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Effect of Tris on catalytic activity of MP-11

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Abstract

The effect of tris(hydroxymethyl)aminomethane (Tris) on the catalytic activity and microstructure of heme undecapeptide, microperoxidase-11 (MP-11) in the aqueous solution was investigated using cyclic voltammetry, circular dichroism (CD) spectroscopy, UV–vis absorption spectroscopy and X-ray photoelectron spectroscopy (XPS). It was found for the first time that Tris would inhibit the catalytic activity and electrochemical reaction of MP-11 at the glassy carbon (GC) electrode. This is mainly due to the fact that Tris would induce more α-helix and β-turn conformations from the random coil conformation of MP-11, cause the asymmetric split-up in the Soret band region of MP-11, increase the non-planarity of the heme of MP-11, and change the electron densities of N, O and S atoms of MP-11. Meanwhile, It was found that the electrochemical reaction of MP-11 with Tris at GC electrode is diffusion-controlled, and the diffusion coefficient of MP-11 and the rate constant for the heterogeneous electron transfer of MP-11 in the presence of Tris are decreased by 19% and 16%, respectively. Further experiments showed that the electrocatalytic current of MP-11 on the reduction of H_2O_2 is decreased by about 25% after the addition of Tris to the MP-11 solution.

Keywords: Tris; MP-11; Catalytic activity; Secondary structure; Asymmetric split-up

1. Introduction

Being traditionally regarded as a buffer solution, tris(hydroxymethyl)aminomethane (Tris) has been widely used for preparation of biomolecular solutions, in which the molar ratio of Tris and the biomolecule is very large (about 100) [1–3]. Although N.E. Brasch had reported that Tris had other functions besides as a buffer, such as the catalyst for the hydrolysis of $Cr_2O_7^{2-}$ [4], there was almost no report about the effect of Tris on biological molecules.

Microperoxidase-11 (MP-11) is a small enzyme obtained by proteolytic digestion of cytochrome *c*, connected to an α-helical undecapeptide via two thioether bonds; owing to its special structure, it has been studied as the model molecule for biomacromolecules [5,6]. According to incomplete statistics, about 6000 papers have been published using MP-11, as the heme model species for nearly half a century, and lots of important and valuable information have been obtained on structure and mechanism of biochemistry [7–15] such as POD, offering the possibility of studying their coordinated complexes [16–18]. Occasionally, it was found that Tris could interact with MP-11 and alter the secondary structure and electrochemical behavior of MP-11.

In this paper, taking the molar ratio of 100 for Tris and MP-11 in physiological solution in vitro as an example, the effect mechanism of Tris on the catalytic activity of MP-11 is discussed by using electrochemical method, circular dichroism (CD) spectroscopy, UV–vis absorption spectro-

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scopy and X-ray photoelectron spectroscopy (XPS). It was found that Tris, as a widely used buffer, could obviously alter the catalytic activity and the microstructure of MP-11, resulting in the decrease in the activity of the heme group of MP-11 and the reversibility in the electrochemical reaction of MP-11. Therefore, before Tris is used as the buffer in solution with biomolecules, especially for small biomolecules, it should be determined if Tris changes the structure and/or properties of the biomolecules.

2. Experimental

Microperoxidase-11 (MP-11) was purchased from Sigma Chemical Co. and was purified further by repeat ion-exchange chromatography on Hiprep-16/10 CM (AKTA Explorer-100). The concentrations of MP-11 were estimated according to [19] and purified samples were stored at 4 °C. All physiological solutions [20] in our experiments in vitro were maintained at pH 7.4 and ionic strength 0.1 M by adding HCl (or NaOH) and NaCl into Tris (or MP-11) aqueous solution. Triple distilled water was used throughout this work. All the measurements were carried out at 25±0.2 °C.

Electrochemical experiments were performed with an EG&G PAR Model 273 Potentiostat/Galvanostat and a model 270 electrochemical software in a three-electrode cell at 25±1 °C. A glass carbon electrode (GCE, apparent surface area of about 0.031 cm^2) was used as the working electrode, which was polished with slurry of 0.03 μm alumina powders and sonicated in triple distilled water for 1–5 min before use. A platinum wire constituted the auxiliary electrode, while a saturated calomel electrode (SCE) was used as the reference electrode. All potentials (mV) in this paper are quoted with respect to SCE. Oxygen was purged from the solution by bubbling with nitrogen for 30 min prior to the electrochemical measurements.

CD spectra were recorded in the far-UV (190 nm–250 nm) and Soret region (360–460 nm) using a JASCO J-715 spectropolarimeter with a quartz cell of 0.2 cm path length. The final spectrum was obtained by averaging over four consecutive scans. The background absorption of the solvent (physiological solution without MP-11) was subtracted for each spectrum. The molar ellipticity (θ) in units of deg cm^2 $dmol^{-1}$ was calculated using a value of 115 as the mean molar mass of the amino acid residues. In order to evaluate the secondary structure of MP-11, the reference spectra for α-helix, β-sheet, β-turn and the random coil conformations were calculated according to methods of Chang et al. [21] and Obert et al. [22].

UV–vis absorption spectra were obtained using a Perkin-Elmer Lambda 16 UV–vis recording spectrophotometer with 0.5 cm path length cell. Water and the relational physiological solution without MP-11 were used as the reference solution. Absorbance difference spectra were obtained between 190 and 700 nm.

XPS analysis was carried out on an ESCALab MK2 X-ray photoelectron spectrophotometer (VG, UK) using Mg Kα (1253.6 eV) radiator. The sample preparation is as follows. The MP-11 solution with or without Tris was dropped on a biological membrane (0.8×0.8 cm microscope slide with a thickness of 0.5 mm) and dried in the vacuum condition overnight.

3. Results and discussion

3.1. Cyclic voltammetric measurements

The voltammetric behavior of MP-11 in the presence of Tris has been investigated, in order to determine if the Tris may affect electron transfer between the heme-iron and the electrode surface. Fig. 1 shows the cyclic voltammograms of the 3 μM MP-11 in a 0.1 M NaCl solution without (Fig. 1, curve a) and with 300 μM Tris (Fig. 1, curve b) at the GC electrode. A pair of the well-defined redox peaks of MP-11 is observed in Fig. 1 (curve a). The anodic and cathodic peaks are located at about −347 and −441 mV, respectively. The anodic peak current is almost equal to the cathodic peak current and the difference between the cathodic and anodic potentials (ΔE_p) is about 94 mV. This indicates that MP-11 undergoes a quasi-reversible electrochemical reaction at the GC electrode [23]. Its formal potential $E^{\circ\prime}$ is −394 mV, which is in agreement with the previous researches [8,24].

In Fig. 1 (curve b), a pair of the redox peaks of MP-11 located at −318 and −436 mV, respectively, is observed and ΔE_p is about 118 mV when Tris is added to MP-11 solution. The anodic peak current is almost equal to the cathodic peak current and the formal potential $E^{\circ\prime}$ is −378 V. In addition, the peak currents I_p are significantly decreased relative to that of the MP-11 solution without Tris; it shows that Tris can inhibit the electrochemical activity of MP-11 [8].

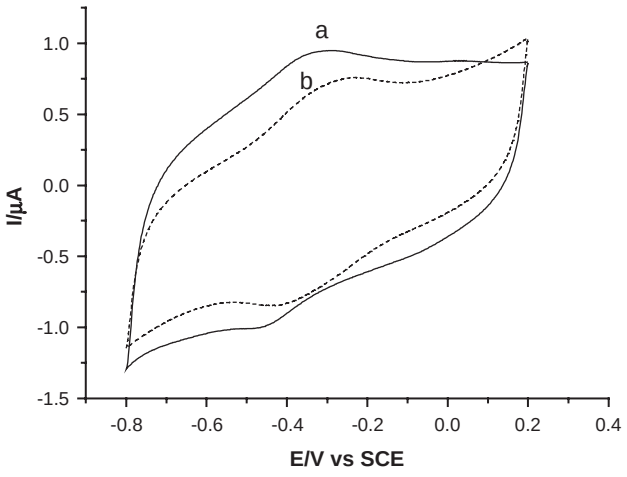

Fig. 1. The cyclic voltammograms of 3 μM MP-11 in a 0.1 M NaCl solution without (a) and with (b) 300 μM Tris at the GC electrode. Scan rate: 100 mV s^{-1}.