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Short communication

Direct electrochemistry of catalase on glassy carbon electrodes

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Abstract

Catalase was investigated as a possible catalyst of the electrochemical reduction of oxygen on glassy carbon electrodes. The presence of catalase dissolved in solution only provoked a moderate current increase, which was fully explained by the catalase-catalysed disproportionation of hydrogen peroxide (Scheme I). When catalase was adsorbed from dimethylsulfoxide on the surface of electrodes that did not undergo any electrochemical pre-treatment (EP), catalase efficiently catalysed oxygen reduction via direct electron transfer from the electrode (Scheme II). The results are discussed with respect to the electrode surface properties and the enzyme structure. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Catalase; Glassy carbon; Oxygen reduction; Direct electronic transfer

1. Introduction

Catalase is made up of four equal subunits each of 57,000 molecular mass and equipped with a Fe(III)-protoporphyrin IX. It catalyses the disproportionation of hydrogen peroxide into oxygen and water without the formation of free radicals:

$$2H_2O_2 \rightarrow O_2 + 2H_2O \qquad \text{(reaction 1)}$$

We know only little about the electrochemistry of catalase. A few studies have shown that a slow direct electron transfer between catalase and pure graphite or soot electrodes should be possible in deoxygenated conditions [1,2].

The purpose of this work was to determine whether catalase is able to ensure direct electron transfer with glassy carbon electrodes, and whether it is able to catalyse the electrochemical reduction of oxygen, because such a catalysis may play a key role in some cases of aerobic microbiologically influenced corrosion [3].

2. Experimental

All experiments were made in 0.1M potassium phosphate buffer at pH 8.0, which also contained 0.15 M NaCl. Experiments in the absence of oxygen were carried out after removing oxygen from the solution by 30-min nitrogen bubbling. Only very gentle bubbling was used when catalase was free in solution. No stirring or gas bubbling was performed during current recording. Catalase from bovine liver ($EC_{1.11.1.6}$, 1990 U/mg solid) was purchased from Sigma. Adsorption of catalase from DMSO was carried out as described elsewhere [4]. The glassy electrode was soaked in pure DMSO for 15 min, then rinsed with phosphate buffer and distilled water. Catalase was dissolved in pure DMSO at 2 mg/ml final concentration. This concentration value is only given as an indication, because catalase was poorly soluble in DMSO. The electrode was immersed in this solution for 15 min, and finally rinsed with buffer solution at pH 8.0.

Glassy carbon working electrodes of 0.3-cm diameter (Le Carbone Lorraine, V25) were connected to a saturated calomel reference electrode (SCE) and a GC auxiliary electrode. All experiments were performed with a Solartron–Schlumberger 1286 interface. The GC working electrodes were polished with emery paper (Norton P320 and A621). When indicated, a preliminary electrochemical pretreatment (EP) consisted of 1 min electrolysis at 1.5 V/SCE,

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followed by potential cycles in the range from 0.1 to − 1.1 V/SCE for 6 min at 100 mV s^{-1}. The solution was then strongly stirred to get a homogeneous oxygen concentration, and a current–potential curve was recorded at 20 mV s^{-1}. The whole procedure was repeated at least four times, until the curves became reproducible.

3. Results

3.1. Catalase dissolved in solution

The current–potential curves obtained at 20 mV s^{-1} with 0.25 mg/ml catalase (500 U/ml) dissolved in the NaCl–phosphate buffer pH 8.0 are reported in Fig. 1. GC electrodes were used just after polishing (Fig. 1A) or with a preliminary EP (Fig. 1B). In both cases, current was firstly recorded in aerated solutions without enzyme (curves a), then with catalase in solution (curves b), and lastly with the enzyme in deoxygenated solution (curves c). According to the literature [5], in the absence of catalase (curves a) the first peak was attributed to the reduction of oxygen into hydrogen peroxide:

$$O_2 + 2H^+ + 2e^- \rightarrow H_2O_2 \qquad \text{(reaction 2)}$$

and the second to the reduction of hydrogen peroxide into water:

$$H_2O_2 + 2H^+ + 2e^- \rightarrow 2H_2O \qquad \text{(reaction 3)}$$

The reactions were written with H_2O_2 rather than HO_2^-, because H_2O_2 is the predominant species at pH 8.0: log $([HO_2^-]/[H_2O_2]) = -11.63 + \text{pH}$.

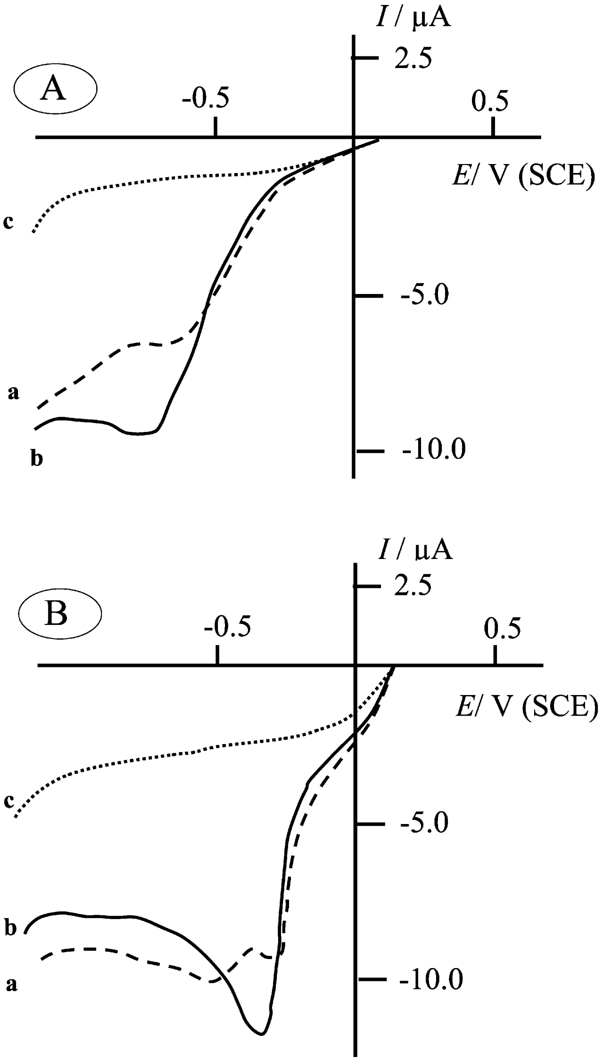

Fig. 1. Current–potential curves obtained with non-EP (A) and EP (B) electrodes with catalase dissolved in solution. Scan rate 20 mV s^{-1}; curves (a) without catalase; curves (b) with 0.25 mg ml^{-1} catalase; curves (c) with 0.25 mg ml^{-1} catalase in deoxygenated solution.

The current–potential curves, which were recorded at different scan rates from 1 to 50 mV s^{-1}, gave the same general shape.

For both EP and non-EP electrodes, adding catalase did not affect the shape of the first part of the current–potential curves (before − 0.3 V/SCE), catalase did consequently not affect the electrochemical kinetics of oxygen reduction. The presence of catalase increased the current in the peak zone (from − 0.3 to − 0.5 V/SCE). In this range, hydrogen peroxide is produced electrochemically (reaction (2)), and its reduction rate (reaction (3)) is lower than its production rate. Catalase catalysed hydrogen peroxide disproportionation, and produced additional oxygen (reaction (1)). The production of 1/2 molecule of oxygen from one molecule of hydrogen peroxide provoked the current increase that was observed around the peak current, according to Scheme I of Fig. 2. At the most cathodic potentials, the addition of catalase had different effects with EP and non-EP electrodes: the current was decreased on EP electrodes, but it was increased on non-EP electrodes. Actually, the balance between the kinetics of hydrogen peroxide production and hydrogen peroxide consumption is different for EP and non-EP electrodes, and this balance controls the concentration of hydrogen peroxide and the effect of catalase. It is generally admitted that the anodization performed during the EP creates carboxyl groups, which promote oxygen reduction into hydrogen peroxide [5]. On EP electrodes, high concentrations of hydrogen peroxide were consequently produced from − 0.3 to − 0.5 V/SCE; in the presence of catalase, this hydrogen peroxide was consumed, and it was no longer available for the electrochemical reduction at more cathodic potentials. At the most cathodic potentials the current was consequently lower in the presence of catalase. On the contrary, on non-EP electrodes, hydrogen peroxide was produced at slower rates, but during the whole potential scan. The current was smaller than on EP electrodes, but the effect of catalase was observed on the whole potential range.

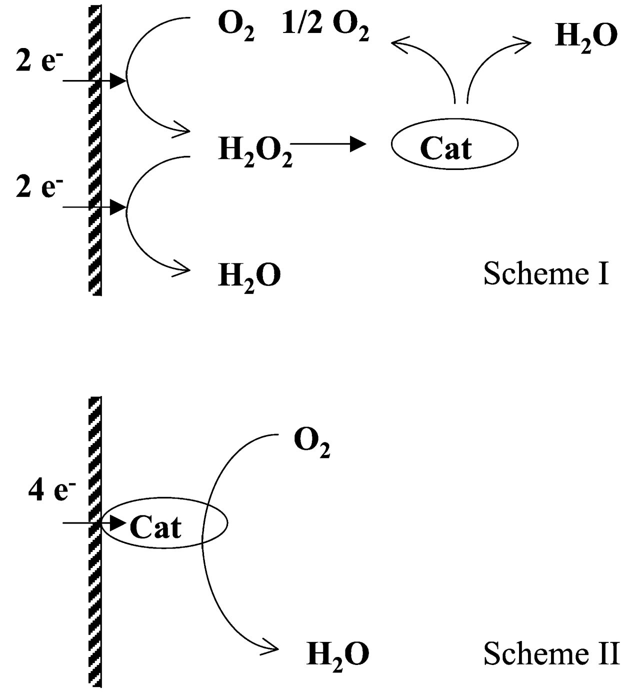

Fig. 2. Schematic representation of the effect of catalase on the electrochemical reduction of oxygen. Scheme I: coupling of electrochemical reduction of oxygen with the regular catalase-catalysed hydrogen peroxide disproportionation; Scheme II: direct catalysis of the electrochemical reduction.

3.2. Catalase deposited on GC electrode surface from DMSO solution

Fig. 3 gives the current–potential curves obtained with the catalase–DMSO-modified electrodes without preliminary EP (Fig. 3A) or with EP (Fig. 3B). The current–potential curves obtained with catalase adsorbed on the electrode surface with EP (Fig. 3B) presented the same shape as those obtained with catalase in solution. On the contrary, adsorption of catalase on the electrodes that did not undergo preliminary EP (Fig. 3A) shifted oxygen reduction towards more anodic potentials, and gave a strong cathodic peak around − 0.3 V/SCE, where the current was increased by about 60%, with respect to the clean electrode. With non-EP catalase–DMSO modified electrodes (Fig. 3A) production of oxygen by the disproportionation of hydrogen peroxide cannot explain the high current increase observed in the first part of the curve from potentials of approximately − 0.15 V/SCE. In this case, catalase adsorbed on glassy carbon from DMSO directly catalysed the electrochemical reduction of oxygen via direct electron transfer from the electrode to catalase, as represented on Scheme II of Fig. 2.

The fine structure of catalase from beef liver (the common source) has been widely studied [6]. The heme group is buried at least 20 Å below the molecular surface. It is accessible by one (or possible two) channel, which is 30 Å long and lined with hydrophobic residues [7]. Interaction with DMSO could modify the morphology of this channel, resulting in easier connection with the electrode surface [4]. This may explain why catalase allows direct electron transfer with the electrode only when it is adsorbed from DMSO, and not when it is randomly adsorbed from aqueous solutions. Analysis by scanning tunneling microscopy of catalase adsorption on highly oriented pyrolytic graphite has led to the conclusion that anodization of graphite creates carboxyl groups, which react with the amine of the polypeptide chain to fix catalase onto the surface [8]. According to this last work, it may be suggested that EP provokes adsorption of catalase in an orientation which prevents electron transfer. Moreover, EP modifies the GC surface from hydrophobic to hydrophilic properties [9]. The con-

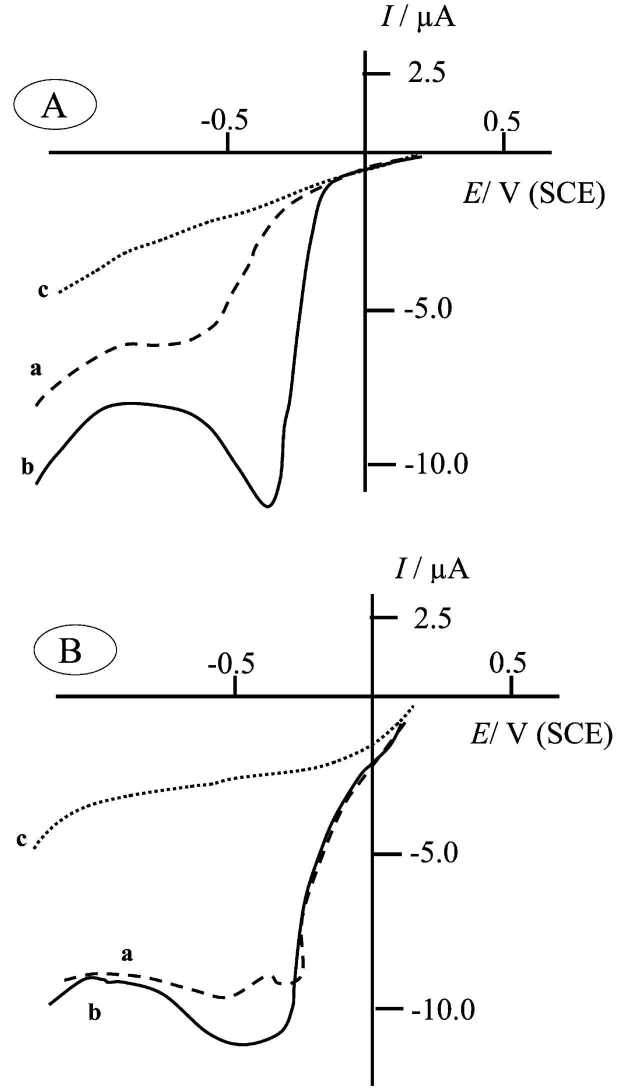

Fig. 3. Current–potential curves obtained with non-EP (A) and EP (B) catalase–DMSO-modified electrodes. Scan rate 20 mV s^{-1}; curves (a) GC electrodes were soaked 15 min in pure DMSO without catalase; curves (b) catalase–DMSO modified electrodes; curves (c) catalase–DMSO modified electrodes in deoxygenated solution.

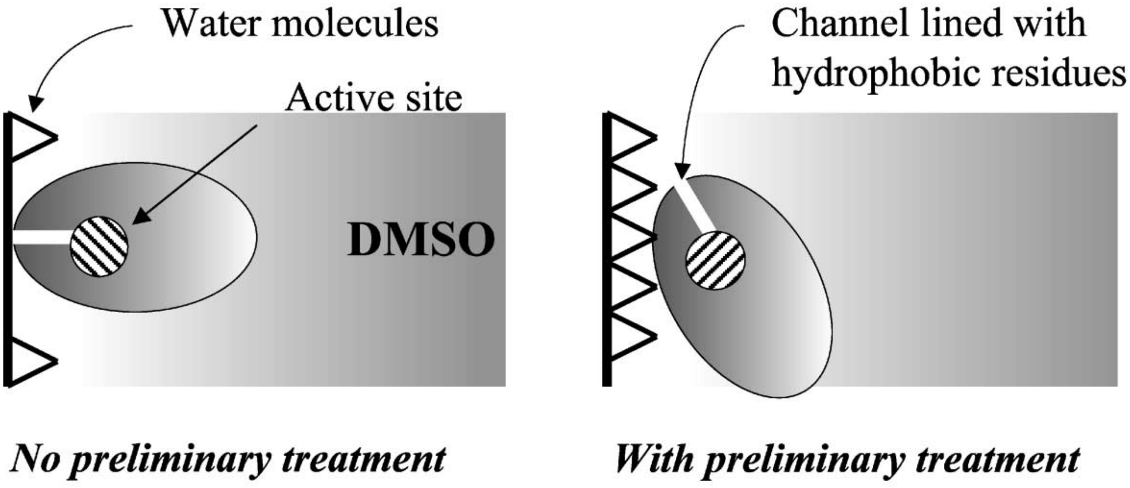

Fig. 4. Schematic representation of hypothesis made on the interaction between the hydrophobic lined channel of catalase and the hydrophobic (non-EP) or hydrophilic (EP) GC electrode surface.

nection of the hydrophobic channel of catalase might consequently be more difficult with the EP electrode than with the non-EP electrode as schematised in Fig. 4.

4. Conclusion

When catalase was adsorbed on non-EP electrodes from DMSO solution, the current increase that was obtained can no longer be explained by Scheme I. In this case it is postulated that a direct electron transfer between catalase and the GC electrode occurs, which catalyses the direct electrochemical reduction of oxygen.

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