Bioelectrochemistry 77 (2009) 26–30

Contents lists available at ScienceDirect

Bioelectrochemistry

journal homepage: www.elsevier.com/locate/bioelechem

Electrochemistry of the interaction of furazolidone and bovine serum albumin

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ARTICLE INFO

Article history:
Received 22 February 2009
Received in revised form 23 May 2009
Accepted 25 May 2009
Available online 30 May 2009

Keywords:
Furazolidone
Cyclic voltammetry
Bovine serum albumin
Determination
Interaction

ABSTRACT

The electrochemical behavior of the interaction of furazolidone (Fu) with bovine serum albumin (BSA) was investigated by cyclic voltammetry and differential pulse voltammetry at a glassy carbon electrode. Fu shows an irreversible reduction at −0.34 V in pH 4.0 Britton–Robinson buffer (B–R) buffer–10% DMF solution. After the addition of BSA into the Fu solution, the reductive peak currents decreased without any significant shift of the peak potential and the appearance of new peaks. The electrochemical parameters of the interaction system were calculated in the absence and presence of BSA. This electrochemical method was further applied to the determination of BSA samples and the results were in good agreement with the traditional cellulose acetate electrophoresis. The linear dynamic range was between 10.0 and 80.0 mg l^{-1}. The detection limit was 7.6 mg l^{-1} and the recoveries were obtained from 97.0% to 104.0%.

1. Introduction

Bovine serum albumin (BSA) is a single-chain 582 amino acid globular nonglycoprotein cross-linked with 17 cystine residues (8 disulfide bonds and one free thiol). As most abundant proteins, BSA plays an important role in the transport and deposition of a variety of endogenous and exogenous ligands in blood. BSA has a wide range of physiological functions involving the binding, transport, and delivery of fatty acids, bilirubin, porphyrins, thyroxine, tryptophan and steroids [1–4]. It is home to specific binding sites for metals, pharmaceuticals, and drugs. The binding ability on the interaction of drug with protein will significantly affect the apparent distribution volume of the drugs and also affect the elimination rate of drugs in most cases.

A key process in the development of new drugs is elucidation of the nature of the interaction between the drug molecule and the target protein. Such knowledge then makes it possible to make systematic structure modifications of the drug molecule to optimize the interaction.

There are a variety of techniques currently available for obtaining information about drug–protein interactions such as the measurement of kinetics and binding affinities. Two traditional methods, X-ray diffraction and NMR spectroscopy, employed to obtain structural information of proteins and drug–protein complexes. Both of these methods have disadvantages: X-ray diffraction requires the preparation of a crystal, which can be time consuming or even impossible; and NMR spectroscopy is not easily applied to larger proteins of more than a few hundred amino acids. Other analytical techniques that can be applied to proteins in solution are spectrophotometry, chemiluminescence, resonance light scattering [5–8].

Compared with spectroscopic methods, electrochemical assay is simple, easily implemented, and has low-cost and fast response. On the other hand, the interpretation of electrochemical data can contribute to elucidation of the interaction of drug with biomolecules. Li et al. [9–11] had studied the interaction of DNA with some electrochemical active compounds, such as 9,10-anthraquinone or porphyrins. However, by comparison with other techniques, the electrochemical methods have been still largely overlooked.

The drug furazolidone (Fu) belongs to the group of nitrofuran antibacterial agents, which has been widely applied as a feed additive in food-producing animals such as cattle, swine, poultry, and cultured fish and shrimps and for prophylactic and therapeutic treatment of diseases causally linked to bacteria or protozoa [12]. Furazolidone forms protein-bond metabolite in vivo [13] leading to a significant decrease in plasma levels of the parent compound [14] and may persist for considerable periods in the tissue of animals after treatment. Although the use of furazolidone is prohibited in numerous countries, there are indications of its illegal use. At present, available data concerning the identity and toxic potential of compounds released from bound furazolidone residues are still insufficient [15].

Investigations of metabolism of radioactive labeled furazolidone had shown that the drug was able to form tissue-bound residues. These metabolites included an intact side-chain that could act as marker residues for furazolidone—namely 3-amino-2-oxazolidone [14]. This marker can be released by mild acid hydrolysis. It was found that the interaction of Fu with BSA could be studied by the voltammetric method according to the changes of peak current of the Fu–BSA reaction in a relatively acidic solution. Thus in this paper electrochemical studies of the interaction of Fu in the presence of BSA at the glassy carbon electrode are reported. In our previous works [16–18] we have reported the voltammetric behavior of nitro radical formation of

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1567-5394/$ – see front matter © 2009 Published by Elsevier B.V.
doi:10.1016/j.bioelechem.2009.05.011

Fu and its kinetic parameters. In continuation of our interest in the electrochemistry of biological active organic compounds [16–20], we developed an electrochemical method as a sensitive and convenient technique for investigation of binding mechanism between Fu and BSA. Additionally this electrochemical behavior was applied to the determination of BSA.

2. Experimental

2.1. Reagent

Bovine serum albumin (BSA, Sigma) was commercially purchased and accepted for use without further purification. The 1.0 g l^{-1} stock solution of BSA was prepared by directly dissolving it in twice distilled water and was stored at 4 °C. The working solutions were obtained by diluting the stock solution with water. Furazolidone was obtained from Sigma. Stock solutions were prepared by dissolving the appropriate amount of Fu in B–R buffer (pH 4.0)–10% DMF solution. A stock B–R buffer solution 0.04 M with respect to boric, ortho-phosphoric and acetic acid was used to control the pH of solutions tested. All other reagents used were of analytical reagent grade.

2.2. Apparatus

Electrochemical measurements were carried out with Metrohm model 746 VA trace analyzer connected to a 747 VA stand. The working electrode was a glassy carbon electrode (GCE) (2 mm diameter). Before use the working electrode was sequentially polished with graded 10 μM alumina powder, and rinsed with doubly distilled water. A platinum wire and a commercial saturated Ag/AgCl from Metrohm were used as the auxiliary and reference electrodes respectively. The scan rate in cyclic voltammetry was 100 mV s^{-1}, with exception of the experiments in which the influence of this variable was studied. All solutions were purged with pure nitrogen for 10 min before the voltammetric runs.

3. Results and discussion

3.1. Electrochemical behavior of Fu

Fu is an electrochemically active nitro compound with a nitro group in its molecular structure, so it can be easily reduced at the glassy carbon electrode.

In our previous reports, we showed that voltammetric reduction of nitro group depends significantly on the solvent system and pH of medium [16–18]. In protic media (low percent of DMF and acidic pH media) Fu is reduced at two reductive potentials at −0.34 and −1.25 V

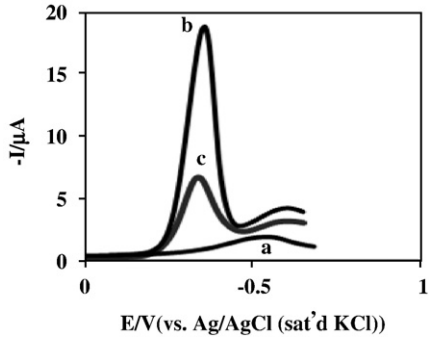

Fig. 1. The differential pulse voltammograms of (a) pH 4.0 B–R buffer–10% DMF solution, (b) 1.0 mM Fu in the absence and (c) 1.0 mM Fu in the presence of 60 mg l^{-1} BSA at glassy carbon electrode.

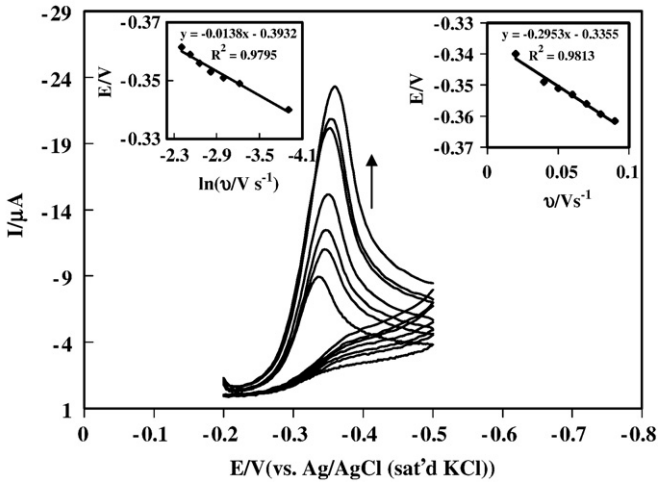

Fig. 2. Cyclic voltammograms of 1.0 mM Fu at various scan rates. Inset right, the linear plot of E_p vs. υ ($y=-0.2953x$–0.3355, $R^2=0.981$). Inset left, the linear plot of E_p vs. ln (υ/V s^{-1}) ($y=-0.0138x$–0.3932, $R^2=0.979$). pH 4.0 B–R buffer–10% DMF solution at glassy carbon electrode. Scan rates: 20, 40, 50, 60, 70, 80, and 90 mV s^{-1}.

due to the following well known mechanism for aromatic nitro compounds.

$$RNO_2 + 4e + 4H^+ \rightarrow RNHOH + H_2O \quad (1)$$

$$RNHOH + 2e + 2H^+ \rightarrow RNH_2 + H_2O \quad (2)$$

However, in this work our current interest is devoted to the study of the interaction of BSA with first reductive peak (0.34 V) corresponding to the reduction of nitro group to hydroxylamine derivatives. Fig. 1 shows the differential pulse voltammograms of Fu in the absence and presence of BSA at glassy carbon electrode in B–R buffer (pH 4.0)–10% DMF solution. On the addition of BSA, a decrease in the cathodic peak current without any significant shift in peak potential was observed. Two factors may be considered for decreasing of the reductive peak current. First, is the competitive adsorption between the Fu and BSA on the GCE and second, the formation of electroinactive complex without the changes of electrochemical parameters. The competitive adsorption factor can be excluded by recording cyclic voltammogram of Fu in the excess of BSA. The peak current of Fu did not disappear completely with the increase of the concentration of BSA, which was not the character of competitive adsorption. The interaction of some biomolecules such as hemoglobin, albumin and DNA showed that in such lower concentration of protein and shorter accumulation time, the coverage of electrode surface was only accounted for about 10% of the total area of electrode, so the competitive absorption between small molecules with protein can hardly exist [9,21]. Thus the decrease in the peak current without any changes in electrochemical parameters is a good indication of BSA.Fu_m electroinactive complex formation (m is Hill coefficient), which results in the decrease of equilibrium concentration of Fu in solution.

The multi-sweep cyclic voltammograms of BSA.Fu_m in B–R buffer (pH 4.0)–10% DMF solution at the glassy carbon electrode indicated that the cathodic peak current decreases in second cycle, which means that the peak current had adsorption behavior. The effect of scan rate on peak current showed that the plot of current vs. υ is linear for a limited short scan rate, indicating that electrochemical process virtually belongs to an adsorption-controlled process. However, the process is also diffusion-controlled due to the linear plot of peak current versus $\upsilon^{1/2}$ at higher scan rates (υ>300 mV s^{-1}) [22–24]. From the observed experiments it can be concluded that the electrochemical process virtually belongs to a mixture of diffusion–adsorption-controlled process, depending on the scan rate.

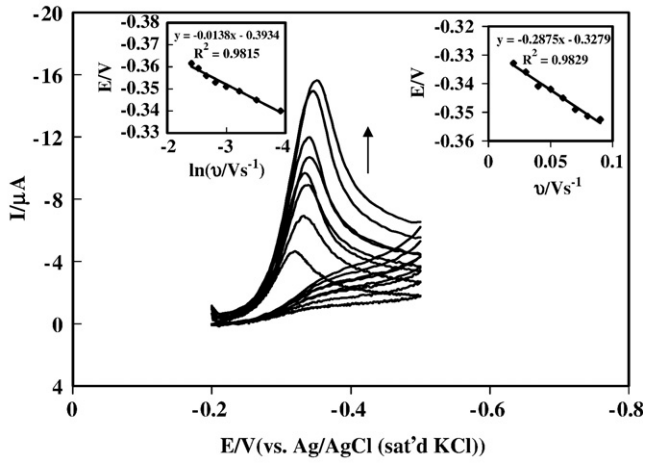

Fig. 3. Cyclic voltammograms of 1.0 mM Fu + 20 mg l^{-1} BSA. Inset right, the linear plot of E_p vs. υ (y = -0.2875x-0.3279, R^2 = 0.983). Inset left, the linear plot of E_p vs. ln(υ/V s^{-1}) (y = −0.0138x–0.3934, R^2 = 0.982). pH 4.0 B–R buffer–10% DMF solution at glassy carbon electrode. Scan rates: 20, 30, 40, 50, 60, 70, 80, and 90 mV s^{-1}.

In order to study the electrochemical parameters of the reduction of Fu, the cyclic voltammograms of Fu in B–R buffer (pH 4.0)–10% DMF solution in the absence (Fig. 2) and presence of BSA (Fig. 3) were recorded at low scan rate. As it is shown by increasing the scan rate, the peak potential is shifted to more negative potential. Because of the irreversible surface electrode process of the reduction of Fu, the following Laviron's equation [24] was used to estimate αn and standard rate constant of the surface reaction, k_s:

$$E_p = E^{0'} + (RT/\alpha F)\ln\left[(RTk_s/\alpha F)/\mathrm{V\ s^{-1}}\right] - (RT/\alpha F)\ln\left[\upsilon/\left(\mathrm{V\ s^{-1}}\right)\right] \quad (3)$$

where R is the universal gas constant (8.314 J K^{-1} mol^{-1}), T is Kelvin temperature, F is the Faraday constant (96487 C mol^{-1}), α is the electron transfer coefficient, k_s is the standard rate constant of the surface reaction, υ is the scan rate and $E^{0'}$ is the formal potential. k_s and αn values can be concluded from the intercept and slope of the linear plot of E_p with respect to ln(υ/V s^{-1}), if the $E^{0'}$ is known.

The $E^{0'}$ value in the absence and presence of BSA can be deduced from the intercept of E_p vs. υ plot on the ordinate by extrapolating the line to υ = 0.0 V s^{-1} (Figs. 2 and 3, insets right). By knowing $E^{0'}$, and graphical representations of E_p vs. ln(υ/V s^{-1}) for Fu in the absence and presence of BSA (Figs. 2 and 3, insets left), the α and k_s values were obtained from the slope and intercept, respectively. The results are shown in Table 1. The value of α of about 2 may be due to a chemical step preceded by the uptake of two electrons in quasi-equilibrium [25,26].

According to the method of Qu et al. [27], it is assumed that BSA and Fu only produced a single complex BSA.Fu_m according to the reaction scheme:

$$\mathrm{BSA} + m\mathrm{Fu} \leftrightarrow \mathrm{BSA.Fu}_m. \quad (4)$$

The height of peak current of the differential pulse voltammogram is most sensitive to the equilibrium constant of BSA.Fu_m complex. The decreasing value of the peak current, I, is proportional to the concentration

Table 1
The k_s and α values of Fu in the absence and presence of BSA.

Parameters	Fu	Fu + BSA
E^0 (V)	−0.335	−0.328
α	2.170	2.130
k_s (s^{-1})	1.940	0.650

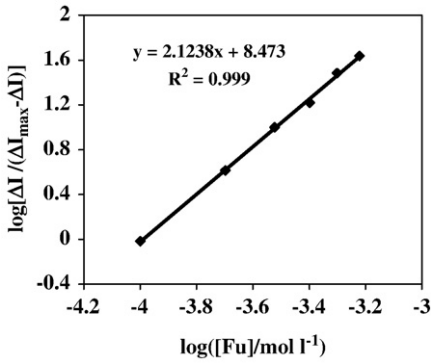

Fig. 4. The linear plot of log($[(\Delta I/(\Delta I - \Delta I_{max})]$) vs. log([Fu]/$M$) ($y$ = 2.1238x–8.473, R^2 = 0.999). Fu concentrations: 0.10–0.60 mM. pH 4.0 B–R buffer–10% DMF solution at glassy carbon electrode, 20 mg l^{-1} BSA.

of BSA.Fu_m complex. In terms of the all-or-none (Hill) cooperative of multiple ligand binding [28,29], the fraction of BSA to which furazolidone is bound as BSA.Fu_m, relative to the total BSA concentration in the supporting electrolyte $[BSA]_0 = [BSA.Fu_m]_{max}$ is given by:

$$f = [\mathrm{BSA.Fu}_m]/[\mathrm{BSA.Fu}_m]_{max} = [\mathrm{Fu}]^m/([\mathrm{Fu}]^m + K_d^m) \quad (5)$$

and

$$K_d^m = [\mathrm{Fu}]^m(1-f)/f \quad (6)$$

where $[BSA.Fu_m]_{max}$ is the maximum concentration of complexed binding sites, [Fu] is the concentration of free furazolidine, K_d is the dissociation equilibrium constant and m is the Hill coefficient.

Note that $K_d = [Fu]_{0.5}$, i.e., at half occupation. The association constant K_a is given by the reciprocity: $K_a = K_d^{-1}$. It is not advisable to use the overall constant $K = (K_a)^m$, which would have the physically meaningless dimension of M^{-m}.

The interaction partners for Fu are the binding sites of BSA. Mass conservation dictates that:

$$[\mathrm{BSA}] = [\mathrm{BSA.Fu}_m]_{max} - [\mathrm{BSA.Fu}_m] \quad (7)$$

$$[\mathrm{Fu}] = [\mathrm{Fu}]_0 - m[\mathrm{BSA.Fu}_m] \quad (8)$$

where $[Fu]_0$ is the total concentration of furazolidone. According to the Ilkovic equation of irreversible electrode process [23]:

$$I = k.[\mathrm{Fu}] \quad (9)$$

where k is a constant. In line with this relationship the current difference ΔI is defined as:

$$\Delta I = k([\mathrm{Fu}_0] - [\mathrm{Fu}]) \quad (10)$$

Insertion of Eqs. (8) and (9) into Eq. (10) yields:

$$\Delta I = k([\mathrm{Fu}_0] - [\mathrm{Fu}]) = k\,.\,m.[\mathrm{BSA.Fu}_m] \quad (11)$$

$$\Delta I_{max} = k.m.[\mathrm{BSA.Fu}_m]_{max}. \quad (12)$$

From Eq. (6), we obtain that:

$$\log[f/(1-f)] = m\log\left(K_a/M^{-1}\right) + m\log([\mathrm{Fu}]/M). \quad (13)$$

Insertion of Eqs. (11) and (12) into Eq. (13) yields:

$$\log[\Delta I/[\Delta I_{max} - \Delta I] = m\log\left(K_a/M^{-1}\right) + m\log([\mathrm{Fu}]/M). \quad (14)$$

Table 2
Effect of foreign substances on the determination of 20 mg l^{-1} BSA.

Foreign substance	Concentration (mM)	Change ΔI (%)
[Ca(II)$(NO_3)_2$]	1.0	0.23
[Ba(II)$(NO_3)_2$]	1.0	3.08
[Cu(II)$(SO_4)_2$]	1.5	−1.81
[Co(II)$(NO_3)_2$]	8.6	2.31
[Fe(II)$(NO_3)_2$]	6.1	1.46
[Ni(II)$(SO_4)_2$]	1.0	3.49
[L-arginine]	0.3	−0.89
[D-tryptophan]	0.2	0.83
[L-glutamine]	0.3	3.53
[L-leucine]	0.4	1.77
[Citronensaure-1-hydrate]	2.5	2.08
[D(+)glucose]	5.5	−2.98

Table 3
Results obtained in the determination of BSA in synthetic samples.

Sample	BSA added (mg l^{-1})	BSA found (mg l^{-1})	RSD (%) ($n=5$)	Recovery (%)
1	5	5.10	1.90	102.10
2	7	6.84	2.20	97.74
3	9	9.27	1.69	130.10

Table 4
The results for the determination of BSA in serum samples obtained from Holstein cows.

Sample	This method (mg l^{-1})	Cellulose acetate electrophoresis (mg l^{-1})
1	10.37	9.75
2	11.24	11.50
3	17.21	17.25
4	15.44	15.25

By keeping the BSA concentration at 20 mg l^{-1} and varying the concentration of Fu, the plot of $\log[\Delta I/(\Delta I_{max}-\Delta I)]$ as a function of $\log([Fu]/M)$ is linear with the slope m. Fig. 4 shows the plot of $\log[\Delta I/(\Delta I_{max}-\Delta I)]$ vs. $\log([Fu]/M)$ from the recording differential pulse voltammograms of Fu at varying concentration (0.10–0.60 mM) and constant concentration of BSA (20 mg l^{-1}). The results of $m=2.1\pm0.1$ and $K_a=1.67\pm0.08\times10^4$ M^{-1} were obtained in the concentration range of the applied furazolidone. The stoichiometry of the cooperative furazolidone binding is thus at least 2 per binding site unit.

3.2. Determination of BSA

3.2.1. The optimal conditions

The effect of the reaction pH was tested at various pH and pH 4.0 was used as the optimum pH throughout. Different addition orders of reagents were tested and no obvious differences were observed. The pulse amplitude was also studied and the optimum value of 50 mV was selected. The effect of Fu concentration on the system was studied with 20 mg l^{-1} BSA. The results showed that ΔI increased firstly and then become constant with increasing the Fu concentration. Therefore the concentration of 1.0 mM was employed for further assay. The effect of the percent of DMF was investigated and the results showed no significant changes in the current peak with varying the DMF percent.

3.2.2. The effect of foreign substance

Under the optimum conditions, the influence of the foreign substance was tested when the concentration of BSA was 20 mg l^{-1}. The interference levels are summarized in Table 2.

3.2.3. Calibration curve and analysis of sample

Differential pulse voltammetric technique was applied to determine the concentration of BSA. Under optimal conditions, a linear relationship was obtained between the difference of current height of Fu in the absence and presence of BSA (ΔI) and the concentration of BSA.

The linear dynamic range was within the BSA concentration from 10 to 80 mg l^{-1} (Fig. 5). For the regression plot of ΔI versus BSA concentration, the slope is 0.158 μA/mg l^{-1}, the intercept is 0.0, and the coefficient of correlation (r^2) is 0.981. To characterize the reproducibility of BSA, repetitive measurements were carried out in 20 mg l^{-1} of BSA. The results of 10 successive measurements show a relative standard deviation of 2.6% mg l^{-1}. The detection limit (3σ) for BSA is found to be 7.60 mg l^{-1}.

The validity of the method was demonstrated by the analysis of both synthetic and real samples. Synthetic samples were prepared based on the interferences of foreign substances and BSA. The analytical results of the determination of BSA in synthetic samples are summarized in Table 3.

The present method also demonstrates the capability to detect and quantify BSA in biological samples, such as serum samples obtained from Holstein cows. The results in Table 4 showed close agreement to those obtained by our method and the cellulose acetate electrophoresis method.

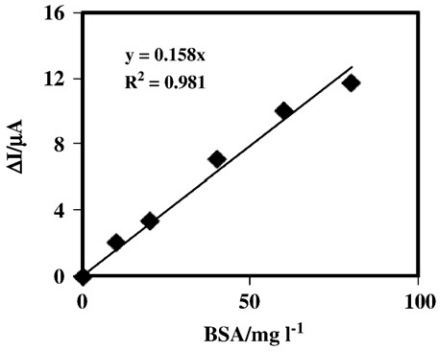

Fig. 5. The plot of ΔI vs. BSA concentration. pH 4.0 B–R buffer–10% DMF solution at glassy carbon electrode.

4. Conclusion

The interaction of BSA and Fu was studied by electrochemistry. The binding mechanism was preliminarily discussed by calculating the binding constant and Hill coefficient. The proposed method was applied to the determination of BSA. This method is simple, rapid, reliable, and cost-effective and can be used for determination of BSA in serum sample.

Acknowledgment

The authors express their gratitude for financial support from the Department of Chemistry of Alzahra University.

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