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Catalytic signal of rabbit liver metallothionein on a mercury electrode: a combination of derivative chronopotentiometry with adsorptive transfer stripping

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Abstract

Constant current chronopotentiometric stripping analysis (CPSA) in combination with adsorptive transfer stripping (AdTS) technique was used to study the rabbit liver metallothionein (MT) on a hanging mercury drop electrode (HMDE). Metallothionein yielded a distinct, sharp chronopotentiometric signal at very negative potentials (about − 1.7 V), also known as the "peak H". The height and potential of this peak were dependent on experimental conditions, such as buffer composition, pH, and the presence oxygen in solutions. The peak H was highest in borate buffer with pH close to the isoelectric point (pI) of MT. The chronopotentiometric results contribute to a deeper understanding of the nature of catalytic hydrogen evolution and demonstrate the usefulness of the peak H in peptide and protein research. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Metallothionein; Chronopotentiometric stripping analysis; Adsorptive transfer stripping; Peak H; Catalytic hydrogen evolution; Effect of aeration

1. Introduction

Metallothioneins (MTs) are cysteine-rich proteins of low molecular weight that bind essential and toxic metal ions with high specificity and form unusual metal-thiolate clusters [1,2]. In alkaline buffer solutions, both in the presence and in the absence of cobalt ions, the rabbit liver MT catalyses the evolution of hydrogen. This catalytic effect is well measurable by the constant current stripping chronopotentiometry [3,4]. In this technique, the stripping is carried out by applying a constant current, and the electrode potential (E) vs. time (t) curve is monitored [5]. However, with respect to the sensitivity of analytical application, the dependence of $(\mathrm{d}E/\mathrm{d}t)^{-1}$ vs. potential E was shown to be more advantageous.

The chronopotentiometric peak H [3,4,6] is related to the DC polarographic "presodium wave" discovered about 70 years ago by Heyrovský and Babička [7]. This wave, resulting from hydrogen evolution catalysed by free -NH_2 and -SH groups in the protein molecule [8,9], was not suitable for analytical purposes because of its distorted shape caused by the discharge of the supporting electrolyte.

The rabbit liver MT was studied on a hanging mercury drop electrode (HMDE) by means of three potentiostatic methods (linear sweep, differential pulse, and square wave voltammetry), and a galvanostatic method (constant current chronopotentiometric stripping analysis—CPSA) [10]. In contrast to voltammetric methods, the CPSA yields a well-developed peak H, which allows the determination of MT at the femtomole level [10]. In this paper, we have used CPSA in connection with the adsorptive transfer stripping (AdTS) technique to study a rabbit liver MT at an HMDE under different conditions.

2. Experimental

The metallothionein from rabbit liver (MT1A containing 5.9% Cd and 0.5% Zn) and other chemicals (components of buffers) were analytical grade reagents purchased from Sigma-Aldrich. All solutions were prepared using deionized water (Millipore, Milli-Q). Constant current chronopotentiometric measurements (CPSA) were performed using the AUTOLAB Electrochemical Instrument (EcoChemie, The Netherlands) connected with the VA-Stand 663 (Metrohm, Zurich, Switzerland). A standard cell with three electrodes was used. The working electrode was a hanging mercury drop electrode (HMDE). The reference electrode was the

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Ag/AgCl/3M KCl electrode, and a platinum wire was used as an auxiliary electrode. Adsorptive transfer procedure was performed in a 5-μl drop of the corresponding buffer solution containing 13.1 nM MT (100 ng/ml) at 0 V. Adsorption of MT and the subsequent CPSA were carried out in a buffer of identical composition. All experiments were carried out at 25 °C. Solutions were deaerated with argon (99.99%) for at least 10 min prior measurement, and blanketed with argon during measurement, except for the experiment conducted in the presence of oxygen. The software GPES 4.4 supplied by EcoChemie was used for smoothing and baseline correction.

3. Results and discussion

The effect of buffer composition on the CPSA signal of MT (peak H) was studied using the adsorptive transfer stripping (AdTS) procedure [11]. This procedure is based on immobilization on HMDE of the analyte from a small

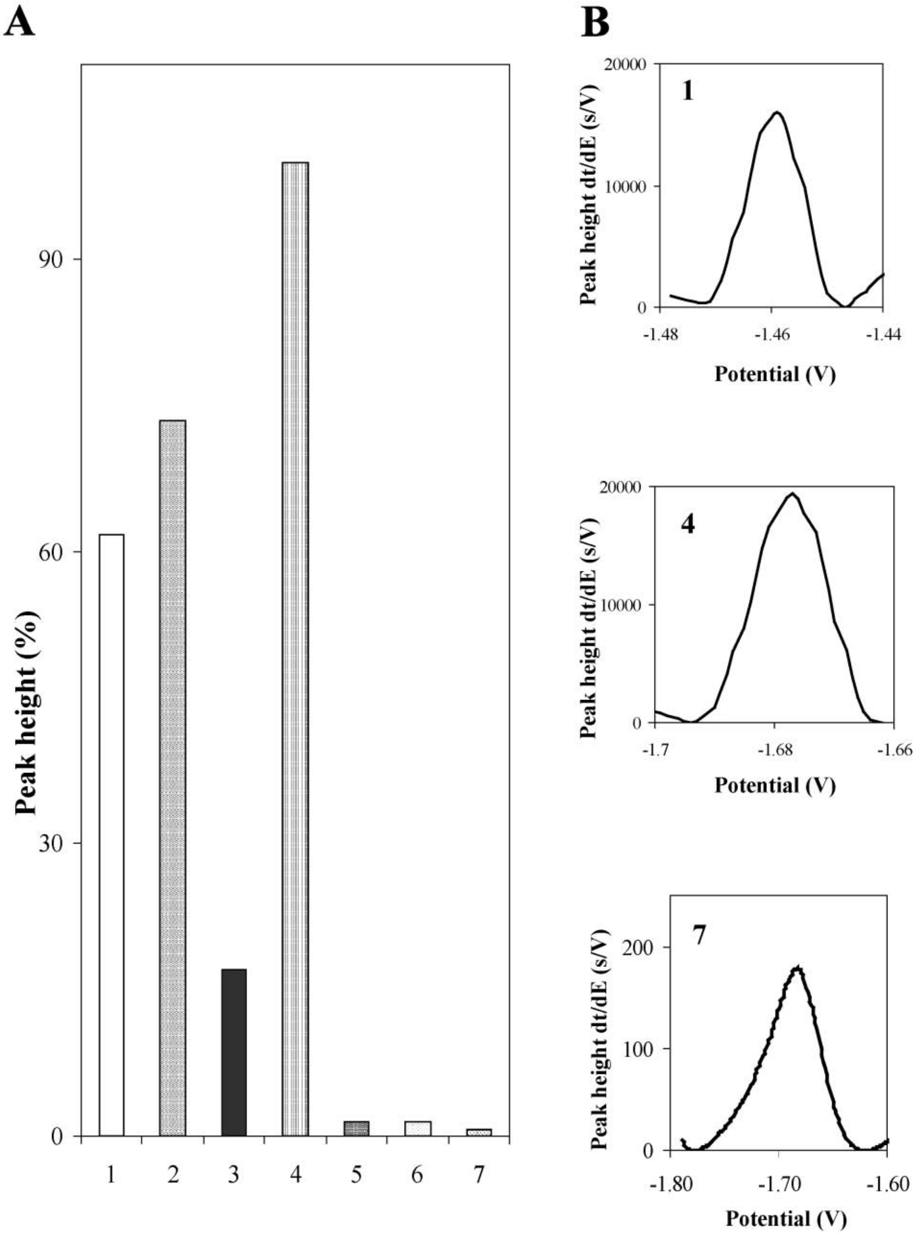

Fig. 1. AdTS CPSA peak heights of rabbit liver MT in various buffers. Time of accumulation of MT (100 ng/ml): 120 s, conditioning potential: 0 V, I_{str}: − 1 μA. (A) Dependence of CPSA peak heights on the composition of the background electrolyte: 1—Britton–Robinson (pH 3.9), 2—0.2 M CH_3COOH + 0.2 M CH_3COONa (pH 5.0), 3—0.1 M NaH_2PO_4 + 0.1 M Na_2HPO_4 (pH 7.0), 4—0.1 M H_3BO_3 + 0.05 M $Na_2B_4O_7$ (pH 8.0), 5—0.1 M $(NH_4)H_2PO_4$ + 0.1 M $(NH_4)_2HPO_4$ (pH 8.3), 6—0.1 M NH_3 + 0.1 M NH_4Cl (pH 8.5) and 7—0.5 M $NaHCO_3$ + 0.5 M K_2CO_3 (pH 9.5). The peak height obtained in borate buffer (pH 8.0) was taken as 100%. (B) Dependence of AdTS CPSA peak potential on pH for three selected buffers: 1—pH 3.9, 4—pH 8.0, 7—pH 9.5.

drop of a solution, followed by washing and electrochemical measurement in a cell containing a buffer. Before each CPSA measurement, MT (13.1 nM) was adsorbed from a buffer solution (5 μl) for accumulation time of 120 s at a potential of 0 V. In seven different buffers, the heights of peak H were obtained (Fig. 1): 1—Britton-Robinson buffer (pH 3.9), 2—0.2 M CH_3COOH + 0.2 M CH_3COONa (pH 5.0), 3—0.1 M NaH_2PO_4 + 0.1 M Na_2HPO_4 (pH 7.0), 4—0.1 M H_3BO_3 + 0.05 M $Na_2B_4O_7$ (pH 7.8), 5—0.1 M $(NH_4)H_2PO_4$ + 0.1 M $(NH_4)_2HPO_4$ (pH 8.3), 6—0.1 M NH_4Cl + 0.1 M NH_3 (pH 8.5), and 7—0.5 M $NaHCO_3$ + 0.5 M K_2CO_3 (pH 9.5). The best CPSA response and good reproducibility was obtained in borate buffer. The height of this peak was taken as 100% for the purpose of comparison of the peak heights in the other buffers. The visible decrease of the peak height (~ 80%) in sodium phosphate at pH 7 (the column 3) can be explained by the competitive adsorption of phosphate ions at the electrode surface. This result is in agreement with the decrease of the peak height observed during adsorptive transfer stripping in phosphate buffer—see Fig. 3D in Ref. [10]. The unexpected dramatic decrease of peak height in ammonium and carbonate buffer solutions (5, 6, 7) is probably connected with the deprotonization of MT. With the increase of pH value, the peak H potential shifts to more negative values (by about 240 mV on going from pH 3.9 to pH 9.5—Fig. 1B).

The highest peak H obtained in borate buffer (Fig. 2) is close to the isoelectric point (pI) of MT1A which is 8.38 [12]. The maximum peak height at about pH 8 in borate buffer corresponds to the most positive potential at this pH. The shift of the peak potential with pH towards positive potentials may be due to the fact that lower energy is required for the catalytic reduction of hydrogen at this pH. The positive effect of borate buffer on the potential and height of the peak H can be attributed to the specific behavior of boric acid which, unlike other acids, acts as an effective proton donor using a water molecule for the proton transfer process [13]:

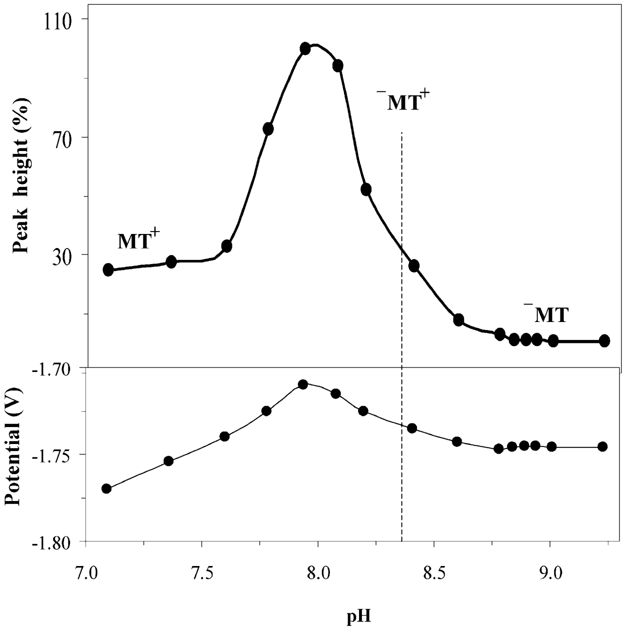

Fig. 2. Dependence of AdTS CPSA peak height (top figure) and potential of rabbit liver MT (bottom figure) on pH in borate buffer. The peak height at pH 8 was taken as 100%. Other conditions are same as in Fig. 1.

$$\mathrm{MT + H - O - H + B(OH)_3 \rightarrow MT - H^+ + B(OH)_4^-}$$

Furthermore, we have studied the dependence of the height of the peak H (without AdTS) on the content of oxygen in borate buffer solution. It was found that oxygen present in solutions substantially increased the peak height. This effect was observed while purging the solutions with argon for various periods of time (Fig. 3A—the dependence of MT peak height on deaeration time, inset—30 and 60 s). The effect of oxygen was confirmed in an alternative experiment in which the deaerated solutions of MT were purged with air. The solution, from which oxygen had been removed (300 s purging with argon), was subsequently enriched in oxygen by bubbling air through it (Fig. 3B—the dependence of the peak height on aeration time, inset—60 s). After purging for 60 s, the peak H still has the value of 700 (s/V). The inset 3B shows the relatively high peak H (250 s/V) in the absence of oxygen. These results demonstrate that the oxygen in the solution has a very positive effect on the derivative CPSA signal of MT (peak H). Our future study will be focused on the explanation of this effect.

4. Conclusions

Constant current chronopotentiometric stripping analysis (CPSA), in combination with the adsorptive transfer stripping procedure, was applied to the electrochemical study of rabbit liver metallothionein (MT) on a hanging mercury drop electrode. In CPSA, rabbit liver MT yields a sharp peak H due to catalytic hydrogen evolution at highly negative potentials (about − 1.7 V). The shape, height, and position of the peak H are influenced by varying experimental conditions, such as stripping current, time of accumulation, pH, buffer components, and the presence of oxygen. The results show that CPSA can provide valuable information about the interaction of MT with buffer components, can yield a deeper insight into the nature of the catalytic processes of hydrogen evolution, and into the role of MT in these processes, and can serve as a useful tool not only for peptide and protein analysis, but also for the study of the structure and functions of MTs.

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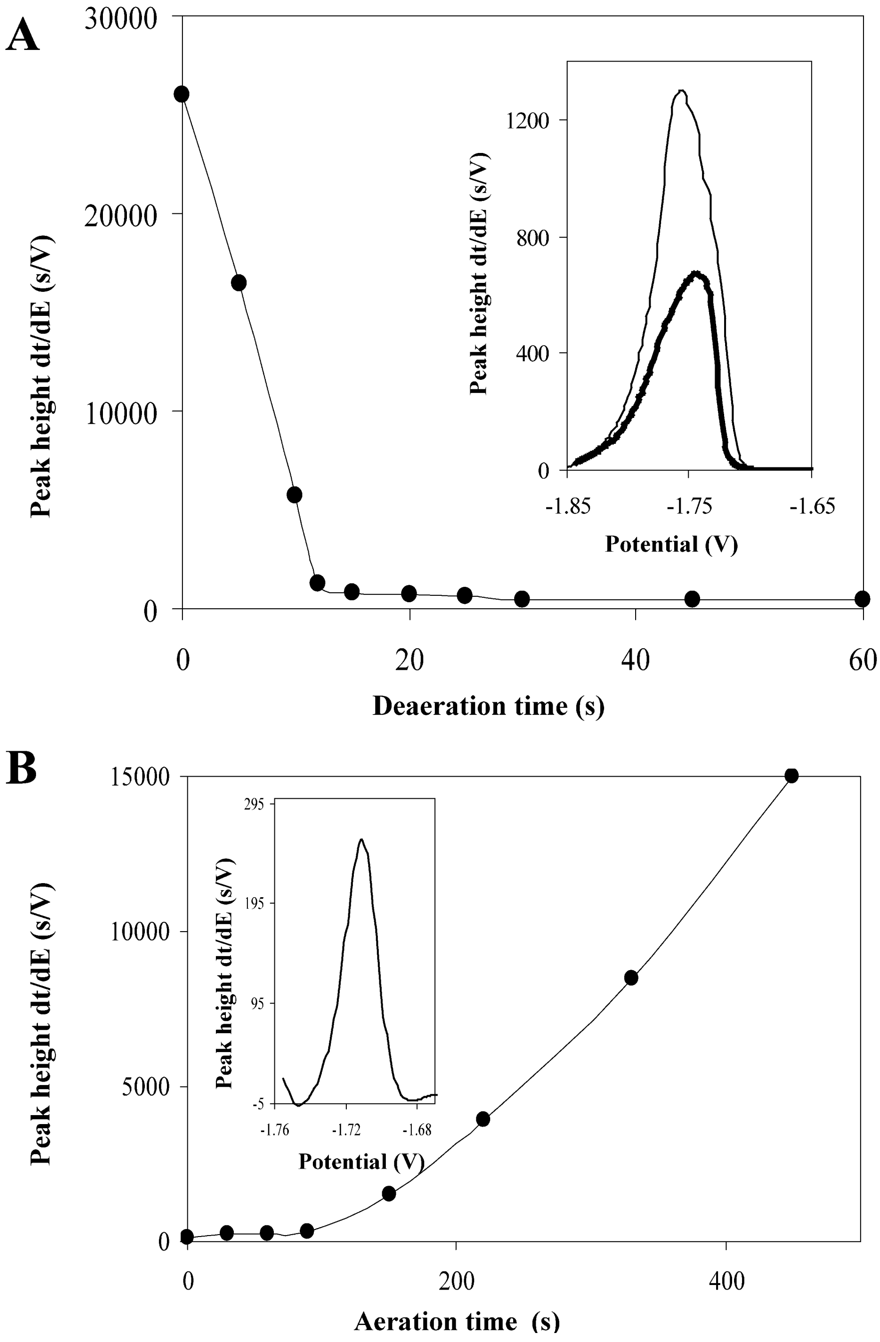

Fig. 3. Effect of oxygen on the CPSA peak height of rabbit liver MT (100 ng/ml). (A) Solution purged by argon for 0–60 s; inset: signals at purging times of — 30 s and ▬ 60 s; (B) solution purged first by argon for 300 s and then by air for 0–400 s; inset: a signal after purging with air for 60 s; I_{str}: − 1 μA, t_A 120 s; conditioning potential: − 0.1 V.

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