_3$ at the carbon (edge) electrode. No mediators nor electrode modifiers were required for the obtaintion of an imediate and reproducible response. The cyclic voltammograms show, as expected, the overlapping of several redox processes during the cathodic and anodic sweeps, suggesting that the individual redox potentials are rather close. As a consequence, the originated voltammogram shows a cathodic/anodic peak potential separation greater than the one expected for a simple reversible situation (around 80 mV), preventing the use of the ΔEp as a criteria for the reversibility of the process.

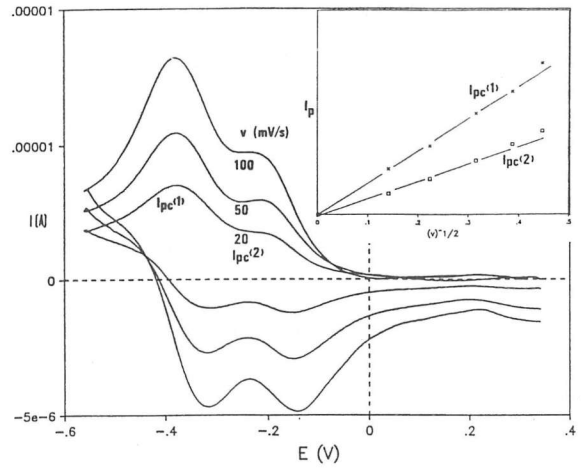

Figure 1.

D.baculatus cytochrome c_3 cyclic voltammograms, at pH 8.0.

Insert: Dependence of the cathodic peaks current on the scan rate.

As the cyclic voltammograms obtained correspond to sucessive one electron processes and the determination of the electrochemical parameters required the deconvolution of the experimental data into the individual components, it was developed a computer program for the theoretical simulation of a complete cv curve, based on the method proposed by Nicholson and Shain(1), using the Gauss-Legendre method for calculation of the integrals involved.

Figure 2 (*Panel 1*) shows the deconvolution of the total voltammogram into the four components. In the same figure, *Panel 2* compares, by superimposition, the experimental and the theoretical voltammograms.

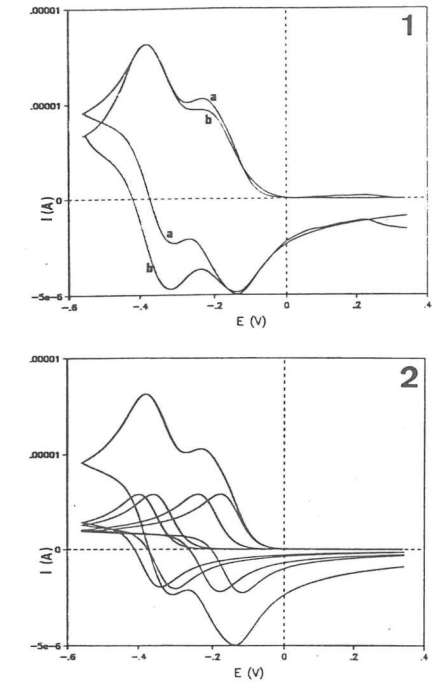

Figure 2

Panel 1 - Simulation of the cyclic voltammogram of D.baculatus cytochrome c_3 (scan rate 100 mV/s at pH=8.0; a-theoretical, b-experimental).

Panel 2 - Deconvolution of the total voltammogram in four components with E^o=-150,-210, -330 and -380 mV vs NHE.

Using the described method, we determine the pH dependence of the redox potentials obtained after deconvolution of the cyclic voltammograms. There is a very pronounced pH dependence of E1 and E2, but E3 and E4 are almost pH independent (2).

In the study of the unmediated electron transfer between a protein and an electrode surface, the interface is an important aspect. In order to be sure that this measurements are independent of the electrode system, different electrodes were used. The following table shows the mid-point redox potentials obtained by deconvolution of the experimental data of D.baculatus c_3 in different electrodes, pH values and electrochemical methods. A reasonable agreement was obtained.

Mid-point redox potentials of multiheme cytochromes determined after deconvolution of cv and dpv experimental data in different electrode systems

Cytochrome c_3	Method	pH	E_1	E_2	E_3	E_4
D.baculatus	CV (carbon-edge)	4.9	-60	-180	-300	-360
		6.3	-130	-190	-320	-360
		7.6	-140	-200	-320	-360
		8.0	-150	-210	-330	-380
		9.2	-150	-210	-330	-380
		10.3	-200	-270	-330	-380
D.baculatus	CV (Indium oxide)	7.6	-150	-270	-340	-370
D.baculatus	DPV (Au)	5.0	-70	-170	-300	-345
		7.0	-155	-260	-310	-355
		9.0	-170	-260	-320	-370
Estimated error (mv)			E_1±10	E_2±20	E_3±15	E_4±10

These redox potentials were determined by independent methods (namely EPR) with a good agreement, giving confidence on the deconvolution method used.

We plan to apply our method to other systems, namely hexaheme proteins (nitrite reductase) where multiredox reaction occurs in paralell with high-spin / low-spin equilibrium that is a determining factor of protein reactivity.

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REACTION OF PYRIDOXAL AND PYRIDOXAL-5'-PHOSPHATE WITH HEXYLAMINE. COMPARATIVE ELECTROCHEMICAL AND SPECTROPHOTOMETRIC STUDIES

T. Pineda, M. Blázquez,* J.M. Sevilla, F. García-Blanco and M. Domínguez

Departamento de Química Física y Termodinámica Aplicada,
Facultad de Ciencias, Universidad de Córdoba, 14004 Córdoba (Spain)

ABSTRACT

A comparative study of the Schiff bases formed by reaction of the pyridoxal (PL) and pyridoxal-5'-phosphate (PLP) with hexylamine in basic media was carried out. Acid-base dissociation constant of the imine group was obtained by spectrophotometric measurements. Apparent formation constant of the Schiff base was obtained as a function of the pH. Electrode processes were considered on the basis of polarographic and kinetic parameters.

INTRODUCTION

PLP is a cofactor of numerous enzymes which catalyze reactions of transamination, decarboxylation, α-β elimination, etc. The binding to protein is via formation of a Schiff base with a lysil residue.[1] Studies of the reaction of PLP with primary amines or amino acids confirmed that the formation of the Schiff base is coupled with multiple acid-base and tautomeric equilibria.[2,3] The Schiff base of PLP and hexylamine was proposed as a simple model to estimate polarities of the microenvironment of the coenzyme on the basis of spectrophotometric properties.[4] Indeed, combined spectrophotometric and electrochemical studies allowed a quantitative characterization of equilibria in solution of this Schiff base.[5,6]

The present work deals with a comparative study of the Schiff base of PL and PLP with hexylamine by DC and DP and cyclic voltammetric in basic media. In addition, a study of the UV-visible spectra of the reaction mixture has been carried out to characterize the protonation equilibrium involved in the electroreduction of the Schiff base.

EXPERIMENTAL

PL and PLP were purchased from Sigma. The Schiff bases were obtained by adding known amounts of hexylamine to PL and PLP solutions. In all cases measurements were carried out after the reaction reaches equilibrium. All other conditions were similar to those described in refs. 7 and 8.

(*) To whom correspondence should be addressed