Polarographic catalytic wave of hydrogen

——Parallel catalytic hydrogen wave of bovine serum albumin in the presence of oxidants

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Abstract A polarographic catalytic hydrogen wave of bovine serum albumin (BSA) at about –1.80 V (vs. SCE) in NH_4Cl-$NH_3 \cdot H_2O$ buffer is further catalyzed by such oxidants as iodate, persulfate and hydrogen peroxide, producing a kinetic wave. Studies show that the kinetic wave is a parallel catalytic wave of hydrogen, which resulted from that hydrogen ion is electrochemically reduced and chemically regenerated through oxidation of its reduction product, atomic hydrogen, by oxidants mentioned above. It is a new type of poralographic catalytic wave of protein, which is suggested to be named as a parallel catalytic hydrogen wave.

Keywords: bovine serum albumin (BSA), catalytic hydrogen wave, parallel catalytic hydrogen wave.

At present, studies and applications on protein in polarography have been mainly concerned with three classes of polarographic waves, its reduction wave, catalytic hydrogen wave and parallel catalytic wave. The protein can yield three reversible reduction waves of disulfide linkages. Among them, two are the surface reduction waves of mercuric and mercurous thiolate resulting from the reaction of the disulfide linkage with mercury electrode, with peak potential about –0.25 and –0.5 V[1,2], respectively. The other is the diffusion-controlled reduction wave of the disulfide linkage, with peak potential about –0.9 V[3–5]. The catalytic hydrogen wave of protein was classified into two types. One is the so-called "sodium pre-wave"[6,7]. It is the catalytic hydrogen wave, which resulted from the decrease of the hydrogen overvoltage and the accelerating of the discharge of the hydrogen ion by protein combined with proton. It appears generally at more positive potential by about 0.2 V than the usual hydrogen discharge of the base solution. The other is the catalytic hydrogen wave of protein in the presence of metal ions, for example, the catalytic hydrogen double wave of protein in cobalt ammonia medium[8–10] and the catalytic hydrogen wave of protein in presence of Rh(III) and Ni(II)[11,12]. Recently, we reported a kind of the parallel catalytic wave of the disulfide linkage of proteins[13–15], in which the disulfide linkage was reduced and regenerated through oxidation reaction of its reduction product sulfhydryl by KIO_3.

In this work, the catalytic hydrogen wave of BSA in NH_4Cl-$NH_3 \cdot H_2O$ medium is further

catalyzed by oxidants such as potassium iodate, potassium persulfate and hydrogen peroxide, producing a new kinetic wave. To our knowledge, this type of the kinetic wave of protein has not been found in literature so far. And the mechanism is discussed.

1 Experimental

1.1 Reagent

1.0×10^{-5} mol/L stock solution of BSA (Sino-American Biotechnology Co., Xi'an, China) was prepared and stored at 4℃ refrigerator. The other standard working solutions of proteins were obtained by diluting the stock solution with water. All chemicals used were of analytical reagent grade. Buffers of different pH values were obtained by mixing 1.0 mol/L NH_4Cl solution and 1.0 mol/L $NH_3 \cdot H_2O$ solution (AR, Xi'an Chemical Reagent Factory, Xi'an, China) at different volume ratios. Twice-distilled water was used throughout this experiment.

1.2 Apparatus

Model JP2 linear-potential scan polarograph (Shandong No. 7 Electric and Communication Factory, China) was equipped with a three-electrode set-up, which includes a dropping mercury working electrode (DME), a saturated calomel reference electrode and a platinum wire counter electrode. Model CH660 electrochemical workstation (CHI Instrument Com., USA) was equipped with a model 303A static mercury drop working electrode (EG&G PARC Com., USA), a saturated calomel reference electrode and a platinum wire counter electrode.

1.3 Procedure

Take an aliquot of standard working solution of BSA into a 25 mL volumetric flask, then add 10 mL of NH_4Cl-$NH_3 \cdot H_2O$ buffer. Dilute it with water to the mark and then shake it to get the uniform solution. Transfer the obtained solution to polarographic cell, and examine the characteristics of the catalytic hydrogen wave of BSA at about –1.80 V in the range of –1.60 — –2.10 V. Moreover, besides adding an aliquot of standard working solution of BSA and 10 mL of NH_4Cl-$NH_3 \cdot H_2O$ buffer, add an aliquot of oxidants solution into a 25 mL volumetric flask. Operate according to these procedures described above and examine the characteristics of the parallel catalytic hydrogen wave of BSA at about –1.80 V.

2 Results and discussion

2.1 Catalytic hydrogen wave of BSA

2.1.1 Characterization of catalytic hydrogen wave of BSA. Several media have been tested in this work. In weak acidic medium such as HOAc-NaOAc (pH 4.7) and tartaric acid-sodium tartrate (pH 3.3) buffers, the usual hydrogen wave of the base solution appears at more positive potential. Any reduction wave of BSA cannot be observed in the potential range of –1.20—–2.10 V. However, BSA yields a reduction wave at about –1.80 V in NH_4Cl-$NH_3 \cdot H_2O$ buffer (pH 8.3).

The polarographic characters of the reduction wave of BSA have been examined. With increasing the pH value of the buffer from 8.0 to 10.2, the peak current i_p of the reduction wave decreases gradually (fig. 1, curve 1) and the peak potential E_p shifts to the negative direction. When pH value is in the range of 8.0—8.89, the i_p decreases slowly with increasing the pH value, and the relationship of E_p with pH is $-E_p = 1.62 + 0.021$ pH. When the pH value is higher than 8.89, the i_p decreases fast with increasing the pH value, and the E_p-pH relationship is $-E_p = 1.13 + 0.075$ pH. With increasing the total concentration of the buffer with constant pH 8.31 from 0.08 to 0.48 mol/L, the i_p increases gradually (fig. 2, curve 1), and the E_p shifts positively from –1.86 to –1.79 V. As seen in cyclic voltammogram (fig. 3, curve 1), there is a reduction wave with peak

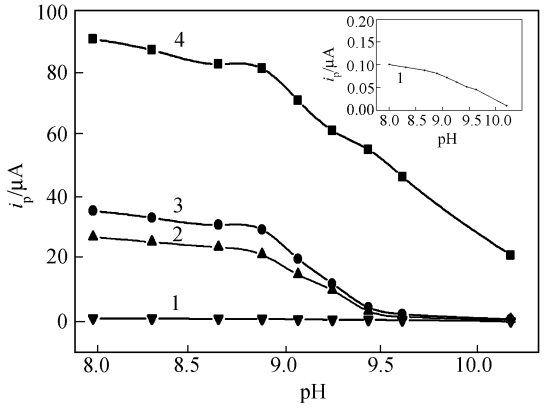

Fig. 1. Effect of pH value on peak current of 4.0×10^{-8} mol/L BSA. 1, NH_4Cl-$NH_3 \cdot H_2O$ buffer; 2, (1) + 1.5×10^{-3} mol/L H_2O_2; 3, (1) + 1.5×10^{-3} mol/L $K_2S_2O_8$; 4, (1) + 1.5×10^{-3} mol/L KIO_3.

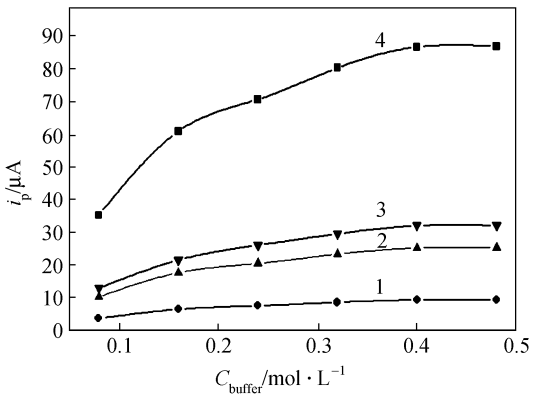

Fig. 2. Effect of total concentration of buffer on peak current of 4.0×10^{-8} mol/L BSA. 1, H_4Cl-$NH_3 \cdot H_2O$ (pH 8.31) buffer; 2, (1) + 1.5×10^{-3} mol/L H_2O_2; 3, (1) + 1.5×10^{-3} mol/L $K_2S_2O_8$; 4, (1) + 1.5×10^{-3} mol/L KIO_3.

potential –1.80 V on cathodic scan and no oxidation wave on anodic scan. Moreover, the current function $i_p v^{-1/2}$ of the reduction wave remains almost unchanged with raising linear-potential scan rate v (fig. 4, curve 1). These results mentioned above show that the reduction wave of BSA at –1.80 V in polarographic character is different from the reduction wave of the disulfide linkage, and is similar to the pre-sodium wave of protein. Therefore, it can be deduced that the reduction wave at –1.80 V is a catalytic hydrogen wave of BSA.

BSA molecule is of a hart-shape stereo-structure, including three domains of I(1—183), II(184—377) and III(378—582)[16]. There are such free residuals as one α-carboxyl group, 99 side chain carboxyl groups, 16 imidazole groups, one α-amino group, 57 ε-amino groups, 19 phenolic groups and 22 guanidine groups per molecule BSA. Their acid dissociation constants pK_a are 3.75, 3.95, 6.9, 7.75, 9.8, 10.35 and >12 respectively. Curve 1 in fig. 1 shows the dependence of the peak current i_p of the catalytic hydrogen wave of BSA on the pH value of the buffer. The dependence coincides nearly with the acid titration curve of BSA obtained by Tanford[17]. In the pH range of 8.0—10.2, the inflection point of the dependence is about pH 8.89, approximating to the second

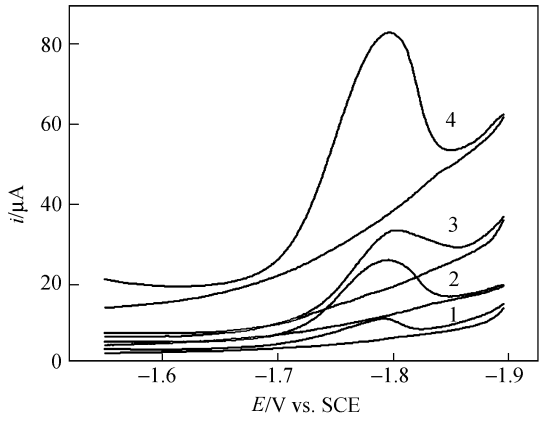

Fig. 3. Cyclic voltammogramm of 4.0×10^{-8}mol/L BSA. 1, 0.36 mol/L NH_4Cl-0.04 mol/L $NH_3 \cdot H_2O$ (pH 8.31) buffer; 2, (1) + 1.5×10^{-3} mol/L H_2O_2; 3, (1) + 1.5×10^{-3} mol/L $K_2S_2O_8$; 4, (1) + 1.5×10^{-3} mol/L KIO_3. Scan rate v = 150 mV/s.

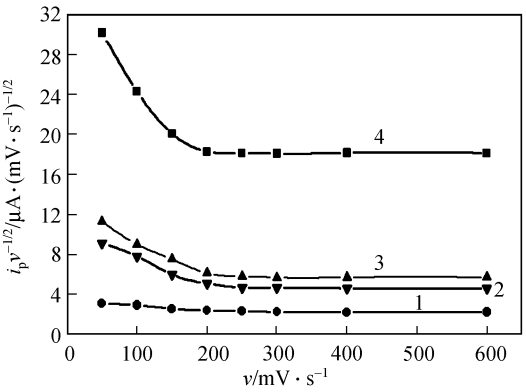

Fig. 4. Current function of 4.0×10^{-8}mol/L BSA. 1, 0.36 mol/L NH_4Cl-0.04 mol/L $NH_3 \cdot H_2O$ (pH 8.31) buffer; 2, (1) + 1.5×10^{-3} mol/L H_2O_2; 3, (1) + 1.5×10^{-3} mol/L $K_2S_2O_8$; 4, (1) + 1.5×10^{-3} mol/L KIO_3.

stoichiometric point, pH 9.5, of the acid titration curve. This demonstrates that the production of the catalytic hydrogen wave is closely related to the chemical forms of these residuals in BSA molecule. When the pH value is in the range of 8.0—8.89, all of these ε-amino groups, guanidine groups and phenolic groups of BSA are protonated. They accelerate the discharge of the combined hydrogen ion, and the i_p of the catalytic hydrogen wave is higher. When the pH is higher than 8.89, only two kinds of free residuals, the guanidine and phenolic groups, are protonated, so the i_p is low and decreases fast with the pH value increasing. The conclusion is further verified with the following two experiments. The first is modifying the free amino groups of BSA molecule with formaldehyde. After certain amount of formaldehyde is added into the buffer containing BSA, the i_p of the catalytic hydrogen wave of BSA decreases. With formaldehyde concentration increasing, the i_p decreases gradually, and finally decreases by about 59.3% and then unchanged. The results are listed in table 1. The final decreased percentage 59.3% is nearly approximate to that, 58.2%, of 57 ε-amino groups in total sum of 57 ε-amino groups, 19 phenolic groups and 22 guanidine groups. The second is denaturing BSA further by anodic surfactant, sodium dodecyl sulfate SDS. When SDS is present, the catalytic hydrogen wave is split into two peaks, meanwhile, the total current of the two peaks increases. The results are showed in table 2. The reason is that the BSA

Table 1 Effect of HCHO concentration on peak current of 4.0×10^{-8} mol/L BSA

C_{HCHO} /mol • L^{-1}	i_p/μA			
	—	KIO_3[a)]	$K_2S_2O_8$[a)]	H_2O_2[a)]
0.00	9.45	87.0	32.3	25.5
0.04	7.33	68.6	24.7	19.9
0.08	4.82	44.2	16.8	14.7
0.10	3.86	36.2	12.4	10.8
0.12	3.85	36.2	12.5	10.9

a) Oxidant concentration is 1.5×10^{-2} mol/L.

Table 2 Effect of SDS concentration on peak current and peak potential of 4.0×10^{-8} mol/L BSA

$C_{SDS} \times 10^{-5}$ /mol • L^{-1}	—		KIO_3 a)		$K_2S_2O_8$ a)		H_2O_2 a)	
	$-E_p$/V	i_p/μA	$-E_p$/V	i_p/μA	$-E_p$/V	i_p/μA	$-E_p$/V	i_p/μA
0.0	1.80	9.45	1.80	87.0	1.81	32.3	1.81	25.5
0.8	1.76, 1.82	4.87, 5.32	1.81	94.2	1.82	36.1	1.82	27.9
1.2	1.77, 1.82	5.43, 6.14	1.81	110.8	1.82	41.5	1.83	31.7

a) Oxidant concentration is 1.5×10^{-2} mol/L.

molecule is further denatured and unfolded by SDS to enlarge the connected area of BSA with electrode surface and to cause the discharge of more hydrogen ions of protonated residuals.

2.1.2 Mechanism of catalytic hydrogen wave of BSA. According to the theory of catalytic hydrogen wave[18], the production process of the catalytic hydrogen wave of BSA involves the following steps. The BSA molecule combines with hydrogen ion to form the protonated BSA, $BSAH^+$. The hydrogen ion at the $BSAH^+$ then accepts one electron to produce an unstable free radical $BSAH^*$ on electrode surface. The unstable $BSAH^*$ enters into a bimolecular interaction to evolve hydrogen molecule subsequently. The process can be described as follows:

$$BSA + NH_4^+ \Leftrightarrow BSAH^+ + NH_3$$

$$BSAH^+ + e^- \rightarrow BSAH^*$$

$$2\,BSAH^* \rightarrow 2BSA + H_2\uparrow$$

2.2 Kinetic wave of BSA in the presence of oxidant

2.2.1 Characterization of kinetic wave. It can be observed that the catalytic hydrogen wave of BSA is further catalyzed by oxidants such as potassium iodate, potassium persulfate and hydrogen peroxide, producing a kinetic wave. The experimental results indicate that three kinetic waves are very similar in polarographic character in the cases that anyone of the three oxidants is present alone. Cyclic voltammetry shows that the peak current of the catalytic hydrogen wave of BSA on cathodic scan increases greatly, the peak potential remains at the original position, and no oxidation wave on anodic scan (fig. 4, curves 2—4). The peak current $i_{p,l}$ of these kinetic waves increases gradually with increasing the oxidant concentrations. The ratio $i_{p,l}/i_p$ of the peak current $i_{p,l}$ of these kinetic waves with that i_p of the corresponding catalytic hydrogen wave without oxidants is proportional to the square root of oxidant concentrations in the range of 1.0×10^{-3}—1.2×10^{-2} mol/L (table 3). In addition, the current function $i_{p,l}\, v^{-1/2}$ of these kinetic waves decreases gradually with raising linear-potential scan rate v (fig. 4, curves 2—4). These results mentioned

Table 3 Linear regressions of $i_{p,l}/i_p$ ratio with $C_{ox}^{1/2}$

Oxidant	Linear regressions	Corr. coefficient r
KIO_3	$i_{p,l}/\,i_p = -13.1 + 633.3\, C_{KIO_3}^{1/2}$	0.9992
$K_2S_2O_8$	$i_{p,l}/\,i_p = -10.6 + 366.7\, C_{K_2S_2O_8}^{1/2}$	0.9989
H_2O_2	$i_{p,l}/\,i_p = -3.3 + 144.1\, C_{H_2O_2}^{1/2}$	0.9919

above demonstrate that these kinetic waves are a parallel catalytic wave related to the catalytic hydrogen wave of BSA.

2.2.2 Mechanism of the kinetic wave of BSA. To ascertain the electrode reaction of the parallel catalytic wave, it is again examined that the pH value of the buffer, total concentration of the buffer, modifying with formaldehyde and denaturing with SDS affect three parallel catalytic waves of BSA. Experiments show that their influent on three parallel catalytic waves is similar. With increasing the pH value of the buffer from 8.0 to 10.2, the peak current $i_{p,l}$ decreases gradually (fig. 1, curves 2—4) and the peak potential $E_{p,l}$ shifts to the negative direction. Furthermore, the dependence of the $i_{p,l}$ on the pH value has the same inflection point of pH 8.89 as the catalytic hydrogen wave of BSA. When the pH value is in the range of 8.0—8.89, the $i_{p,l}$ decreases slowly with increasing the pH value, and the $E_{p,l}$ -pH relationship is $-E_{p,l} = 1.62 + 0.022$ pH. When the pH value is higher than 8.89, the $i_{p,l}$ decreases fast with increasing the pH value, and the $E_{p,l}$ -pH relationship is $-E_{p,l} = 1.13 + 0.077$ pH. With increasing the total concentration of the buffer with constant pH 8.31 from 0.08 to 0.48 mol/L, the $i_{p,l}$ increases gradually (fig. 2, curves 2—4), and the $E_{p,l}$ shifts positively from –1.87 to –1.79 V. Modifying free amino groups of BSA with HCHO leads to the decrease of the $i_{p,l}$ also. The $i_{p,l}$ of three parallel catalytic waves in the presence of KIO_3, $K_2S_2O_8$ and H_2O_2 alone is finally decreased by 58.4%, 61.6% and 57.6%, respectively (table 1). SDS makes the $i_{p,l}$ of three parallel catalytic waves increase apparently (table 2). The effects of these factors on three parallel catalytic waves are almost similar to that on the catalytic hydrogen wave, demonstrating that the electrode reaction of three parallel catalytic waves is the same as that of the catalytic hydrogen wave, which is still the discharge of the protonated BSA, $BSAH^+$, on electrode surface.

The one-electron reduction of the $BSAH^+$ on electrode surface produces an intermediate product, free radical $BSAH^*$. At the same time, the partial H^* that carried by the free radical $BSAH^*$ is an atomic hydrogen. The atomic hydrogen H^* is very active in chemical properties. It can be oxidized by IO_3^-[19]. Undoubtedly, it is sure that the atomic hydrogen that carried with the free radical BSAH* can be oxidized by potassium iodate, potassium persulfate and hydrogen peroxide on electrode surface to regenerate the $BSAH^+$ under the condition of this work. The cycle of both the electrochemical reduction and chemical regeneration of hydrogen ion leads to the production of the parallel catalytic wave that related to the catalytic hydrogen wave. Moreover, it should be pointed out that the reduction of these oxidants used is multi-electron successive processes via their corresponding intermediate radicals. For example, the reduction of IO_3^- is a six-electron successive transfer, in which these radicals such as $IO^{-\bullet}$ and $IO_2^{-\bullet}$ are produced[20]. The reduction of $S_2O_8^{2-}$ is a two-electron successive exchange, in which the product from one-electron reduction of $S_2O_8^{2-}$ is the sulfate radical $SO_4^{-\bullet}$.

$$S_2O_8^{2-} + e^- \rightarrow SO_4^{2-} + SO_4^{-\bullet} \qquad E^\circ \leqslant 0.6\ \text{V},$$

$$SO_4^{-\bullet} + e^- \rightarrow SO_4^{2-} \qquad E^\circ \geqslant 3.4\ \text{V}.$$

The two-electron successive reduction of H_2O_2 involves the hydroxyl radical $OH^\bullet$[21].

$$H_2O_2 + e^- \rightarrow OH^- + OH^\bullet \qquad E^\circ \leqslant 0.6\ \text{V},$$

$$OH^\bullet + e^- \rightarrow OH^- \qquad E^\circ \geqslant 2.9\ \text{V}.$$

These radicals possess higher oxidation ability. Therefore, it is sure that they take part in the oxidation reaction of the $BSAH^*$ by these oxidants. These oxidation processes involve a series of successive reaction steps, which can be simplified as follows, respectively:

$$6\ BSAH^* + IO_3^- + 3H_2O \rightarrow 6BSAH^+ + 6OH^- + I^-$$

$$2\ BSAH^* + S_2O_8^{2-} \rightarrow 2\ BSAH^+ + 2\ SO_4^{2-}$$

$$2\ BSAH^* + H_2O_2 \rightarrow 2\ BSAH^+ + 2OH^-$$

In summary, the kinetic wave of BSA resulted from the further catalysis of the catalytic hydrogen wave of BSA by oxidants is the parallel catalytic wave of hydrogen in nature. Its production is due to that the hydrogen ion H^+ carried by the protonated $BSAH^+$ is electrochemically reduced and chemically regenerated on electrode surface. The new type of the poralographic catalytic wave of protein has not been reported so far. Therefore, it is suggested to name it as a parallel catalytic hydrogen wave.

2.3 Analytical application

The parallel catalytic hydrogen wave of BSA reported in this work is of theoretical significance and practical application. It is more sensitive than the catalytic hydrogen wave with high sensitivity, and permits the determination of trace of BSA. The results show that the sensitivity of the parallel catalytic hydrogen wave of BSA in the presence of KIO_3 is the highest in the systems containing three oxidants tested. When KIO_3 concentration is 1.2×10^{-2} mol/L, the peak current of the parallel catalytic hydrogen wave of 4.0×10^{-8} mol/L BSA is about 60 times higher than that of the corresponding catalytic hydrogen wave. In 0.36 mol/L NH_4Cl-0.04 mol/L $NH_3 \cdot H_2O$ (pH 8.3 ± 0.3)-1.2×10^{-2} mol/L KIO_3 optimal supporting electrolyte, the peak current $i_{p,l}$ is linearly proportional to BSA concentration in the range of 2.4×10^{-9}—9.6×10^{-9} mol/L. The linear regression equation can be shown as

$$i_{p,l}(\mu A) = -4.75 + 5.38\times10^{10} C_{BSA}\,(\text{mol/L})$$

with linear regression coefficient $r = 0.9991$ ($n = 5$).

Because the limit of detection is determined by the initial adsorption amount of BSA on electrode surface, if the pre-adsorption time of BSA is prolonged, the analytical sensitivity could be improved further.

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