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On the mechanism of hydrogen evolution catalysis by proteins: A case study with bovine serum albumin

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ABSTRACT

The catalysis of the hydrogen evolution reaction (HER) by proteins has been known for decades but was only recently found to be useful for electroanalytical purposes. The mechanism of the catalytic process is investigated at hanging mercury drop electrodes by cyclic voltammetry, with bovine serum albumin as a model system. It is shown that the catalyst is the protein in the adsorbed state. The influence of various parameters such as the accumulation time, scan rate or buffer concentration is studied, and interpreted in the framework of a surface catalytic mechanism. Under the experimental conditions used in the work, a "total catalysis" phenomenon takes place, the rate of HER being limited by the diffusion of the proton donor. The adequacy of the existing models is discussed, leading to a call for the development of more refined models.

1. Introduction

The hydrogen evolution reaction (HER) and its reverse process the hydrogen oxidation reaction (HOR) have attracted for decades a huge attention from the electrochemical community [1–4]. Not only is it an archetypal system to understand at the fundamental level the electrochemical reaction mechanisms, the catalytic and electrocatalytic processes or the influence of the electrode materials, but it is also of paramount importance for future application in fuel cell technologies or hydrogen production plants. A lot of work is thus aimed at improving the efficiency of the HER by electrocatalysis and bioelectrocatalysis [5]. Quite naturally, bioelectrocatalysis of HER and HOR is essentially focused on the catalytic properties of hydrogenase enzymes and their constituents, whether dissolved in solution or immobilised at electrode surfaces [6–10]. Although hydrogenases are, among proteins, the most obvious candidates for large scale hydrogen production, it is well established that many if not all proteins are able to catalyse the HER in certain conditions [11–16]. This property of proteins was discovered by polarographic experiments at the dropping mercury electrode eight decades ago in the laboratory of Heyrovský [17]. Because the polarographic characteristics of the catalytic wave were of limited analytical interest, very little attention was paid to the phenomenon in the following decades, in contrast to the closely related though distinct "Brdička reaction" of cysteine containing proteins, yielding a characteristic "double wave" in the presence of cobalt ions [15]. Recently, however, it has been shown that the catalytic hydrogen evolution can be of great interest in the analysis of proteins, by taking advantage of adsorptive methodologies at hanging mercury drop or solid amalgam electrodes in conjunction with fast electrochemical methods such as chronopotentiometric stripping analysis [12–14]. Under these experimental conditions, where the direct reduction of the solution component is largely suppressed [10], the characteristics of the chronopotentiometric catalytic peak (so-called "peak H") were found to be extremely sensitive to the nature of the investigated protein [18–20], its redox state [21], native or denatured state [22–27] and to changes in protein structures resulting from single amino acid exchange in mutant proteins [28], allowing protein determination down to nanomolar and subnanomolar concentrations [29,30].

Interestingly, it should be possible to use the catalytic HER to get information about protein structures, allowing the development of new electrochemical tools in proteomics. Such a goal requires a good understanding of the catalytic mechanism, which is currently lacking. To gain some insights in this topic, few laboratories have studied in the recent years the behaviour of model systems made of simple peptides [31–34] and proteins [21–27,29,35]. In the present work, we investigate the catalytic properties of a protein, bovine serum albumin (BSA), at the hanging mercury drop electrode, and

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the results are analysed in the framework of a mediated electrochemical catalysis, based on the acid–base properties of the protein. Indeed, in contrast to proteins for which data are relatively scarce, HER catalysis by organic and inorganic bases has been comparatively well explored, on mechanistic [36–38] as well as analytical aspects [11,39]. BSA was chosen as a model protein because many data are available regarding its behaviour on mercury electrodes [40–45], and its catalytic properties towards HER have been already the subject of some investigations [22–26,46,47].

2. Experimental

The experiments were conducted in a three-electrode cell connected to an Autolab PGSTAT 20 potentiostat (Metrohm-Autolab) equipped with a frequency response analyser (FRA) module. The working electrode was a mercury drop (area: 0.4 mm^2) controlled by a Metrohm VA-stand 663 (Metrohm), while the counter electrode was a platinum wire and a Ag|AgCl|3 M KCl was used as the reference electrode. The electrolyte was purged with dry nitrogen for 20 min before starting the experiments, and a nitrogen blanket was kept above the solution throughout the measurement. Microliters amounts from a stock solution of bovine serum albumin were inserted directly into the stirred electrolyte to reach the desired concentration, but no further nitrogen purging was performed to prevent the protein denaturation [41]. When an accumulation procedure was applied, the solution was stirred at 1000 rpm during the accumulation. The chemicals $Na_2HPO_4{\cdot}7H_2O$ (Sigma–Aldrich, >98.0%), $NaH_2PO_4{\cdot}H_2O$ (Sigma–Aldrich, >98.0%) and KCl (Sigma–Aldrich, >99.0%) were of analytical grade and used as received. The electrochemical experiments were conducted in 1 M KCl containing different concentrations of phosphate buffer, prepared with equimolar quantities of the acid and conjugate base, resulting in a solution pH of 6.5. The "buffer concentration" expresses the total phosphate concentration. For the determination of the differential capacity, impedance measurements were conducted at 237 Hz with a 10 mV (rms) amplitude, the scan rate being *ca* 5 mV s^{-1}. The in-phase and out-of-phase components of the impedance were measured, and converted to the differential capacity by assuming that the system behaves as a simple series RC circuit. Stock solutions of bovine serum albumin (>96%, Sigma–Aldrich) were prepared by weighing and dissolution in 0.05 M sodium phosphate buffer or in 1 M KCl (for the experiment in the complete absence of buffer). All solutions were prepared with ultrapure water from a Milli-Q system (Millipore).

3. Results and discussion

It was mentioned in Section 1 that the polarographic wave associated with the HER catalysis by proteins was of little analytical interest. This problem was inherent to the dropping mercury electrode configuration. Because the catalyst could be either the protein dissolved in solution or adsorbed at the mercury surface, relatively large protein concentrations were required to reach a measurable catalytic current. In the first case, a "bulk wave" was obtained, whose intensity increased with the catalyst bulk concentration. The second case led to the appearance of a "surface wave", proportional to the amount of protein adsorbed on the electrode, hence on both the drop time and the protein bulk concentration [37]. The problem is largely overcome by the modern use of the hanging mercury drop electrode, for which long adsorption times can be used, allowing for the utilisation of smaller protein concentrations. This is illustrated for BSA in Fig. 1, which presents the cyclic voltammograms obtained in an electrolyte solution containing 100 nM BSA. In this set of experiments, the potential was held at the accumulation potential $E_A = -0.1$ V for different accumulation times t_A before

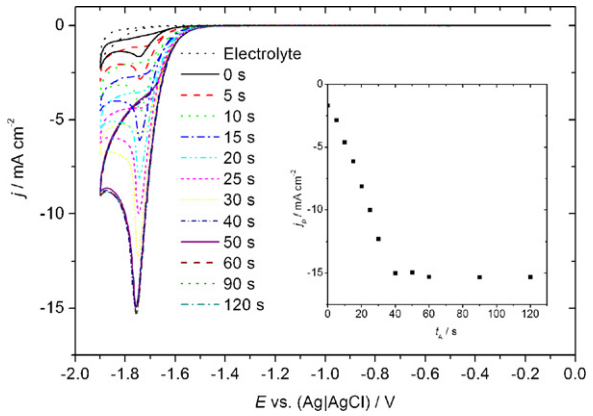

Fig. 1. Cyclic voltammograms recorded in 1 M KCl + 0.05 M phosphate buffer (pH 6.5) in the presence of 100 nM of BSA. The potential was held at $E_A = -0.1$ V for the accumulation time t_A indicated in the legend, before starting the linear potential sweep at 1 V s^{-1}. Inset: plot of the peak current j_p as a function of the accumulation time t_A.

starting the potential sweep. A scan rate of 1 V s^{-1} was used to minimise the time elapsed during the potential sweep as compared to the accumulation time. Under these experimental conditions, the voltammogram obtained in the presence of BSA exhibits a well-defined peak with a maximum around −1.75 V, whose intensity increases progressively with the accumulation time until stabilising at a constant value. The time dependence is more clearly represented in the inset of Fig. 1, where the peak current vs. accumulation time is plotted. The peak is obviously associated with the presence of BSA in the electrochemical cell, but the protein concentration in solution does not vary with time, in contrast to the amount of the protein adsorbed at the surface which is expected to reach a saturation coverage at long accumulation times, in good agreement with the observed time dependence. This strongly suggests a correlation between the peak and the adsorption of BSA, this latter phenomenon being well established on mercury [40–45].

The adsorption of the protein was confirmed by measuring the interfacial capacitance as a function of the potential and the time. Fig. 2A shows the differential capacity curves recorded in the absence and presence of BSA, the latter being recorded after a long equilibration time (300 s) at the initial potential of −0.1 V. The protein is clearly adsorbed in the potential range −1.5 to −0.1 V, as inferred from the lower capacity measured in this domain as compared to that of the pure electrolyte. Around −1.6 V, a small hump is noticed, which can be indicative of a reorientation or a desorption of the adsorbed layer, or be of Faradaic nature and correspond to the cathodic peak seen in the voltammograms. In any case, at very negative potentials, the differential capacity curves recorded in the presence and absence of the protein do not merge together, suggesting that BSA is not fully desorbed from the surface. In Fig. 2B, the evolution of the differential capacity with time is presented. For this experiment, the potential was set at −0.4 V, where the difference in capacity is the largest, but a similar trend was observed for other potentials including −0.1 V: a regular decrease of C_d is observed at short times, then a plateau is reached for longer t_A. Interestingly, the stabilisation of the differential capacity occurs around $t_A = 40$ s, which is in very good agreement with the value where the cathodic peak observed in the previous experiment reaches its maximum (see Fig. 1 and its inset). This fact nicely confirms that this is the protein in the adsorbed state which is responsible for the appearance of the peak. In the forthcoming experiments, the accumulation time is always taken in the plateau region.

At this stage, the issue of protein denaturation at the surface should be briefly evoked. Interactions between proteins and