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Kinetics and Thermodynamics of the Mediator Function of 9,10-Anthraquinone Derivatives in Electrochemical Reactions with Peroxidase

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Abstract—On the basis of the results of studying bioelectrocatalysis induced by electrochemical reduction of *N*-(9,10-anthraquinon-1- and -2-yl)oxamides under conditions close to physiological in the presence of peroxidase, new criteria for estimation of the cholate–cholesterol coefficient of these compounds have been proposed; these are the rate constants for homogeneous electron transfer from the reduced form of the quinone to the active center of peroxidase and thermodynamic parameters of the process.

It is known that enhancement of lipoperoxidation, which leads to accumulation of excess peroxide compounds in cells, is a factor responsible for pathogenesis of some acute and chronic diseases [1]. Experimental studies have shown that peroxidation of lipids increases in particular in patients infected with hepatitis [2]. Therefore, up-to-date approaches to treatment of, e.g., hepatic diseases should include search for compounds stabilizing cell membranes, which possess antioxidant properties [3]. Administration of such compounds (hepatoprotectors) should stimulate choleresis and synthesis of cholates and prevent cholestasis. These properties are intrinsic in polybasic phenols, including 9,10-dihydroxyanthracene (QH_2) [4].

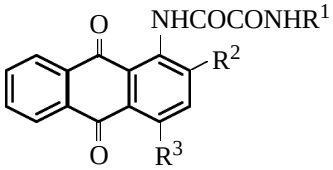
While studying models of bioelectrocatalysis processes [5, 6] we have found that 9,10-dihydroxyanthracene formed by electrochemical reduction of 9,10-anthraquinone (Q) participates in further transformations involving peroxidase active center (E^{3+}).

$$Q + 2e + 2H_2O \longrightarrow QH_2 + 2OH^-,$$

$$QH_2 + 2E^{3+} \xrightarrow{k} Q + 2E^{2+} + 2H^+.$$

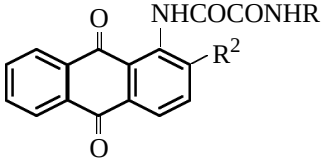
This scheme is also valid for *N*-(9,10-anthraquinon-1- and -2-yl)oxamides, since electrochemical reduction of these compounds occurs primarily at the quinoid fragment [7, 8]. Therefore, such amides should act as specific mediators in electron transfer to peroxidase. This also follows from the appearance of a kinetic component of the depolarizer reduction current upon addition of the enzyme to the system.

On the basis of the electrocatalytic effect with respect to mediator (i.e., the compound under study) we calculated the rate constants *k* [9]. Also, the corresponding standard thermodynamic parameters of the process were estimated using the Eyring equation [10]. The following compounds were examined:



I–XVIII

I, IV, XI, $R^1 = C_2H_4OH$; **II,** R^1 = *i*-Pr; **III**, R^1 = Me; **V**, $R^1 = (CH_2)_2NEt_2$; **VI, IX, XV,** R^1 = Bu; **VII, X,** $R^1 = C_6H_{11}$; **VIII, XIV, XVIII,** R^1 = H; **XII**, $R^1 = CH_2CH{=}CH_2$; **XIII, XVI,** $R^1 = CH_2Ph$; **XVII,** $R^1 = C_5H_{11}$; **I, II, VI, VII, XVI, XVIII,** R^2 = OMe; **III–V, VIII–XV, XVIII, R^2 = H; I, II, VI–IX, XI, XVI, XVIII,** R^3 = OH; **III–V, X, XII–XV, XVII,** R^3 = H.



XIX–XXII

XIX, R = *i*-Pr; **XX**, R = C_2H_4OH; **XXI**, R = H; **XXII**, R = CH_2Ph.

Our results allowed us to compare the kinetic and thermodynamic parameters of the electrocatalytic

Kinetic and thermodynamic parameters of the mediator function and biological effect of *N*-(9,10-anthraquinon-1- and -2-yl)oxamides **I**–**XXII**

Comp. no.	$k \times 10^{-6}$, s^{-1} [a]	$\Delta G^{\neq}$, kJ mol^{-1}	$\Delta H^{\neq}$, kJ mol^{-1}	$\Delta S^{\neq}$, J mol^{-1} K^{-1}	Cholate–cholesterol coefficient
I	7.03 ± 0.07	36.1 ± 0.3	64.7 ± 0.6	95.3 ± 0.9	79 ± 5
II	6.74 ± 0.07	36.5 ± 0.3	64.3 ± 0.6	92.7 ± 0.9	77 ± 5
III	6.40 ± 0.07	37.5 ± 0.3	64.0 ± 0.6	90.0 ± 0.9	73 ± 5
IV	6.38 ± 0.07	37.1 ± 0.3	64.1 ± 0.6	89.8 ± 0.9	73 ± 5
V	6.40 ± 0.07	37.2 ± 0.3	63.9 ± 0.6	89.0 ± 0.9	73 ± 5
VI	6.29 ± 0.06	37.5 ± 0.3	64.1 ± 0.6	88.8 ± 0.9	72 ± 5
VII	6.30 ± 0.06	37.5 ± 0.3	64.1 ± 0.6	88.6 ± 0.9	72 ± 5
VIII	6.30 ± 0.06	37.7 ± 0.3	64.2 ± 0.6	88.3 ± 0.9	72 ± 5
IX	6.31 ± 0.06	37.9 ± 0.3	64.3 ± 0.6	88.0 ± 0.9	72 ± 5
X	6.27 ± 0.06	38.2 ± 0.3	64.4 ± 0.6	87.4 ± 0.8	71 ± 5
XI	6.13 ± 0.06	38.5 ± 0.3	64.5 ± 0.6	86.9 ± 0.8	71 ± 5
XII	5.86 ± 0.06	38.6 ± 0.3	64.2 ± 0.6	85.5 ± 0.8	67 ± 5
XIII	5.82 ± 0.06	38.8 ± 0.4	64.2 ± 0.6	84.6 ± 0.8	66 ± 5
XIV	5.71 ± 0.06	38.8 ± 0.4	64.0 ± 0.6	84.0 ± 0.8	66 ± 5
XV	5.53 ± 0.06	39.5 ± 0.4	64.4 ± 0.6	83.1 ± 0.8	63 ± 5
XVI	5.31 ± 0.06	39.9 ± 0.4	64.6 ± 0.6	82.4 ± 0.8	60 ± 5
XVII	5.20 ± 0.05	40.8 ± 0.4	64.4 ± 0.6	83.1 ± 0.8	59 ± 5
XVIII	5.03 ± 0.05	41.1 ± 0.4	65.3 ± 0.6	78.1 ± 0.8	57 ± 5
XIX	6.29 ± 0.06	37.5 ± 0.3	64.1 ± 0.6	88.8 ± 0.8	72 ± 5
XX	6.10 ± 0.06	38.4 ± 0.3	64.2 ± 0.6	86.0 ± 0.8	69 ± 5
XXI	5.93 ± 0.06	38.5 ± 0.3	64.2 ± 0.6	85.8 ± 0.8	67 ± 5
XXII	5.50 ± 0.05	39.6 ± 0.4	64.4 ± 0.6	82.7 ± 0.8	62 ± 5

[a] At 310 ± 0.5 K.

reduction of *N*-(9,10-anthraquinon-1- and -2-yl)oxamides in the presence of peroxidase with the biological (antioxidant) effect of these compounds. The rate constants *k*, Gibbs energies $\Delta G^{\neq}$, entalpies $\Delta H^{\neq}$, and entropies $\Delta S^{\neq}$ of the homogeneous electrocatalytic reactions of 9,10-anthraquinone derivatives **I**–**XXII** and their cholate–cholesterol coefficients are given in the table. It is seen that compounds exhibiting a greater catalytic effect are characterized by a higher hepatoprotecting activity. Here, the rate constants *k* and the biological activity change in parallel in the ranges from $(5.03 \pm 0.05) \times 10^6$ to $(7.03 \pm 0.07) \times 10^6$ s^{-1} and from 57 ± 5 to 79 ± 5, respectively. This tendency almost does not depend on the position of the oxamide fragment in the anthraquinone core, which also evidences in favor of the determining role of the quinoid structure in electron transfer to the enzyme.

A similar trend is observed while comparing the biological effect with variation of the entropy component of the enzymatic reaction. Here, increase in the cholate–cholesterol coefficient is accompanied by the rise in $\Delta S^{\neq}$ from 78.1 ± 0.8 to 95.3 ± 0.9 J mol^{-1} K^{-1}. Taking into account that the entropy factor reflects the probability of formation of activated complex, a conclusion can be drawn that the above relation indicates increase in the probability of the enzymatic reaction. Therefore, $\Delta S^{\neq}$, as well as *k*, may be regarded as a parameter characterizing the antioxidant efficiency of *N*-(9,10-anthraquinon-1- and -2-yl)-oxamides.

On the other hand, there is a persistent reverse relation between $\Delta G^{\neq}$ and biological activity. The rise in $\Delta G^{\neq}$ from 36.1 ± 0.3 to 41.1 ± 0.4 kJ mol^{-1} is accompanied by the decrease of the cholate–cholesterol coefficient from 79 ± 5 to 57 ± 5 (see table), i.e., the enzymatic reaction is inhibited. Thus the quantity $\Delta G^{\neq}$ can be regarded as one more thermodynamic criterion for estimation of biological effect under the given conditions. Unlike the thermodynamic parameters of the peroxidase reaction, almost no directed drift of the enthalpy component is observed. Presumably, in our case the enthalpy factor is not a direct criterion of the biological effect.

The proposed relations between the physicochemical parameters and cholate–cholesterol coefficient in the series of *N*-(9,10-anthraquinon-1- and -2-yl)-

oxamides were analyzed by statistical methods. For the initial sample parameters $\alpha = 0.95$ and $n = 6$, the analytical value of t_α (1.96) exceeds that calculated from our experimental data (1.47). Therefore, the advanced hypothesis may be accepted with the assumed confidence probability [11].

Thus, the relative biological activity (characterized by the cholate–cholesterol coefficient) in the series of *N*-(9,10-anthraquinon-1- and -2-yl)oxamides can be estimated using both a kinetic parameter, rate constant of the enzymatic peroxidase reaction, and thermodynamic functions $\Delta G^{\neq}$ and $\Delta S^{\neq}$ of the process.

EXPERIMENTAL

Kinetic studies were performed by polarography (using an LP-7e instrument) at a dropping mercury electrode ($m^{2/3}\tau^{1/6} = 1.62$ mg$^{2/3}$ s$^{-1/2}$; polarization potential –1.20 eV relative to a saturated calomel electrode) in a cell maintained at a constant temperature (within 0.05 K); temperature range 293–313K. In all experiments, nearly physiological conditions were maintained using a phosphate buffer (pH 6.85). The substrate concentration was 5×10^{-5} M. A stream of electrolytic hydrogen was passed through the working solution.

N-(9,10-Anthraquinon-1- and -2-yl)oxamides were synthesized as described in [8]. The compounds were purified by recrystallization from dioxane and were identified by paper chromatography, IR spectroscopy, and elemental analysis.

Peroxidase from Armoracia Gaerth-Mey-Schreb had a specific activity of 50 units per mg protein.

The cholate–cholesterol coefficients were determined by L.I. Filipova according to the procedure described in [12].

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