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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].

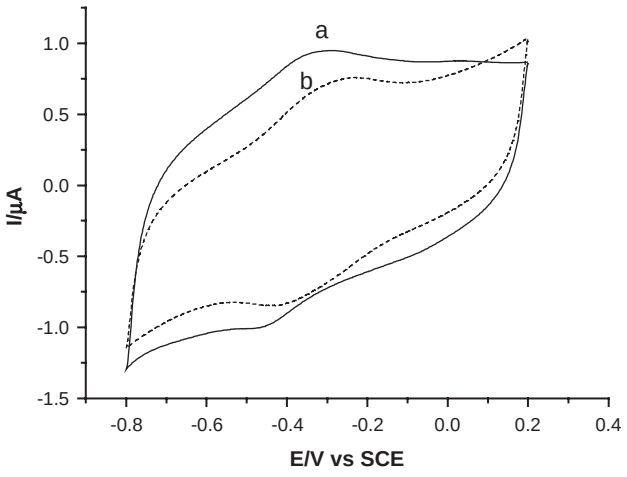

Fig. 1. The cyclic voltammograms of 3 μM MP-11 in a 0.1 M NaCl solution without (a) and with (b) 300 μM Tris at the GC electrode. Scan rate: 100 mV s^{-1}.

Table 1
Electrochemical data of 3 μM MP-11 at different concentration of Tris

Molar ratio of Tris and MP-11	$E^{\circ\prime}$ (mV)	E_{pa} (mV)	E_{pc} (mV)	ΔE_p (mV)	$I_{cat}(H_2O_2)$ (μA*)
0	−394	−347	−441	94	2.89
100	−378	−318	−436	118	2.13

$C(H_2O_2)$: 2 mM, scan rate: 100 mV/s, potential: − 0.56 V.

All the above results demonstrate that the reversibility of the electrochemical reaction of MP-11 is decreased after Tris is added to the MP-11 solution. Electrochemical data of MP-11 summarized in Table 1 shows the increase in ΔE_p and the decrease in the peak current is by 25% and 26%, respectively, compared with that in absence of Tris. This data clearly indicate that Tris can inhibit the electrochemical activity and the catalytic activity of MP-11.

Fig. 2 shows the cyclic voltammograms of the 3 μM MP-11 in a 0.1 M NaCl solution with 300 μM Tris (Fig. 1, curve b) at the GC electrode for the different scan rates ranging from 50 to 400 mV s^{-1}. It was found from Fig. 2 that the ΔE_p increased with the scan rate and I_{pa} is proportional to the square root of the scan rate (Fig. 3). This indicates that the electrochemical reaction of MP-11 in the presence of Tris at the GC electrode is diffusion-controlled [25]. From the slope of the curve in Fig. 3, the diffusion coefficient of MP-11 in the presence of Tris was calculated to be $D_o = 2.7 \times 10^{-6}$ cm^2 s^{-1}. According to the procedure of Nicholson [26], the rate constant for the heterogeneous electron transfer of MP-11 in the presence of Tris was calculated to be $k_s = 2.2 \times 10^{-3}$ cm s^{-1}, assuming $n = 1$, $\alpha = 0.5$ and $T = 25$ °C. The cyclic voltammetric measurements of MP-11 without Tris for the different scan rates were also carried out. The D_o and k_s obtained in the absence of Tris are 3.2×10^{-6} cm^2 s^{-1} and 2.7×10^{-3} cm s^{-1}, respectively, which are similar values to those reported by Santucci et al. [8]. This indicates that the D_o and k_s of MP-11 in the presence of Tris are decreased by nearly 19% and 16%, respectively. Further experiments showed that the addition of Tris to the MP-11 solution decreased the electrocatalytic current of MP-11 on the reduction of H_2O_2 (Table 1). All the above results prove that the catalytic activity of MP-11 can be decreased in the presence of Tris.

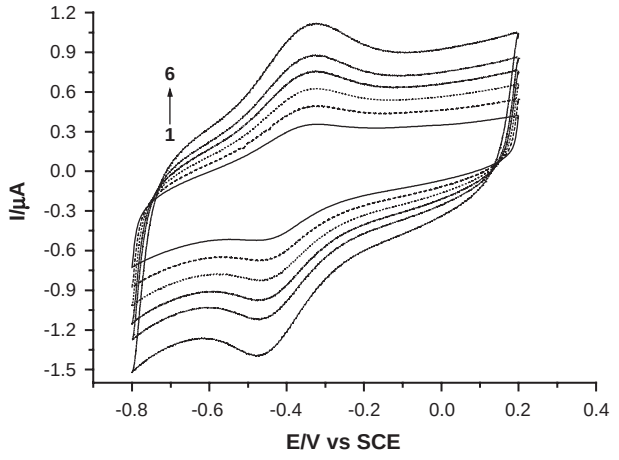

Fig. 2. The cyclic voltammograms of 3 μM MP-11 in a 0.1 M NaCl solution with 300 μM Tris at the GC electrode. Scan rate: (1) 50 mV s^{-1}, (2) 100 mV s^{-1}, (3) 150 mV s^{-1}, (4) 200 mV s^{-1}, (5) 300 mV s^{-1}, (6) 400 mV s^{-1}.

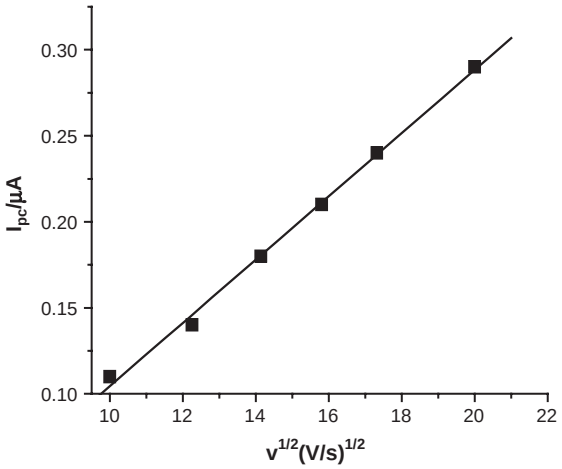

Fig. 3. The relation between the square root of the scan rate and positive peak current from Fig. 2.

Many factors, such as the type of the six axial ligand and the hydrophobicity of its environment of the heme group, can affect $E^{\circ\prime}$ of the heme group. An increase in the hydrophobicity of its environment of the heme group or the replacement of the original six axial ligand of the heme group would shift $E^{\circ\prime}$ to the positive direction [27–30]. When Tris is added into the aqueous solution, the hydrophobicity would be increased because Tris has the hydrophobic groups; meanwhile, the $E^{\circ\prime}$ of MP-11 is shifted to the positive direction by 16 mV. This indicates that Tris would increase the hydrophobicity of the environment of the heme group in MP-11 or replaces the original six axial ligand of the heme group in MP-11 [30].

3.2. XPS spectra

XPS has become a powerful tool for providing precise information concerning the core-level binding energies and linewidths, and the valence electronic structure of macromolecules [31,32]. Fig. 4 shows the XPS spectra of MP-11 in the absence of Tris (Fig. 4, curve a) and MP-11 in the presence of Tris (Fig. 4, curve b). The average binding energies of C1s, O1s, N1s and S2p of MP-11 are listed in Table 2. It was reported that the binding energy of N1s for C–N bonds is at about 399 eV and the binding energy of O1s for C–O bonds is at about 530 eV [33]. Thus, the average binding energies of O1s and N1s in Table 2 are mainly contributed from C–O and C–N bonds. The average binding energy of S2p near 163 eV for MP-11 corresponds to thioether bonds [34]. The binding energy of C1s at 284.6 eV was used as the standard.

Figs. 5 and 6 clearly show XPS of N1s and O1s, respectively, for MP-11 in the absence of Tris (curve a) and MP-11 in the presence of Tris (curve b). It was clearly

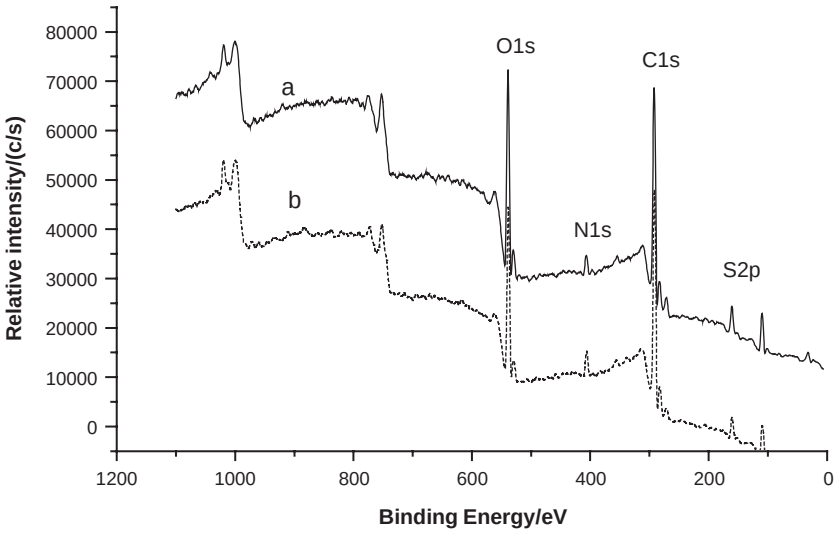

Fig. 4. X-ray photoelectron spectra of 3 μM MP-11 solutions with the molar ratio of Tris and MP-11: (a) 0 and (b) 100.

observed from Table 2, Figs. 5 and 6 that the average binding energies of N1s and O1s decrease by about 0.54 and 0.50 eV, respectively. In addition, the average binding energy of S2p increases by 0.20 eV.

In the structure of MP-11, it consists of the heme group linked with an undecapeptide chain via two thioether bonds of the cysteine residues and one of the axial coordination sites of iron(III) occupied by a His residue. The six axial ligand of a MP-11 molecule is the $-NH_2$ group of the valine-11 or lysine-13 residue in another MP-11 molecule [35]. The XPS results mentioned above may indicate that the six axial ligand of the MP-11 molecule, the $-NH_2$ group of the valine-11 or lysine-13 residue from another MP-11 molecule is replaced with the $-NH_2$ group of Tris, leading to the change in the average binding energies of N and O atoms in MP-11 [36,37]. Thus, the dimer of the MP-11 molecules would become monomer and the secondary structure of the MP-11 molecule may be changed.

3.3. CD measurement

Fig. 7 shows the far-UV CD spectra of MP-11 in the absence of Tris (Fig. 7, curve a) and in the presence of Tris (Fig. 7, curve b). When the solution has no Tris, a distinct negative band at 201 nm and a wide negative band at 218 nm can be observed in Fig. 1, curve a, which represents the random coil conformation of MP-11 [38].

Table 2
Binding energies of C1s, O1s, N1s and S2p for MP-11 as well as MP-11 and Tris mixture with the molar ratio of 100

Molar ratio of Tris and MP-11	C(1s) (eV)	O(1s) (eV)	N(1s) (eV)	S(2p) (eV)
0	284.6	531.95	400.15	162.55
100	284.6	531.45	399.61	162.75

C1s standard: 284.6 eV.

After Tris is added into the MP-11 solution, a weak positive band near 192 nm, which is π–π* transition of amide in polypeptide chain, has been changed. Meanwhile, an obvious negative band at 222 nm has been observed from the CD spectrum (Fig. 7, curve b), in which the 222 nm band is predominantly associated with α-helix n-π* amide transitions [39]. The results revealed that Tris could change the secondary structure of MP-11.

The effect of Tris on the secondary structure of MP-11 is shown in Table 1. When the molar ratio of Tris and MP-11 is 100, the percentages of α-helix and β-turn conformations are increased, and the percentage of random coil is decreased. It is obvious that Tris can change the secondary structure of MP-11. It may be due to the change in the equilibrium between the hydrophilicity and hydrophobicity in the MP-11 solution with adding Tris into the solution [40] (Table 3).

It was reported that the far-UV CD spectroscopy could provide the information about the secondary structure of

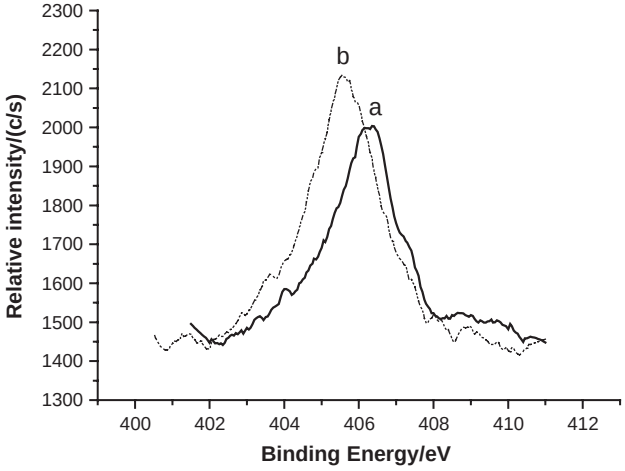

Fig. 5. X-ray photoelectron spectra of 3 μM MP-11 solutions with the molar ratio of Tris and MP-11: (a) 0 and (b) 100.

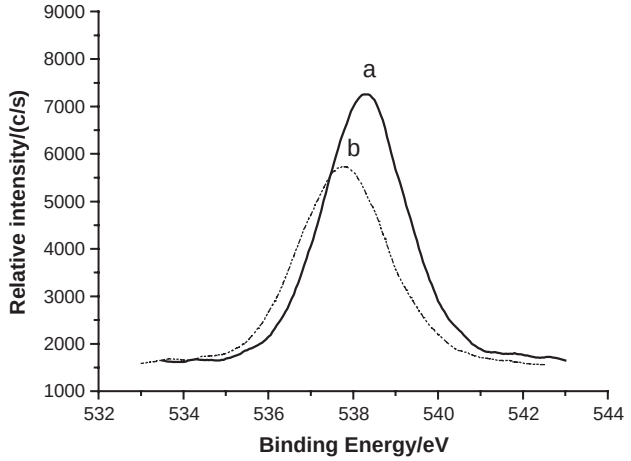

Fig. 6. The N1s X-ray photoelectron spectra of 3 μM MP-11 solution with the molar ratio of Tris and MP-11: (a) 0 and (b) 100.

biomolecules [41], while the CD spectra of the heme-containing biomolecules in the Soret region could further offer the microstructure information of the heme group [42]. The heme is CD inactive in its free form, but, when the heme-containing biomolecules are in the asymmetric environments, the heme group becomes CD active [43].

Fig. 8 shows the CD spectra of MP-11 in the absence of Tris (Fig. 8, curve a) and in the presence of Tris (Fig. 8, curve b). Firstly, a positive band at 401 nm is observed in Fig. 8, curve a, corresponding to π−π* transition of heme porphyrin cycle in the Soret band region [43]. Secondly, after adding Tris to MP-11 solution (Fig. 8, curve b), the Soret band near 401 nm is split into the two new bands of 395 nm and 410 nm; the molar ellipticity is significantly decreased by about 38% and 28%, respectively. This indicates that the non-planarity of the heme group of the MP-11 molecule in the presence of Tris is increased [44,45]. This asymmetric split-up in the Soret band region provides evidence that there is altering of the secondary structure and yet loosing the tertiary structure from MP-11 [43,46].

Table 3
The data of the secondary structure of 3 μM MP-11 solutions at different concentration of Tris

Molar ratio of Tris and MP-11	α-Helix (%)	β-Turn (%)	Random coil (%)
0	3.5	2.1	94.4
100	12.0	13.9	74.0

As is well known, the heme distortion is mainly controlled by the polypeptide of the protein [47]. For this reason, the variation of the Soret band region from CD spectra just coincides with that of far-UV CD of MP-11, both of which would demonstrate that the conformations of MP-11 protein have been altered and the heme of the enzymatic protein have been in a new asymmetric environment by adding Tris to MP-11 solution [42,43]. The observed alteration in the Soret region after the MP-11 interaction with Tris would clearly embody that the microenvironment of the protein around the heme has been greatly changed by Tris as well. Therefore, from the above CD results, it can be concluded that Tris can alter the secondary and tertiary structure (microstructure) of the MP-11 molecule and lead to an increase in the non-planarity of the heme group of the MP-11 molecule.

3.4. UV–vis absorption spectra measurements

Fig. 9 shows the UV–vis absorption spectra of MP-11 in the absence of Tris (Fig. 9, curve a) and in the presence of Tris (Fig. 9, curve b). When the molar ratio of Tris and MP-11 is 0 (Fig. 9, curve a), the Soret band at 403 nm was observed. It corresponds to the π−π* transition of the porphyrin cycle in the heme group of the MP-11 molecule [42]. By adding Tris to MP-11 solution (Fig. 9, curve b), the Soret band at about 403 nm is also split to two bands at 396 nm and 407 nm, respectively. At the same time, the value of absorption is decreased by 23% and 12%,

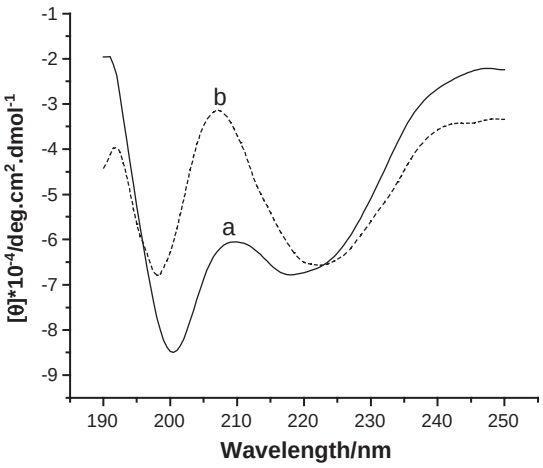

Fig. 7. The far-UV CD spectra of 3 μM MP-11 solution without (a) and with (b) 300 μM Tris. Wavelength region: 190–250 nm.

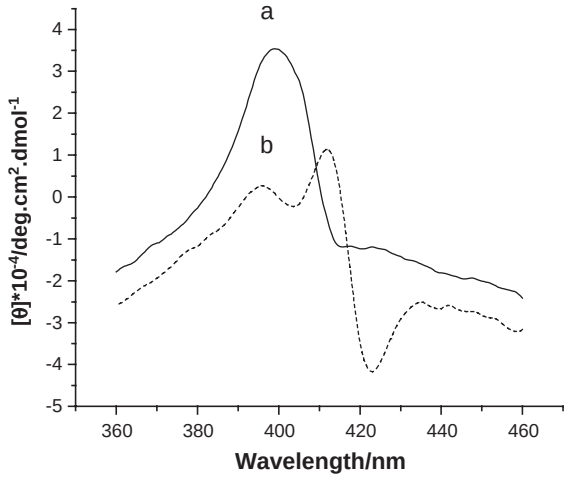

Fig. 8. The CD spectra of 3 μM MP-11 solution without (a) and with (b) 300 μM Tris. Wavelength region: 360–460 nm.

respectively. This phenomenon is similar to that in the CD spectra (Fig. 8).

The above results also reflected that Tris could affect the microstructure of the heme group of the MP-11 molecule and increase the non-planarity of the heme group of the MP-11 molecule. It may be because MP-11 is a small biomolecule and the exposure extent of the heme group is too large in the presence of Tris [48]. Therefore, the $E^{\circ\prime}$ of MP-11 largely shifts to the positive direction and ΔE_p increases after the interaction between Tris and MP-11, leading to the decrease in the electrochemical activity and the catalytic activity of MP-11.

Fig. 10 shows the absorption difference spectrum (ΔA) of 3 μM MP-11 solution at the Soret band with increasing concentration of Tris. With the increase in the concentration of Tris, the maximum absorption at Soret band decreased until the molar ratio of Tris and MP-11 became 150, and then it was increased. The changes in the absorption of Soret spectra are the results of the alteration in the conformation of MP-11 in the present of Tris. The non-planar distortion in heme group of MP-11 is increased with decreasing intensity of the Soret band till the molar ratio of Tris and MP-11 became 150. When the molar ratio of Tris and MP-11 increases from 0 to 50, the ΔA of MP-11 solution is not clearly altered and the hydrophobicity of the solution does not change a lot. Thus, Tris does not change obviously the planarity of the heme group of the MP-11 molecule and the microstructure of the heme group of the MP-11 molecule because the concentration of Tris is low. From the above discussion, it is suggested that, if it is necessary to use Tris as the buffer, the molar ratio of Tris and MP-11 should not be higher than 50.

Our spectral results clearly demonstrated that, after Tris is added into the MP-11 solution, it could interact with MP-11 molecule, inducing the conformational change of MP-11 and altering the secondary structure of MP-11, and yet loosing the tertiary structure of MP-11. The electrochemical results indicated that Tris could inhibit the electrochemical reaction of MP-11. The electrochemical behavior of MP-11

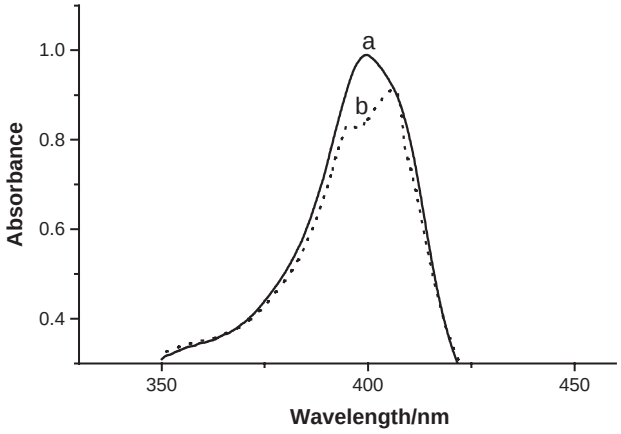

Fig. 9. The UV–vis absorption spectra of 3 μM MP-11 solution without (a) and with (b) 300 μM Tris. Wavelength region: 300–460 nm.

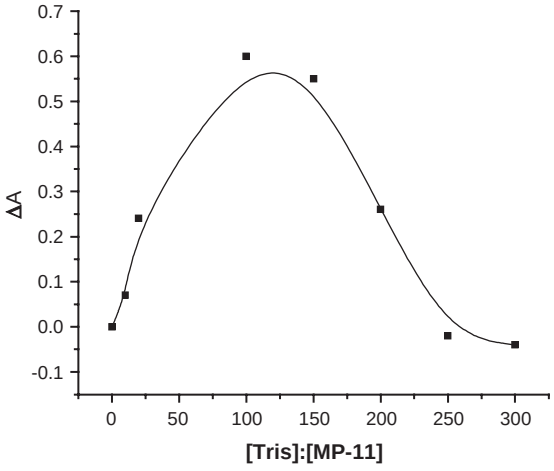

Fig. 10. The absorption difference spectrum (ΔA) of 3 μM MP-11 solution at the Soret band with increasing concentration of Tris.

could be affected by the hydrophobicity of the medium, the axial ligands, the pH of the solution, the cation, etc. [27–30]. So there is a certain relationship between these factors and the electrochemical reaction of MP-11 in the presence of Tris. When the molar ratio of Tris and MP-11 is 100 (especially for the large molar ratio of Tris and MP-11), Tris could interact with MP-11, inducing more α-helix and β-turn conformations from the random coil conformation of MP-11 and altering the non-planarity of the heme. Meanwhile, the anisotropy character of the heme group is obviously altered and the electrochemical activity is also changed due to the split-up from the Soret band of MP-11 in the presence of Tris. Therefore, Tris could change the conformation of MP-11 and reduce the activity of the heme group of MP-11, resulting in the decrease in the reversibility in the electrochemical reaction of MP-11 and inhibition of the electrochemical reaction of MP-11. This further reveals that the conformational changes in the enzymatic proteins of MP-11 are possible rate-controlling steps in biological electron transfer of MP-11.

4. Conclusions

From the above electrochemical and spectroscopic results, it can be concluded that Tris, as the widely used buffer, would alter the microstructure of MP-11 and the catalytic activity of MP-11 with its hydrophobicity and ability to replace the original six axial ligand of MP-11, especially for the large molar ratio of Tris and MP-11. It was further found 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 nearly 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. This further reveals that the conformational changes in the enzymatic proteins of MP-11 are possible rate-controlling steps in biological electron transfer of MP-11. Therefore, before Tris is used as the buffer in the solution with biomolecules, especially for the small biomolecules, it should be paid attention if Tris changes in the structure and the properties of the biomolecules.

Acknowledgments

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