A CHOLESTEROL BIOSENSOR

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The role of cholesterol in health is associated with the incidence of heart diseases. Cholesterol is essential for life, being a structural component of biological membranes. When the metabolism of cholesterol is disturbed it accumulates as the cholesteryl ester in the artery walls. These deposits lead to a disease, atherosclerosis, which is now an epidemic in western society due to genetic, dietary and social factors, such as smoking.

Electrochemical sensors [1] can play an important part in rapidly quantifying cholesterol in biological fluids. While *in vivo* determinations using microminiature sensors still present some problems, *in vitro* measurements can be accomplished using amperometric sensors such as the modified standard oxygen electrode.

In order to determine cholesterol at an oxygen electrode the reactions of two enzymes were used: cholesterol oxidase and catalase, both immobilised directly onto a Teflon membrane [2]. The first enzyme oxidises cholesterol and produces hydrogen peroxide, and the second enzyme oxidises peroxide to oxygen which is determined at the gold electrode.

The enzymes, catalase (from bovine liver) and cholesterol oxidase (from streptomyces species), cholesterol and 1-cyclohexyl-3-(2-morpholinoethyl) carbodiimide metho-p-tolueno-sulphonate (CMC) were obtained from Sigma Chemical Company, U.K. Acrylic acid from Merck, FRG, was used without purification. All other chemicals were of analytical grade and were obtained commercially.

Teflon membranes (0.02g) were supplied by Metrohm, EA 1092/2. These supports were grafted with acrylic acid in order to increase their hydrophilicity and to create reactive groups. The films were immersed in 30cm^3 of 10% aqueous acrylic acid solution and irradiated at 18.3 rad s^{-1},

with a ^{60}Co source, for 72 hours at room temperature in the presence of air. The homopolymer was removed by extraction with water. The graft copolymers were dried in vacuo at 313K to constant weight.

CMC was used as activating agent to couple the enzymes to the -COOH groups of the grafted poly(acrylic acid). The presence of poly(acrylic acid) was confirmed by infrared spectroscopy as in ref. [3] and the percentage of grafting was determined gravimetrically to be 22% graft.

This membrane was used first to immobilise catalase according to previous procedure [3], washed with phosphate buffer and then used to immobilise cholesterol oxidase. The activity of both enzymes was measured chemically.

The amount of immobilised enzymes, catalase and cholesterol oxidase, was determined by the Lowry method. The catalase activity was determined following the method of Beers and Sizer using hydrogen peroxide and the cholesterol oxidase activity was determined by determination of cholesterol - all measurements were done on a Jasco 7800 Spectrophotometer. The activity of both enzymes after being immobilised is indicated in Table 1.

TABLE 1

Immobilisation of enzymes catalase and cholesterol oxidase onto Teflon-g.co-acrylic acid 22% graft supports.

Enzyme	mg $E.g^{-1}$ of copolymer	Coupling yield* /%	Retention of activity** /%
catalase	24.1	0.96	7.6
cholesterol oxidase	26.2	10.50	17.6

* mg of enzyme attached / mg of enzyme used.
** mg active enzyme / mg of immobilised enzyme.

The Teflon membrane containing immobilised catalase and cholesterol oxidase was placed over the gold electrode of a standard Clark-type oxygen electrode (Metrohm EA-541) in the usual way and connected to a Metrohm E627 O_2-meter. The characteristics of the Teflon membrane with the two immobilised enzymes were studied and the kinetics, Michaelis-Menten parameters, optimum temperature, ionic strength and pH were determined.

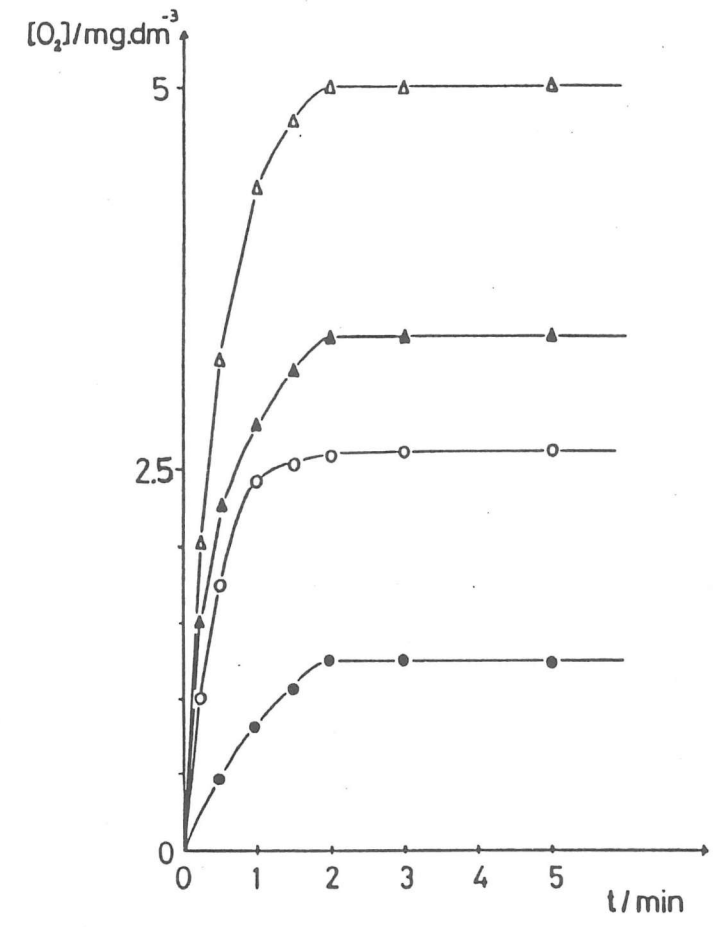

Fig. 1 Biosensor response time for different concentrations of cholesterol in 0.05M phosphate buffer pH=7.0, day 1: ● 0.12mM; o 0.28mM; ▲ 0.37mM; Δ 0.44mM (1mM $O_2 \equiv 32 mg.dm^{-3}$).

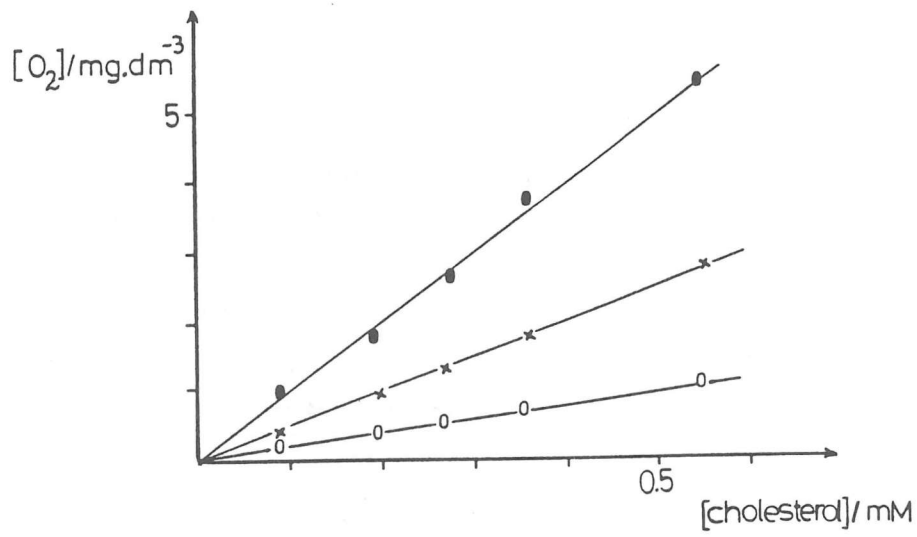

Fig. 2 Biosensor response on: ● day 1, X day 10 and 0 day 30.

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.

REFERENCES

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CURRENT/POTENTIAL STUDIES ON TETRAHEME CYTOCHROMES c_3.

SIMULATION OF THE ELECTROCHEMICAL BEHAVIOR OF MULTIREDOX SYSTEMS

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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.