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ORIGINAL ARTICLE

Voltammetric investigation on interaction of protein with chromotrope 2R and its analytical application

Ni Hui · Xue-Liang Niu · Jun-Ying Han · Wei Sun · Kui Jiao

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Abstract The electrochemical behaviors of the interaction of chromotrope 2R (CH2R) with human serum albumin (HSA) are investigated on the hanging mercury drop electrode with linear sweep voltammetry. In the acidic buffer solution (pH 2.5) CH2R has a well-defined voltammetric reductive wave at −0.34 V (SCE). On the addition of HSA into the CH2R solution, the reductive peak current of CH2R decreases with little movement of the peak potential. The voltammetric study shows that the electrochemical parameters of interaction solution do not change and a new electrochemically non-active complex is formed via interaction of CH2R with HSA, which cannot be reduced on the Hg electrode and results in the decrease of the free concentration of CH2R. The decrease of reductive peak current is proportional to HSA concentration and further used for protein detection. The binding ratio and the binding constant are further calculated with the experimental voltammetric data.

Keywords Chromotrope 2R · Human serum albumin · Linear sweep voltammetry · Interaction · Protein

N. Hui
College of Chemistry and Pharmacy, Qingdao Agriculture University, 266109 Qingdao, People's Republic of China

X.-L. Niu · J.-Y. Han · W. Sun (✉) · K. Jiao
Key Laboratory of Eco-Chemical Engineering of Ministry of Education, College of Chemistry and Molecular Engineering, Qingdao University of Science and Technology, 266042 Qingdao, People's Republic of China
e-mail: sunwei@qust.edu.cn

Introduction

Investigations of the small molecules such as organic dyes, drugs, toxic chemicals with biomacromolecules such as nucleic acids, proteins and glycosaminoglycans have aroused great interests in recent years. The qualitative and quantitative analysis of protein is also an important factor in biochemical research and clinical tests. Up to now, many methods have been proposed on this topic and a great deal of analytical methods have been proposed including spectrophotometry, fluorometry, chemiluminesence, resonance and Rayleigh light scattering technique (Smith et al. 1985; Compton and Jones 1985; Yao et al. 1999a, b; Liu and Liu 2001). Among all these proposed methods for the determination of proteins, spectrophotometry is commonly accepted, which is often based on the interaction of organic dyes with proteins, such as Lowry method (Lowry et al. 1951), Coomassie brilliant blue G-250 (CBB G-250) (Bradford 1976), Bromophenol blue (Wei et al. 1996a) and Bromocresol green (Rodkey 1965). However, there are some disadvantages with sensitivity and selectivity. For example, Lowry method is insensitive with poor selectivity and often suffers serious interferences from coexisting substances. As to Coomassie brilliant blue G-250 method, nonlinearity exists between the absorbance of the CBB G-250 dye-protein complex and inconvenience in operation occurs. The Bromophenol blue method can be only used for protein concentrations more than 10 mg/l and the Bromocresol green procedure is liable to disturbance by turbidity and is not sensitive. These limitations can be solved by some new analytical methods and many improved methods had been developed in recent years.

Electrochemical techniques have been widely used in biological sample determination. The electrochemical activity of different proteins and peptides had been

reviewed recently (Paleček and Ostatna 2007) and the first paper about the polarography of proteins was published in the early 1930s (Heyrovsky and Babicka 1930). After the discovery of the Brdička's catalytic response of Cys-containing proteins in 1933 (Brdička 1933), the electrochemistry of protein had been widely studied. Generally speaking there are three models for protein electrochemistry, (1) the direct electrochemistry of proteins on solid electrode; (2) the catalytic hydrogen wave on mercury electrode; (3) electrochemical studies on the interaction of small molecules with proteins. For example Brabec (1980) and Reynaud et al. (1980) investigated the direct electrooxidation of Tyr and Trp residues on the carbon electrode. Guo et al. studied a parallel catalytic hydrogen wave of HSA (Guo et al. 2000a, b) and BSA (Guo et al. 2002) in the presence of different kinds of oxidants. Luo et al. also reported a polarographic system of Co(II)-HSA complex in the presence of guanidine modifier for the protein assay. The applications of electrochemical methods to the studies of the interaction of small molecules with biomacromolecules also provide a useful complement to other analytical methods. Some binding reactions of small molecules with protein that are not amenable to above detection methods can be potentially studied by voltammetric techniques. Li et al. had studied the interaction of some electro-active small molecules such as 9,10-anthraquinone, tetraphenylporphyrin tetrasulfonate (TPPS) with different kinds of protein such as hemoglobin, albumin and antibody (Zhu and Li 1999; Zhang et al. 1999; Zeng et al. 2002). Our group had used alizarin red S and acid chrome blue K to investigate the protein binding interaction and applied to the serum sample determination (Sun and Jiao 2002; Sun et al. 2006). Girault et al. presented a quantitative method for proteins by differential pulse anodic stripping voltammetry based on the determination of Cu^{2+} released from the Biuret complexes (Schwarz et al. 2000).

In this paper, our focus is to develop a new electrochemical method as a sensitive and convenient technique for the determination of microamounts of protein based on its interaction with chromotrope 2R (CH2R). CH2R has been used as a spectrophotometric reagent for protein determination (Hu et al. 2000) and in this paper it is used as the electrochemical probe for the determination of protein. CH2R is an azo dye with its molecular shown in Fig. 1 and

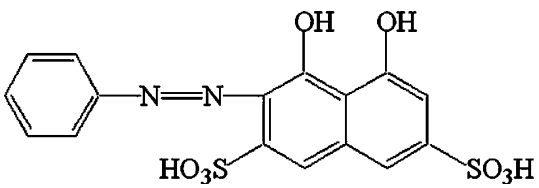

Fig. 1 The molecular structure of chromotrope 2R

the reduction process on the mercury electrode can easily take place. In the selected pH 2.5 Britton–Robinson buffer solution, the sulfonic and hydroxyl groups on the CH2R molecules can dissociate to take negative charges, while the HSA molecules (isoelectric point, $pI = 4.7$) is in positive charge, so it is easy for them to bind together by electrostatic force. The experimental results indicate that the interaction of CH2R with protein can form an electroinactive biocomplex and the reductive peak current of the reaction solution decreased. Based on the decrease of the peak current, a sensitive electrochemical method is proposed for the determination of different kinds of proteins and further applied to the determination of human serum samples with satisfactory results.

Materials and methods

Reagents

Human serum albumin (HSA, 99%, Shanghai Biomedical Products Research Institute), bovine serum albumin (BSA, 99%, Sigma), oval albumin (OVA, Sigma), lipase (Sigma) and bovine hemoglobin (BHb, Tianjin Chuanye Biotechnology Company) were used as received without further purification. The stock solutions of different proteins were prepared by directly dissolving proteins in water and stored at 0–4°C. The working solutions were obtained by diluting the stock solutions with water just before use. The human serum samples were kindly offered by the Hospital of Qingdao University of Science and Technology.

Chromotrope 2R (CH2R, Shanghai No.3 Reagent Factory) stock standard solution (1.0×10^{-3} mol/l) was prepared by dissolving 0.04680 g CH2R in 100 ml water. The operating solution of CH2R was prepared by diluting the stock solution with water properly. 0.2 mol/l Britton–Robinson (B–R) buffer solution was used to control the acidity of solution. It was prepared by mixing 12.35 g H_3BO_3, 13.55 ml 85% H_3PO_4 and 11.80 ml HOAC, diluted to 1000 ml and adjusted by 0.5 mol/l NaOH solution. CBB G-250 (Fluka) solution was prepared by the common procedure for spectrophotometric determination. All the chemicals used were of analytical grade and doubly distilled water was used throughout.

Apparatus

A Cary 50 UV–visible spectrophotometer (Varian, Australia) was used for recording absorption spectra. Cyclic voltammetry was performed on a DS model 2004 electrochemical analyzer (Shandong Dongsheng Electronic Instrument, China) with a DS-991 hanging mercury drop electrode (HMDE, Shandong Dongsheng Electronic

Instrument, China) as working electrode, a saturated calomel electrode (SCE) as reference electrode and a platinum wire as counter electrode. Linear sweep voltammetric experiments were carried out by using a model JP-303 polarographic analyzer (Chengdu Apparatus Factory, China) with a dropping mercury working electrode (DME), a SCE reference electrode and a platinum-wire counter electrode. All the pH values were measured with a pHS-25 acidity meter (Shanghai Leici Instrument Plant).

General procedure

To a dry 10 ml colorimetric tube, solutions were added in the following order: 3.0 ml of 1.0×10^{-4} mol/l CH2R, 1.0 ml of 0.2 mol/l pH 2.5 B–R buffer solution and an appropriate amount of standard protein solution or human serum sample solution. The mixtures were diluted to 10 ml with water, mixed homogeneously and allowed to stand for 15 min. The second order derivative linear sweep voltammetric peak currents (I''_p) were recorded in the potential range from 0 to −0.80 V (vs. SCE). Under the same conditions, the voltammetric peak current (I''_{p0}) of the blank solution without protein was obtained, and then the difference of peak current ($\Delta I''_p = I''_{p0} - I''_p$) was used to determine the concentration of HSA.

Results

Spectral characteristics

Figure 2 shows the absorption spectra of CH2R and its mixture with different amounts of HSA at pH 2.5 buffer solution, which was obtained by keeping the CH2R concentration and pH value constant and changing the HSA concentration. In the wavelength range from 300 to 700 nm, CH2R has a maximum absorption at 508 nm (curve 2) and HSA shows no absorbance (curve 1). On the addition of HSA, the absorbance of CH2R at 508 nm decreases with a red shift of the absorption peak position and no new peak appears (curves 3 and 4). An isobestic point is formed at 542 nm. The results indicate that the free dye species are transformed into bound one and the interactions between CH2R and HSA had taken place. According to the references (Wei et al. 1996b; Pal et al. 2000), the hypochromic and bathochromic effect is due to the changes of the structure of the dye molecule upon binding.

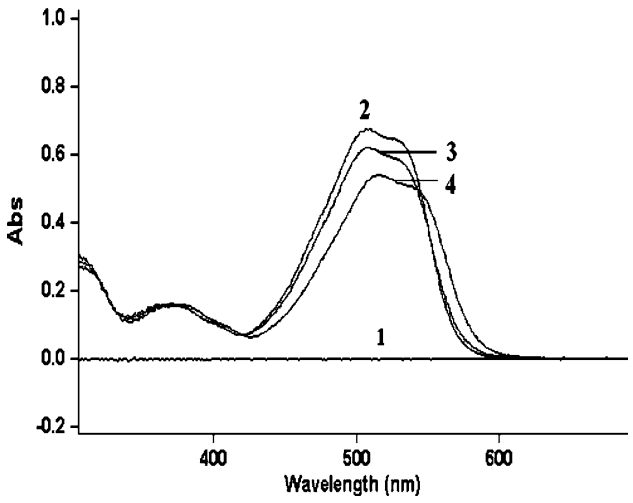

Fig. 2 Absorption spectra of CH2R–HSA reaction system. *1* pH 2.5 B–R + 100.0 mg/l HSA; *2* pH 2.5 B–R + 3.0×10^{-5} mol/l CH2R;3. 2 + 20.0 mg/l HSA;4. 2 + 30.0 mg/l HSA

Electrochemical behavior of CH2R

chromotrope 2R is an electroactive azo dye and can easily take place two electron reduction process on the Hg electrode. It has been used as a chelating agent for metals in polarographic adsorption wave. Figure 3 shows the cyclic voltammogram of CH2R under the selected conditions, which had a reductive peak at −0.34 V (vs. SCE) and did not have any oxidative peak in the potential range of 0 to −0.80 V. The results indicated that the electrochemical behavior of CH2R on Hg working electrode was irreversible at pH 2.5 B–R buffer solution. The multi-sweep cyclic voltammograms (shown in Fig. 3) indicated that with the increase of the scan cycles, the reductive peak currents decreased greatly, which exhibited the strong adsorption of CH2R on the Hg electrode. The relationship of the reductive peak potential against pH of buffer solution was tested. With the increase of buffer pH, the reductive peak potential moved negatively, and the linear relationship was got in the pH range of 1.5–5.5 with the equation as $E_p = -0.069\ \text{pH} - 0.095$ ($n = 8, \gamma = 0.995$). According to

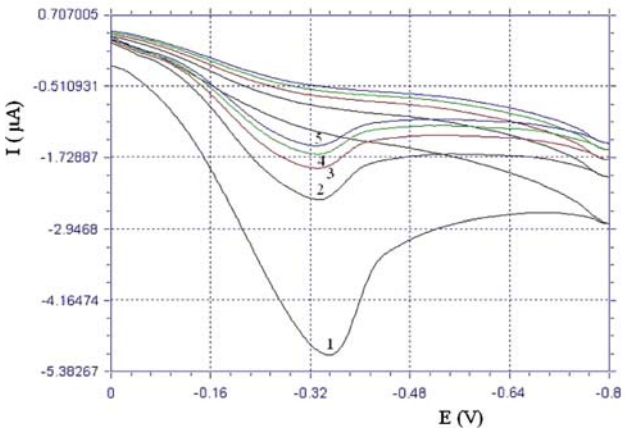

Fig. 3 Multi-sweep cyclic voltammograms of CH2R. pH 2.5 B–R + 3.0×10^{-5} mol/l CH2R; scan rate: 150.0 mV/s

the formula: $-0.059x/n = -0.069$, where n is the number of electron transfer and x is the number of hydrogen ion participating in the reaction, so the uptake of electron was accompanied by an equal number of hydrogen ion and $x = n = 2$.

Electrochemical behavior of CH2R in the presence of HSA

Under the selected conditions, the cyclic voltammograms of CH2R in the absence (curve 1) and presence (curve 2) of HSA were recorded and shown in Fig. 4. After the addition of HSA, the reductive peak current of CH2R decreased obviously, but the reductive peak potential changed a little from −0.34 to −0.32 V (vs. SCE) and no new peaks were found in the potential range. The results also showed that there were interactions between HSA and CH2R.

As for the reason for the decrease of the reductive peak current and a little change of the peak potential after the reaction of CH2R with HSA, there maybe three possible different explanations: (1) the competitive adsorption of CH2R and HSA on the mercury electrode; (2) the formation of electrochemical active supramolecular complex and the changes of electrochemical parameters; (3) the formation of electro-inactive complex and no changes of electrochemical parameters. Firstly the variation of electrochemical parameters of CH2R in the absence and presence of HSA were calculated and compared.

Because of the strong adsorption behavior and the irreversible electrochemical reductive process of CH2R on the mercury electrode, the following Laviron's equation (Laviron 1974a; Laviron 1974b; Laviron 1979) may be used to calculate the electrochemical parameters of the reductive reaction of CH2R on mercury electrode.

$$E_p = E^0 + RT/(\alpha n\mathrm{F})[\ln(\mathrm{RT}k_s)/(\alpha n\mathrm{F}v) - \ln v] \quad (1)$$

where α is the electron transfer coefficient, k_s is the standard rate constant of the surface reaction, v is the scan rate and E^0 is the formal potential.

According to Eq. 1, if the E^0 is known, E_p is in linear with ln$v$ and the value of αn can be calculated from the slope and k_s from the intercept. The E^0 value can be got from the intercept of E_p versus v plot on the ordinate by extrapolating the value of $v = 0$. According to this method, the electrochemical parameters were calculated for CH2R-HSA reaction system.

The relationship of E_p of CH2R solution with ln$v$ is shown in Fig. 5 (curve l), which is a well-defined straight line. From the slope, the αn value of CH2R can be deduced, and from the intercepts, the k_s value can be calculated, if the value of E^0 is known. The values E^0 of CH2R can be determined from the plot of E_p with v by extrapolating the line to $v = 0$ on the ordinate (shown as curve 1 of Fig. 6). The results are shown in Table 1.

Since the electrochemical behavior of CH2R–HSA mixture solution is also an irreversible adsorption electrode process, so the Laviron's equation is also used to evaluate the electrochemical parameters of the CH2R–HSA reaction solution. With the same method, the results are got from curve 2 of Figs. 5 and 6, respectively. All the results are compared and listed in Table 1. Obviously the values of αn and k_s of CH2R before and after the addition of HSA do not change. So the conclusion can be drawn that an electroinactive biocomplex is formed in the mixed solution.

The experimental results of Li et al. (Zhu and Li 1999; Zhang et al. 1999, 2002) showed that the coverage of

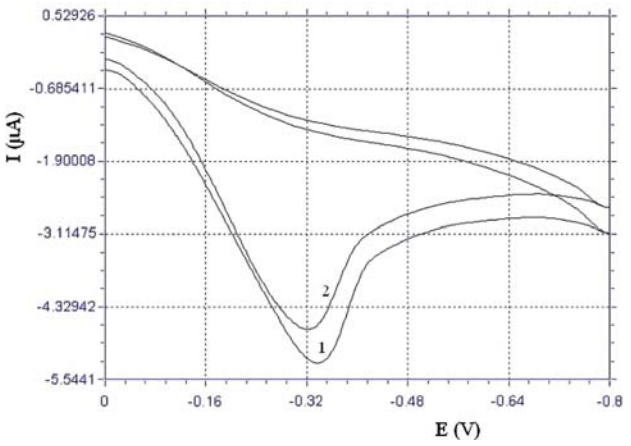

Fig. 4 Cyclic voltammograms of CH2R–HSA binding reaction system. *1* 3.0×10^{-5} mol/l CH2R in pH 2.5 B–R buffer; *2* 1 + 3.0 mg/l HSA; scan rate: 150.0 mV/s

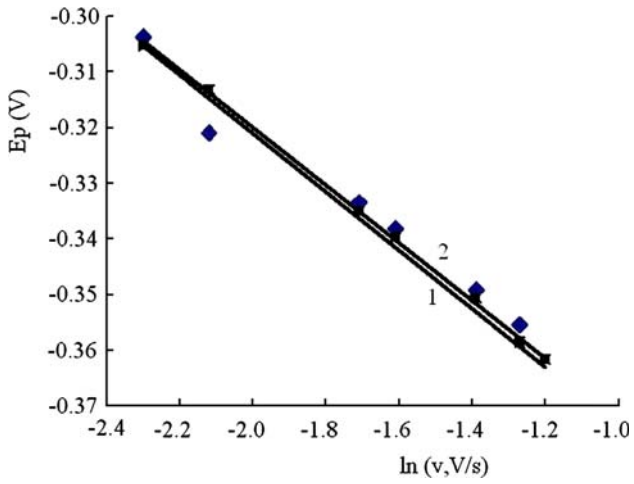

Fig. 5 Semilogarithmic dependence of the peak potential E_p on the potential scan rate (ln$v$). *1* pH 2.5 B–R buffer + 3.0×10^{-5} mol/l CH2R (*filled diamond*); *2* 1 +3.0 mg/L HSA (*filled square*)

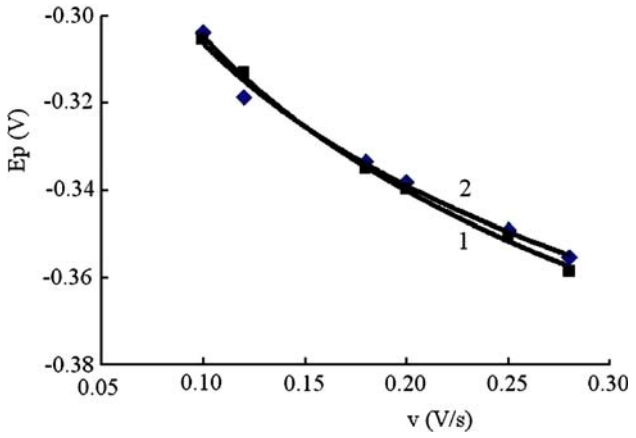

Fig. 6 Dependence of the peak potential E_p on the potential scan rate (v). *1* pH 2.5 B–R buffer + 3.0 × 10^{-5} mol/L CH2R(*filled diamond*); *2* 1 + 3.0 mg/l HSA(*filled square*)

Table 1 Electrochemical parameters of CH2R in the absence and presence of HSA

Parameters	Chromotrope 2R	Chromotrope 2R-HSA
E^0 (V)	−0.2801	−0.2805
αn	0.4899	0.4965
k_s (s^{-1})	1.1788	1.2193

mercury electrode surface was only accounted for about 10% or less of the total electrode area at lower protein concentration and shorter accumulated time, so the competitive adsorption between small molecules and protein can hardly exist. The electrochemical parameters have been calculated and the results show no significant changes, so the CH2R interacting with HSA forms an electroinactive supramolecular complex, which cannot be reduced on the Hg electrode surface and results in the decrease of equilibrium concentration of free CH2R in solution, and the decrease of the reductive peak current.

Stoichiometry of HSA–CH2R supramolecular complex

According to the method proposed by Li et al. (Li and Min 1989), the binding number and the equilibrium constant of CH2R–HSA complex could be calculated by the changes of reductive peak current. It is assumed that CH2R and HSA combined to form the single complex of HSA–xCH2R.

$$\mathrm{HSA} + x\ \mathrm{CH2R} \leftrightarrow \mathrm{HSA}{-}x\mathrm{CH2R} \tag{2}$$

The equilibrium constant is expressed as follows:

$$\beta_s = \frac{[\mathrm{HSA}{-}x\mathrm{CH2R}]}{[\mathrm{HSA}][\mathrm{CH2R}]^x} \tag{3}$$

And the following equations can be deduced:

$$\Delta I_{\max} = kC_{\mathrm{HSA}} \tag{4}$$

$$\Delta I = k[\mathrm{HSA}{-}x\mathrm{CH2R}] \tag{5}$$

$$[\mathrm{HSA}] + [\mathrm{HSA}{-}x\mathrm{CH2R}] = C_{\mathrm{HSA}} \tag{6}$$

Therefore:

$$\Delta I_{\max} - \Delta I = k(C_{\mathrm{HSA}} - [\mathrm{HSA}{-}x\mathrm{CH2R}]) = k[\mathrm{HSA}] \tag{7}$$

Introducing Eqs. 2, 4 and 6 gives:

$$\lg[\Delta I/(\Delta I_{\max} - \Delta I)] = \lg\beta_s + x\lg[\mathrm{CH2R}] \tag{8}$$

where ΔI is the difference of peak current in the presence and absence of HSA, $\Delta I_{\max}$ corresponds to the experimental value when the concentration of CH2R is extremely higher than that of HSA. C_{HSA}, [HSA], [HSA–xCH2R] are referred to the total, free and bound concentration of protein in the solution, respectively.

For minimizing the reading error the second order derivative linear sweep voltammetric data is used and the results are shown in Fig. 7. Curve 1 is the relationship of I_p'' with the concentration of CH2R, curve 2 represents the change of peak current with 100.0 mg/l HSA on varying the concentration of CH2R. Curve 3 is the differences between curve 1 and curve 2, which represents the relationship between $\Delta I_p''$ ($I_{p1}'' - I_{p2}''$) and the concentration of CH2R. From the Eq. 7 the relation of $\lg[\Delta I/(\Delta I_{\max} - \Delta I)]$ with lg[CH2R] is got as a linear with the equation as $\lg[\Delta I/(\Delta I_{\max} - \Delta I)] = 0.944\ \lg[\mathrm{CH2R}] + 4.617$ ($n = 6$, $\gamma = 0.995$). From the intercept and slope the $x = 1$ and $\beta_s = 4.14 \times 10^4$ are deduced, which indicated that a stable 1:1 complex of HSA–CH2R was formed in the selected conditions.

Eq. 7 also leads to

$$1/\Delta I = 1/\Delta I_{\max} + (1/\beta_s \Delta I_{\max})(1/[\mathrm{CH2R}]^x) \tag{9}$$

It is assumed that CH2R and HSA form a single complex and that x is 1, 2 and 3. If the plot $1/\Delta I$ versus $1/[\mathrm{CH2R}]^x$ is linear (Eq. 8), the assumed value of x is

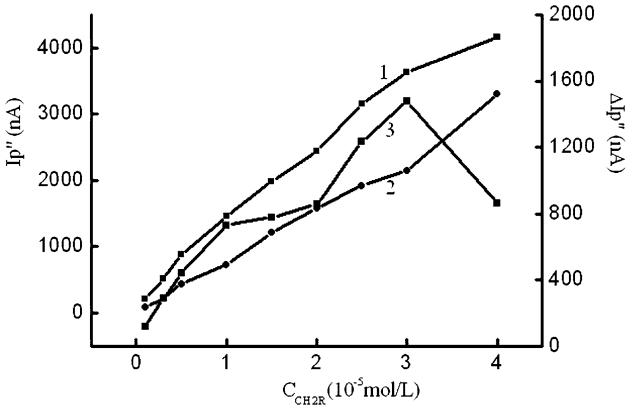

Fig. 7 Relationship between I_p'' and C_{CH2R} (*1*, *2*), $\Delta I_p''$ and C_{CH2R} (*3*). *1* C_{HSA}=0; *2* C_{HSA}=100.0 mg/l; *3* $\Delta I_p'' = I_{p1}'' - I_{p2}''$

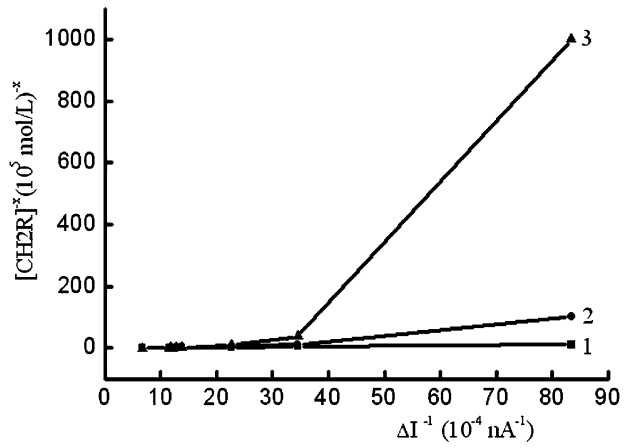

Fig. 8 The relationship of ΔI^{-1} with $[CH2R]^{-x}$. *1* $x = 1$; *2* $x = 2$; *3* $x = 3$

reasonable. The result of $x = 1$ is obtained from the experimental data (Fig. 8), which means that CH2R binding to HSA forms a 1:1 complex of HSA–CH2R.

Optimization of reaction conditions

Effects of pH and buffers

The pH of buffer greatly affects the formation of complex between HSA and CH2R. As shown in Fig. 9, $\Delta I_p''$ reaches its maximum at pH 2.5, so pH 2.5 is chosen as the optimal pH. Experiments indicate that different kinds of buffers also have effect on the system. Among the tested buffers such as B–R, HOAc–NaOAc, Na_2HPO_4–KH_2PO_4, B–R buffer is most suitable for the detection of HSA. In a final 10 ml solution, 0.5–2.0 ml of B–R buffer is suitable, so 1.0 ml B–R buffer is selected as the reaction buffer.

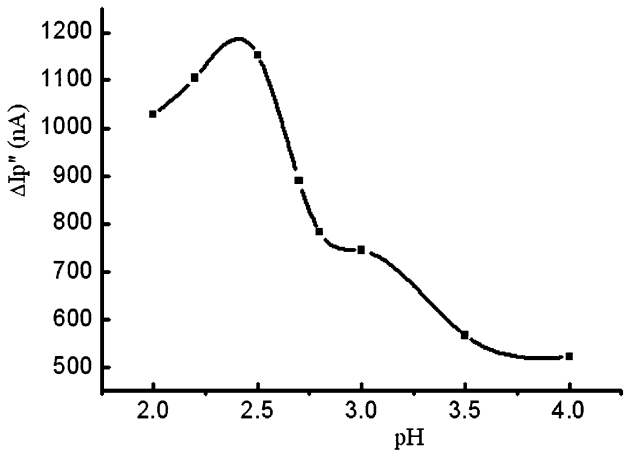

Fig. 9 The influence of pH on the decrease of peak current. 2.0×10^{-5} mol/l CH2R + 100.0 mg/l HSA

Effect of CH2R concentration

The effect of CH2R concentration is studied with 100.0 mg/l HSA. It can be found that a final CH2R concentration of 3.0×10^{-5} mol/l gives the increase of $\Delta I_p''$. When CH2R concentration is more than 3.0×10^{-5} mol/l, the $\Delta I_p''$ begins to decrease. So 3.0×10^{-5} mol/l of CH2R is recommended in subsequent studies.

Reaction time, temperature and stability

The binding reaction between HSA and CH2R occurs rapidly at room temperature and reaches the equilibrium within 5 min, and the system remains constant for at least 4 h. Therefore the system gives enough time to measure the samples. The influence of temperature on the system is also investigated at 15°C, room temperature and 30°C, respectively. The result shows that there is no obviously difference with the increase of temperature.

Optimal of instrumental conditions

The scan rate and the dropping mercury standing time of the instrument for the assay are studied. The peak current of the reaction solution is increased with the increase of the potential scan rate in the range from 200 to 800 mV/s and reaches maximum at 800 mV/s. The dropping mercury standing time for the assay is also optimized and selected at 14 s.

Effect of coexisting substances

As listed in Table 2, the influences of coexisting substances such as various common metal ions and amino acids are tested with 10.0 mg/l HSA, which were premixed with interfering substances. From the Table 2, it can be seen that few of them interfere with this assay and good selectivity can be obtained in this method except L-Cysteine, which may be due to the electrochemical activity of L-Cysteine on the mercury electrode disturbed the determination.

Calibration curve and detection limit

Under the optimal conditions the linear regression equations and other analytical parameters for different kinds of proteins such as HSA, BSA, BHb, OVA and Lipase are summarized in Table 3. It can be seen that different results were got, which may be due to the different isoelectric points of protein. At the same time the size, weight, shape and electric charges of the molecules are also different with each other. So the electrochemical responses for various proteins are different, which indicate a protein-to-protein variability dependent.

Table 2 Effect of interference substances on the determination of 10.0 mg/l HSA

Coexisting substance	Concentration (mg/l)	Relative error (%)	Coexisting substance	Concentration (μmol/l)	Relative error (%)
L-Valine	0.05	−5.62	Mg^{2+}	200.0	2.44
L-Glutamine	0.05	1.02	Ba^{2+}	200.0	0.98
L-Arginine	0.05	3.27	Ni^{2+}	200.0	0.73
L-Leucine	0.05	2.18	Zn^{2+}	200.0	5.51
L-Serine	0.05	6.76	Mn^{2+}	200.0	−1.05
L-Cysteine	0.05	−18.31	NH_4^+	200.0	−4.33
L-Tryptophan	0.05	2.26	Co^{2+}	200.0	−0.75
L-Lysine	0.05	5.23	K^+	200.0	−0.54

Table 3 Analytical parameters for proteins by linear sweep voltammetry

Protein	Linear range (mg/l)	Linear regression equation ($\Delta I_p''$, nA; C, mg/l)	Detection limit (3σ, mg/l)	Correlation coefficient (γ)
HSA	1.0–80.0	$\Delta I_p'' = 11.30\ C + 42.95$	1.0	0.997
BSA	2.0–80.0	$\Delta I_p'' = 11.37\ C + 40.10$	2.0	0.995
BHb	4.0–70.0	$\Delta I_p'' = 9.64\ C + 45.14$	4.0	0.995
OVA	5.0–100.0	$\Delta I_p'' = 12.39\ C + 39.84$	5.0	0.994
Lipase	5.0–80.0	$\Delta I_p'' = 8.23\ C + 31.68$	5.0	0.995

The comparison between this method and some existing electrochemical methods for the protein determination was summarized in Table 4. It can be seen that the proposed method had a larger linear range though the sensitivity of this method was not higher than that of single sweep polargraphy (Luo et al. 2003). On the other hand, the proposed method has the advantages such as low cost apparatus, easy to carry out and so on. Therefore, this method is valuable for routine measurements.

Table 4 Comparison of this method with other reported electrochemical methods

Method	Probe	Linear range (mg/l)	LOD (mg/l)	Reference
Linear sweep voltammetry	ARS	2.0–40.0	1.0	Jiao 2002
Cyclic voltammetry	ACBK	3.0–20.0	1.93	Sun 2006
Cyclic voltammetry	TPPS	2.6–32.5	2.6	Li 1999
Single sweep polarography	Co^{2+}	0.005–20.0	0.002	Luo 2003
Linear sweep voltammetry	CH2R	1.0–80.0	1.0	This paper

ARS Alizarin Red S, *TPPS* tetraphenylporphyrin tetrasulfonate, *ACBK* Acid Chrome Blue K, *CH2R* chromotrope 2R

Sample determination and recovery test

The human serum samples were kindly provided by the Hospital of Qingdao University of Science and Technology and diluted 10,000 fold with doubly distilled water as the total protein samples. As indicated in most references for protein assay (Guo et al. 2000a, b; Li et al. 2000; Dong et al. 2002), human serum albumin (HSA) is commonly chosen as the standard for the detection. Huang (1998) had demonstrated that when the content of γ-globulin (γ-IgG) is lower than 30% for a mixture containing HSA and γ-IgG, an acceptable determination error of 4.8% appeared. So the HSA can be used as the standard for the real human serum samples detection.

Under the recommended conditions, the present method was applied to the determination of the content of the total protein in human serum and the results were compared with the traditional CBB G-250 spectrophotometric method. Table 5 summarizes the determination results and good agreements are got with the recovery in the range of 98.55–104.81%, indicating that the total protein contents measured by the proposed method was in good agreement with CBB G-250 method. So this method is reliable and practical in clinical application.

Table 5 The results for the determination of HSA in human serum samples

Sample	This method (g/l)	RSD (%, $n = 3$)	Recovery (%)	CBB G-250 method (g/l)	RSD (%, $n = 3$)
1	63.51	3.26	102.92	63.70	2.54
2	79.38	4.58	104.81	84.20	2.62
3	100.86	2.28	98.55	104.00	4.08

Discussion

This paper describes a new method with CH2R as an electrochemical probe for the determination of HSA. CH2R has a sensitive linear sweep voltammetric peak at −0.34 V (vs. SCE) and the presence of HSA causes a decrease of reductive peak current. The binding of HSA with CH2R results in the formation of a new electrochemically non-active complex by electrostatic interaction of negatively charged CH2R with positively charged HSA. Based on the decrease of the peak currents, the proposed method can be further applied to the determination of micro amount of HSA with good selectivity and high sensitivity. This method can also be used for other different kinds of proteins such as BSA, BHb, OVA and human serum samples with satisfactory results, which shows the potential of practical application.

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