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Hydrogenases from the hyperthermophilic bacterium *Aquifex aeolicus*: electrocatalysis of the hydrogen production/consumption reactions at carbon electrodes

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Abstract

Two [NiFe] hydrogenases extracted from the hyperthermophilic bacterium *Aquifex aeolicus* (*Aa*) are studied at a pyrolytic graphite electrode using cyclic voltammetry. The two hydrogenases have been shown to exhibit very similar behavior. They are able to catalyze under a nitrogen or a hydrogen atmosphere, the direct hydrogen production from protons and the reverse reaction of hydrogen oxidation directly in the absence of any promoter. The effect of experimental parameters (enzyme concentration, pH, ionic strength) are investigated. The electrocatalytic activity has been shown to increase when the temperature is increased within the range 22–65 °C in agreement with the evolution of the enzymatic activity. Using electrochemical techniques concomitantly with quartz crystal microgravimetry, it is demonstrated that the electrocatalysis is largely governed by the strong adsorption of hydrogenases on the electrode surface. The similarity between the two *Aa* hydrogenases and the hydrogenase from another hyperthermophilic bacterium, *Pyrococcus furiosus*, is discussed.

Keywords: *Aquifex aeolicus*; Hyperthermophiles; Voltammetry; Hydrogenase

1. Introduction

Extremophilic microorganisms are able to live and develop in environments hostile to mammals and especially humans. Depending on their growth conditions, they are referred to as thermophiles, acidophiles, halophiles, etc. Among these, hyperthermophiles have the property of growing at high temperatures, up to about 110 °C [1]. They have been identified in natural or artificial hot aqueous environments such as volcanic springs, shallow or abyssal hydrothermal systems, and smoldering coal refuse piles [2–4]. Most of these microorganisms belong to the domain of Archaea and are presumed to represent the most slowly evolving form of life. The Aquificales are considered as the earliest branching order of the phylogenetic tree of the Bacteria [5]. *Aquifex aeolicus* is a representative member of the genus *Aquifex*. This organism is a hydrogen-oxidizing, microaerophilic, and obligatory chemolithoautotrophic bacterium [6]. Recently, several components of the electron transfer chain in *A. aeolicus* have been studied, in particular the structure of a soluble ferredoxin [7], a sulfide:quinone oxidoreductase [8], monohemic cytochromes c_{555} [9,10], [NiFe] hydrogenases, hydrogenase–cytochrome *b* complex, cytochrome bc_1 complex, and cytochrome oxidase [11]. It has been proposed [12] that specific structural factors in terms of number of ion pairs and cavities, polarity of the exposed surface, and secondary structure could contribute to the remarkable stability of proteins which employ different devices

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to adapt their function to high temperatures close to 100 °C.

At least 13 families of hydrogenases are known [13,14]. All but one are involved directly or indirectly in energy metabolism. Generally speaking, hydrogenases are a class of enzymes that catalyze the reversible reaction $2H^+ + 2e^- \Longleftrightarrow H_2$ in the presence of appropriate electron donors or acceptors, with the ability either to evolve or oxidize hydrogen. Although the oxidation of molecular hydrogen or the reduction of proton can be regarded as a simple redox process, it should be pointed out that the intimate overall reaction results from a very complex mechanism. It is fairly well established that activation of hydrogen by hydrogenase involves the heterolytic cleavage of the molecule with the formation of hydride and proton [13]. It has been assumed that the polarization of the H–H bond required for bond cleavage involves the participation of adjacent residues [13]. The majority of hydrogenases are classified in two main groups on the basis of the nature of their catalytic sites [13,15], namely [Fe] and [NiFe] hydrogenases.

Three distinct [NiFe] hydrogenases have been identified in *A. aeolicus*: two of these hydrogenases (hydrogenase I and II) have been found to interact with the membrane, and a third one soluble in the cytoplasm (hydrogenase III) has been detected. Hydrogenase I was obtained under two different states, i.e., either soluble or involved in a complex with cytochrome *b*. Each of the two hydrogenases contains one [Ni–Fe] center which is the active catalytic site, one [3Fe–4S] cluster and two [4Fe–4S] clusters. The nature and the organization of the metal centers have been shown to be very similar in the two enzymes [11]. The molar masses of hydrogenase I and hydrogenase III are 110 and 80 kDa, respectively [11]. Several possible pathways of electron transfer in hyperthermophilic bacteria have been proposed, e.g., [11,16]. In the case of *A. aeolicus*, it seems that hydrogenase III transfers electrons directly to soluble cytoplasmic ferredoxin. Hydrogenase III would be involved in the CO_2-fixation pathway and hydrogenase I in the electron transfer to cytochrome bc_1 and cytochrome oxidase.

Because of the key role of these enzymes in the hydrogen metabolism of *A. aeolicus*, it was stimulating to study their physico-chemical properties. Electrochemistry offers attractive possibilities for studying redox proteins. In such an approach, the electrode acts as the electron donor/acceptor provided that electron exchanges are rapid. Some drawbacks (e.g., the size of the enzyme, burying of the active centers, the need for pre-activation) can limit the efficiency of electron transfer. That can be true for hydrogenases which are relatively heavy molecules (with molar masses of more than 50 kDa). Because of the possible double role of hydrogenases (i.e., H_2 production/consumption), varying the applied potential in the forward/backward direction is a convenient way to investigate electron exchange between hydrogenase and an electrode. The electrochemical approach has been exploited so far to study hydrogenases in the presence of physiological partners such as *c*-type cytochromes [17–22] or of polycationic species facilitating the electron transfers (promoting effect) [23,24]. Evidence of direct H_2 evolution at an electrode acting as an electron donor has also been demonstrated in the absence of a promoting agent [25–27]. In this paper, we present results on the direct H_2 evolution/consumption via the two soluble hydrogenases (the soluble form of hydrogenase I and hydrogenase III, respectively) from *A. aeolicus* at a basal pyrolytic graphite electrode in the absence of any promoter without the need for pre-activation. Several experimental parameters controlling the electrocatalytic efficiency, including the effect of temperature have been investigated.

2. Experimental

2.1. Materials

A. aeolicus (abbreviated as *Aa*) hydrogenase I and hydrogenase III have been purified in our laboratory as previously described [11]. All the other chemicals were of reagent grade. All solutions were prepared with permuted (Milli-Q) water.

2.2. Apparatus and procedure

Cyclic voltammetry (CV) experiments were carried out using an EG&G 6310 electrochemical impedance analyzer modulated by EG&G PAR M 270/250 software. A three-electrode system consisting of a Metrohm Ag|AgCl|NaCl(sat.) reference electrode, a gold wire auxiliary and the working electrode was used throughout. The working electrode, constructed from a 4 mm diameter rod of graphite (Le Carbone Lorraine, Paris) housed in an epoxy sheath cut with the disk face parallel to the basal plane (*a–b*), was first polished with ultrafine emery paper, and then with 0.05 μm alumina slurry. Unless otherwise specified, all potentials reported in this work refer to the Ag|AgCl|NaCl(sat.) reference electrode. Potentials vs. the SHE can be obtained by adding 210 mV. Prior to each experiment, the solutions were deoxygenated by bubbling high-purity nitrogen. All measurements were carried out at room temperature (about 23 °C).

Quartz crystal microgravimetry (QCM) studies were performed using a MAXTEK PM 710 plating monitor coupled with a MPS-550 sensor probe. The MAXTEK quartz resonators were made from AT-cut quartz crystals (resonance frequency, 5 MHz) covered by