ELECTRODE REACTION OF ADRIAMYCIN INTERPRETED AS TWO CONSECUTIVE ELECTRON TRANSFERS WITH STABILIZATION OF THE INTERMEDIATE

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In cyclic voltammetry of adriamycin (doxorubicin hydrochloride) adsorbed on graphite electrode surface, the response of quinone/hydroquinone redox centers is split into two pairs of peaks because of inactivation of semiquinone intermediates by the hydrogen bonds.
Keywords: Adriamycin; Doxorubicin; Surface ECE mechanism; Quinone/hydroquinone redox couple; Cyclic voltammetry; Graphite electrode; Electroreduction; Electrochemistry.

A molecule containing a number of identical, noninteracting, electroactive centers exhibits a current-potential response having the same shape as the response of the molecule with a single redox center, but the current is enhanced by the presence of additional centers[1–6]. If the centers are connected with π-conjugated bonds, the molecule may exhibit mixed-valence states and the response in cyclic voltammetry may split into several peaks[7–10]. Two peaks may also appear if each electroactive center can exchange two electrons[11,12]. Generally, the two-electron electrode reaction occurs through two consecutive steps with a more or less stable intermediate (the simple EE mechanism)[13–16]. The homogeneous chemical reaction can be coupled to any step of the EE mechanism[17–20]. In this communication the electrode reaction of adsorbed adriamycin is interpreted as two consecutive electron transfers with stabilization of the intermediate.

Adriamycin is the commercial name for doxorubicin hydrochloride which consists of the tetracenequinone ring system linked through a glycosidic bond to an amino sugar, daunosamine (Chart 1). Doxorubicin is a cytotoxic anthracycline antibiotic isolated from cultures of *Streptomyces peucetius* var. *caesius*[21]. Adriamycin is electroactive substance. The redox centers are quinone and hydroquinone moieties[22–31]. The molecule is a weak base: pK_a = 8.2 ± 0.1 for both OH groups in hydroquinone ring 2,

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pK_a = 9.0 ± 0.2 for NH_3^+ group of daunosamine moiety, and pK_a > 14 for the third and the fourth OH groups in fully reduced form of adriamycin[32,33]. Also, adriamycin is strongly adsorbed to the surface of mercury, graphite, carbon paste and glassy carbon electrodes[22,23,26–29,31]. Quinone and hydroquinone are well known redox couple[34]. In a buffered water solution, its polarographic response is a single, reversible, two-electron wave[35,36]: $Q + 2\,e + 2\,H^+ \rightleftarrows H_2Q$.

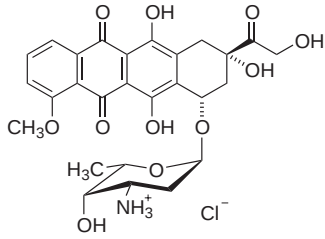


CHART 1
Structural formula of adriamycin

EXPERIMENTAL

Adriamycin (doxorubicin hydrochloride, 2 mg/ml in 0.9% NaCl, pH 3 (HCl) aqueous solution) was obtained from the Cell Pharm GmbH (Hannover, Germany) and used without further purification. KNO_3 and 0.1 M buffer solution (pH 4.65, sodium citrate–HCl), both Kemika, Zagreb, analytical grade, were used as received. Water was demineralized by ionic exchangers Millipore Milli Q until its resistivity was 18.2 MΩ cm. Supporting electrolyte was prepared by adding 1 ml of buffer solution to 9 ml of 1 M KNO_3.

The voltammetric measurements were performed with a multimode polarograph Autolab 30 (EcoChemie, Utrecht, The Netherlands). The working electrode was a spectral-grade paraffin-impregnated graphite rod (diameter 5 mm, length 5 cm). The Pt wire was an auxiliary electrode and Ag|AgCl|3 M KCl (Metrohm) was a reference electrode (E = 0.208 V versus standard hydrogen electrode).

On paraffin-impregnated graphite electrode (PIGE), the adsorbed layer of adriamycin was prepared by immersing PIGE into a stock solution of adriamycin for 3 min. Then the electrode was rinsed with water and transferred into the electrochemical cell filled with a pure supporting electrolyte. This method is superior to the accumulation in situ[28,29,31].

THEORETICAL

A reversible, two-electron electrode reaction with inactivation of intermediate is considered.

$$(Ox)_{ads} + e \rightleftarrows (Int)_{ads} \qquad (1)$$

$$(\mathrm{Int})_{ads} \rightleftarrows (\mathrm{Inact})_{ads} \tag{2}$$

$$(\mathrm{Int})_{ads} + e \rightleftarrows (\mathrm{Red})_{ads} \tag{3}$$

It is assumed that all species are irreversibly adsorbed on the working electrode surface.

$$t = 0: \quad \Gamma_{Ox} = \Gamma^* = 2\,\Gamma_{adriamycin} \tag{4}$$

$$\Gamma_{Int} = \Gamma_{Inact} = \Gamma_{Red} = 0 \tag{5}$$

$$t > 0: \quad \Gamma^* = \Gamma_{Ox} + \Gamma_{Int} + \Gamma_{Inact} + \Gamma_{Red} \tag{6}$$

$$\Gamma_{Ox} = \Gamma_{Int} \exp(\varphi_1) \tag{7}$$

$$\varphi_1 = (F/RT)(E - E^{o}{}_1) \tag{8}$$

$$\Gamma_{Int} = \Gamma_{Red} \exp(\varphi_2) \tag{9}$$

$$\varphi_2 = (F/RT)(E - E^{o}{}_2) \tag{10}$$

$$K = \Gamma_{Inact}/\Gamma_{Int} \tag{11}$$

$$\Gamma_{Ox} = \Gamma^* + \int_0^t (I_1/FS)\,\mathrm{d}\tau \tag{12}$$

$$\Gamma_{Red} = -\int_0^t (I_2/FS)\,\mathrm{d}\tau \tag{13}$$

$$I = I_1 + I_2 \tag{14}$$

Here, Γ_{Ox}, Γ_{Int}, Γ_{Inact} and Γ_{Red} are surface concentrations of the reactant, intermediate, inactivated intermediate and product, respectively. Initial concentration Γ^* is twice as high as the surface concentration of adriamycin. K is the stability constant of inactivated intermediate. $E^{o}{}_1$ and $E^{o}{}_2$ are stan-

dard potentials of the first and the second charge transfer, respectively. S is the electrode surface area, F is Faraday constant and I_1, I_2 and I are currents.

Equations (*12*) and (*13*) were solved by numerical integration. Dimensionless current $\Phi = I/FS\Gamma^*(F/RT)|v|$ was calculated for cyclic voltammetry.

RESULTS AND DISCUSSION

A cyclic voltammogram of adriamycin adsorbed on PIGE is shown in Fig. 1. Two pairs of peaks, with median potentials –0.682 and +0.344 V, can be observed. In the acidic medium both median potentials are linear functions of pH, with the slope –59 mV [27]. Formally, these responses correspond to the reduction of quinon (1) and oxidation of hydroquinone (2) centers, respectively[24,31]. However, in the fully oxidized and fully reduced states adriamycin contains two identical quinone and hydroquinone moieties, respectively. So, one would expect a single, two-electron cyclic voltammogram. The observed response can be explained by assuming that semiquinone intermediates are stabilized by hydrogen bonds. The proposed mechanism is shown in Scheme 1. A simple theoretical model is developed to verify this hypothesis. Considering that there was no dissolved adriamycin in the electrolyte, this experiment shows that all three redox forms of adriamycin are strongly adsorbed on the electrode surface.

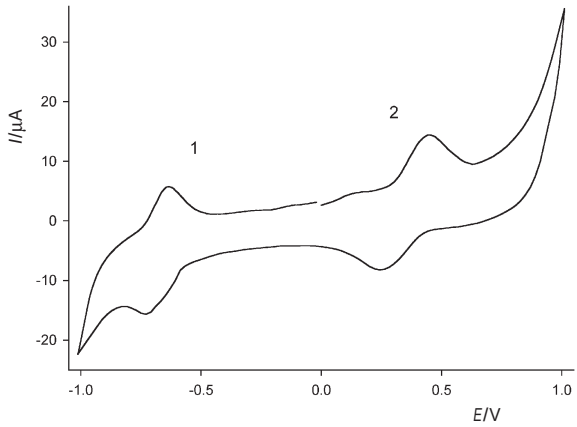
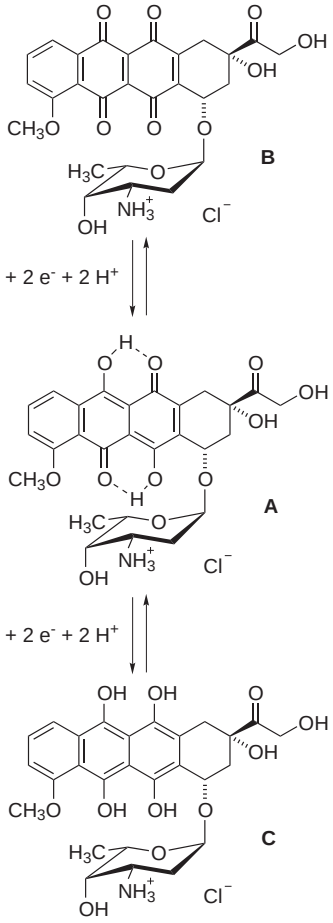


FIG. 1
Staircase cyclic voltammogram of adriamycin adsorbed on the surface of PIGE and immersed into 0.9 M KNO_3, pH 4.65. The starting potential is 0 V vs Ag|AgCl|3 M KCl and the scan rate is 0.1 V/s. Reduction of quinone (1), oxidation of hydroquinone centers (2)

Figure 2 shows theoretical cyclic voltammograms of two-electron electrode reaction of adsorbed reactant and product, with thermodynamically unstable intermediate. As the difference in standard potentials of the second and the first charge transfer ($E^o_2 - E^o_1$) increases, the half-peak width decreases from 65.4 mV for $E^o_1 = E^o_2$ to 47.9 mV for $E^o_2 - E^o_1 = 0.1$ V and 45.3 mV for $E^o_2 - E^o_1 = 0.3$ V. At 25 °C the theoretical value is $90.53/n$ mV, where n is the number of simultaneously transferred electrons. If $E^o_2 - E^o_1 = 0.3$ V, the peak current is $\Phi_p = -0.99854$, which is close to the theoretical



SCHEME 1
Electrode reactions of adriamycin (A) and its oxidized (B) and reduced (C) forms

value $\Phi_p = -0.25 \times n^2$, for $n = 2$. The peak potential is a median of standard potentials: $E_p = (E^o{}_2 + E^o{}_1)/2$.

Under the influence of inactivation of intermediate, the theoretical response is split into two equal peaks, which is shown in Fig. 3. The minimum of split response is the median of standard potentials. If $E^o{}_2 - E^o{}_1 =$

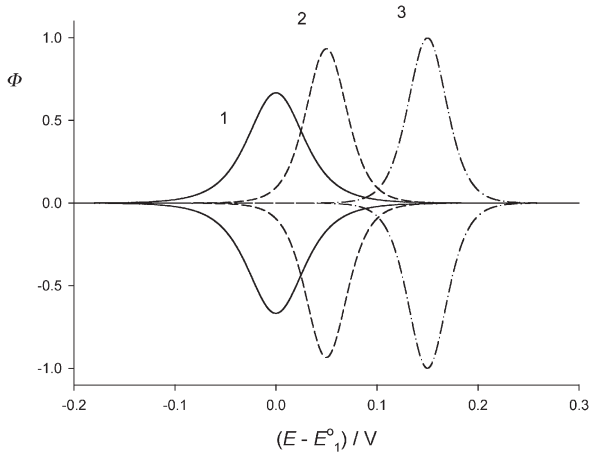


FIG. 2
Cyclic voltammetry of surface EE mechanism. $E^o{}_2 - E^o{}_1 = 0$ (*1*), 0.1 (*2*) and 0.3 V (*3*)

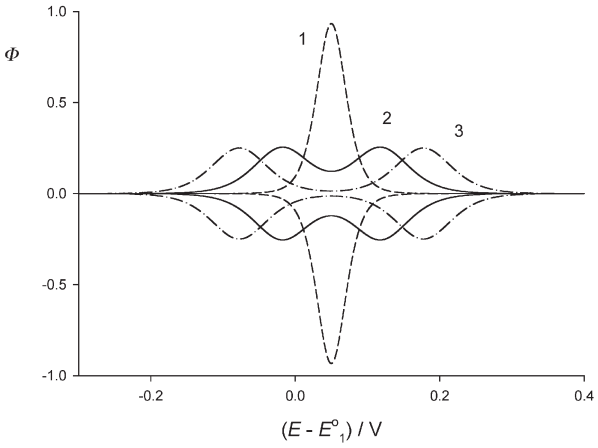


Fig. 3
Cyclic voltammetry of surface ECE mechanism. $E^o{}_2 - E^o{}_1 = 0.1$ V, $K = 0$ (*1*), 10^2 (*2*) and 10^3 (*3*)

0.1 V and $K > 10^3$, both dimensionless peak currents are equal to 0.25, which means that the split response appears as two consecutive one-electron transfers. The separation between peak potentials of the split response ($\Delta E_p = E_{p,1} - E_{p,2}$) depends on the logarithm of stability constant K and the difference between standard potentials $E^o{}_2$ and $E^o{}_1$. This is shown in Figs 4 and 5. For $E^o{}_2 - E^o{}_1 = 0.1$ V, the relationship between ΔE_p and log K is linear, with the slope 0.1182 V if log $K > 2$. For $E^o{}_2 - E^o{}_1 = 0.3$ V, this relationship is linear if log $K > 4$. In the range of linear dependence of ΔE_p on log K, the relationship between ΔE_p and $E^o{}_2 - E^o{}_1$ is linear, with the slope –1. These two linear relationships are unified in the following equation:

$$\Delta E_p = 0.1182 \log K - E^o{}_2 + E^o{}_1. \qquad (15)$$

The separation of median potentials of CV response of adriamycin is 1.026 V. Considering Eq. (*15*) and assuming that $E^o{}_2 - E^o{}_1 = 0.2$ V, the stability constant of the semiquinone form A in Scheme 1 is 2.36×10^{10}. The energy of hydrogen bond is about –60 kJ/mol (ref.[37]), which means that the stability constant is 3.3×10^{10}. However, the difference $E^o{}_2 - E^o{}_1$ is not known and the kinetics of electrode reaction of adsorbed adriamycin is neglected in this estimation. For this reason the stability constant cannot be determined exactly. Furthermore, it is possible that the assumed hydrogen bonds can exist only in the adsorbed state. Moreover, the stabilization of

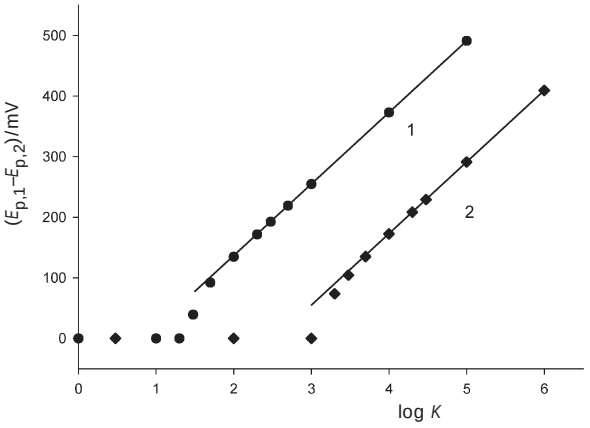


FIG. 4
Dependence of peak separation on the logarithm of stability constant. $E^o{}_2 - E^o{}_1 = 0.1$ (1) and 0.3 V (2)

semiquinone form A can be also achieved by the interactions with the electrode surface. These possibilities need more investigation. Nevertheless, it can be concluded that the semiquinone form A, with or without hydrogen bonds, is a better representation of adriamycin than the quinhydrone form (Chart 1) that is usually found in the literature. This is in agreement with theoretically predicted tautomerism in the structure of adriamycin molecule[38].

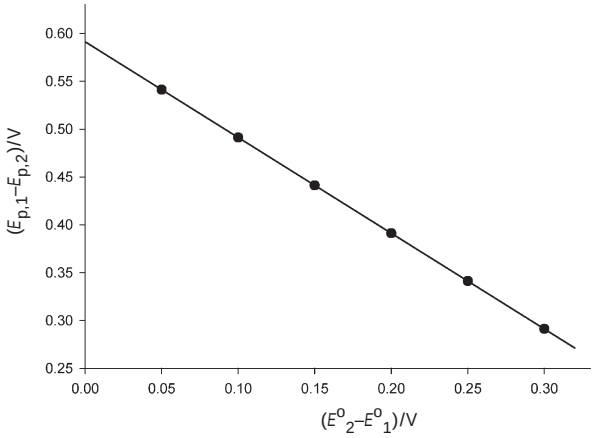


FIG. 5
Dependence of peak separation on the difference in standard potentials. $K = 10^5$

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