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The role of hydrogenases in the anaerobic microbiologically influenced corrosion of steels

S. Da Silva *, R. Basséguy, A. Bergel

Université Paul Sabatier, Laboratoire de Génie Chimique, CNRS, 118, route de Narbonne, 31062 Toulouse, France

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Abstract

The direct electron transfer between 316 L stainless steel and the NAD-dependent hydrogenase from *Ralstonia eutropha* was studied by spectroelectrochemistry. The presence of hydrogenase and NAD^+ clearly increased the quantity of electricity, which was consumed during the electrolysis performed at potential lower than -0.70 V/SCE. The involvement of hydrogenase in the cathodic depolarisation theory was discussed in the light of these results. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Hydrogenase; Stainless steel; Direct electron transfer; Corrosion

1. Introduction

Von Wolzogen Khür and Van Der Vlugt [1] proposed the theory of cathodic depolarisation to explain the role of Sulphate Reducing Bacteria (SRB) in anaerobic microbiologically influenced corrosion of steels. SRB, which consume hydrogen in their metabolisms, could shift a possible equilibrium between proton and hydrogen in solution and consequently increase the rate of proton reduction, that should result in enhancing the oxidation of iron. At the end of the century, a lot of authors have agreed or disagreed with this theory and a large and sharp debate is still a topical question [2,3]. Some of them argued that corrosion is a kinetically controlled process and not an equilibrated process. Consequently, the corrosion rate cannot be influenced by the consumption of hydrogen, which is the end product [4]. Nevertheless, Chatelus et al. [5] and Bryant and Laishley [6] thought that hydrogenase localised inside SRB, and exocellular hydrogenase entrapped in the biofilm, too, could induce a cathodic depolarisation phenomenon. In the present paper, thin layer spectroelectrochemistry was used to highlight the direct electron transfer between an NAD-dependent hydrogenase from *Ralstonia eutropha* and 316 L stainless steel. Our work attempts to propose a new mechanism of the cathodic depolarisation.

2. Experimental

The working electrode was a 316 L stainless steel grid inserted between two glass slides. The thin layer cell was filled with 50 mM potassium phosphate solution pH 8.0. The auxiliary electrode was a platinum grid and the reference electrode was a saturated calomel electrode (SCE). The reduction current was recorded as a function of time during successive electrolyses performed at different potentials. NADH production was simultaneously measured spectrophotometrically at $\lambda = 340$ nm.

3. Results

Fig. 1 represents current versus time resulting from electrolyses performed at different potentials with 2 mM NAD^+, either without hydrogenase, or with 10 U/ml hydrogenase. Two different potential ranges can be distinguished. In the first domain, from -0.60 to -0.65 V/SCE, reduction currents obtained with or without hydrogenase in the cell were equal. In the second domain, from -0.70 to -0.90 V/SCE, currents obtained in presence of enzyme were greater

* Corresponding author. Tel.: +33-561-55-83-02; fax: +33-561-55-61-39.

E-mail address: dasilva@chimie.ups-tlse.fr (S. Da Silva).

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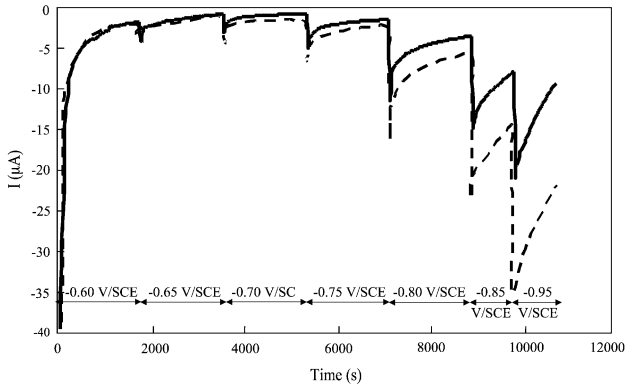

Fig. 1. Electrolyses performed by spectroelectrochemistry at different potentials with 2 mM NAD^+. —: Without hydrogenase and – – –: with 20 U/ml hydrogenase. Current versus time answer.

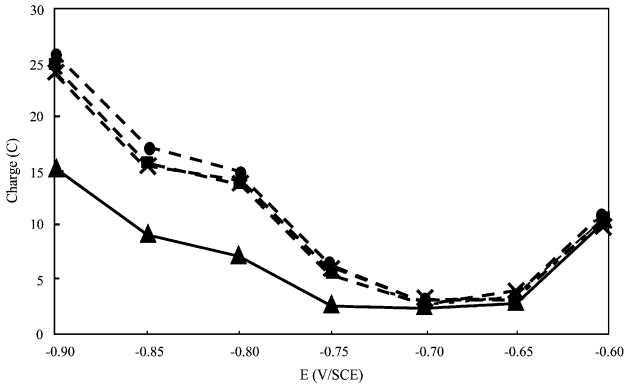

Fig. 3. Electrolyses performed by spectroelectrochemistry at different potentials with 5 mM NAD^+. -▲-: Without hydrogenase, -■-: with 20 U/ml hydrogenase, -●-: with 10 U/ml hydrogenase and -✚-: 5 U/ml hydrogenase.

than in the absence of enzyme. Fig. 2 shows the results of the spectrophotometric measurements performed during the different electrolyses. NADH concentration is reported as a function of time. There are two domains, too, in this graph. A first domain from -0.60 to -0.75 V/ECS, where NADH production is nil, and a second domain where the NADH production rate obtained with hydrogenase was greater than in the absence of hydrogenase. In conclusion, from -0.70 V/SCE, the presence of hydrogenase in the cell increased the charge transferred. This charge was clearly linked to the reduction of NAD^+ into NADH. Fig. 3 gives the charge transferred through the system during electrolyses versus potential. These charges were calculated by integration of the previous current–time curves. The experiments were performed with 5 mM NAD^+ initial concentration, successively without hydrogenase and with several hydrogenase amounts (5, 10 and 20 U/ml). When the enzyme and NAD^+ were in solution, there was an increase of the charge transferred.

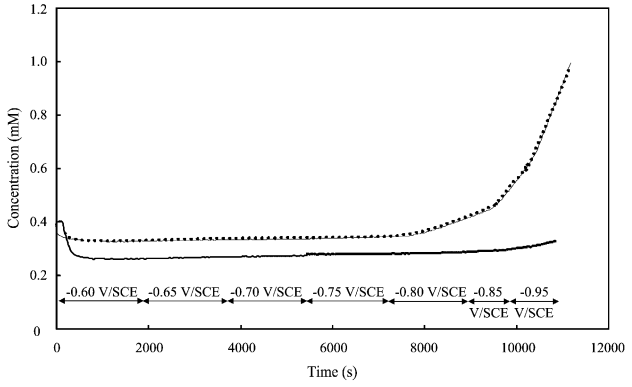

Fig. 2. Electrolyses performed by spectroelectrochemistry at different potentials with 2 mM NAD^+. —: Without hydrogenase and · · · · · ·: with 20 U/ml hydrogenase. NADH concentration versus time answer.

However, the variation of the initial concentration of hydrogenase did not affect this behaviour.

4. Discussion

The *R. eutropha* hydrogenase is an NAD-dependent enzyme that uses NAD^+ as electron acceptor to catalyse hydrogen oxidation. The direct electron transfer on a platinum electrode led to the reaction: $NAD^+ + H^+ + 2e^- \rightarrow NADH$. To date, the mechanism on stainless steel is not so obvious. Three possible mechanisms are reported in Fig. 4 to explain the catalysis of the solvent reduction. In mechanism A, the proton (or solvent in alkaline media) is reduced electrochemically. Hydrogenase consumes the hydrogen produced and so enhances the reduction rate. This mechanism exactly corresponds to the so-called theory of cathodic depolarisation. However, this mechanism has been severely criticised. The most likely mechanism should consequently be a direct electron transfer between hydrogenase and stainless steel. This mechanism can be written in different ways (mechanisms B or C). In mechanism B, hydrogenase is adsorbed directly on the stainless steel while in mechanism C, the electron transfer occurs via an

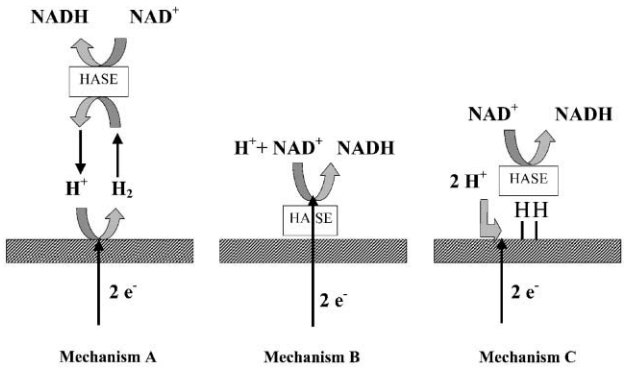

Fig. 4. Possible mechanisms to explain the cathodic depolarisation.

adsorbed intermediate species of hydrogen. Roughly speaking, these mechanisms might be considered as identical because the results are similar: the enhancement of the charges exchanged should correspond to a cathodic depolarisation, which occurs through direct electron transfer from stainless steel to hydrogenase.

5. Conclusion

Influence of the presence of hydrogenase on 316 L stainless steel was experimentally demonstrated here. Because the "old" mechanism of cathodic depolarisation is not theoretically sound, we proposed here to explain this effect by a direct electron transfer from stainless steel to hydrogenase. This could be considered a direct cathodic depolarisation phenomena.

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