POLAROGRAPHIC PROPERTIES OF UBIQUINONES WITH ISOPRENOID CHAINS OF DIFFERENT LENGTHS

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It has been shown that in aqueous ethanolic solutions the polarographic potentials $E_1/_2$ for ubiquinones with isoprenoid chains with different lengths are the same, −0.235 V (relative to a normal calomel electrode) at pH 7.9, and correspond to quasi-irreversible reduction with the addition of $2e^-/2H^+$. It has been found that the potential $E_1/_2$ for the ubiquinone UQ-0 having no isoprenoid chain is more positive by 57 mV relative to the potentials of the other ubiquinones.

Ubiquinones (UQ-n) are widely distributed in nature and fulfill important physiological functions in bacterial, plant, and animal cells. They also find various uses, beginning with medicine [1] and going as far as the creation of photobioelectrochemical energy converters [2]. Information on the redox properties of the ubiquinones is necessary both for understanding the mechanisms of their functioning in living systems and for their effective use. The electrochemical properties of the ubiquinones have been well studied in aprotic and aqueous ethanolic solutions [3]. It is known that ubiquinones differing by the lengths of their isoprenoid chains have the same redox potential, which has been determined polarographically for the three ubiquinones UQ-4, UQ-6,and UQ-7 in aqueous ethanolic buffer solution [4]. However, the appearance of new statements [5] on the considerable dependence of the polarographic potential, $E_1/_2$, on the length of the isoprenoid chains of the uniquinones has made it necessary to investigate this question further. Polarograms of a large set of ubiquinones containing isoprenoid chains of different lengths (1, 2, 6, 8, and 10 units) showed complete coincidence of the $E_1/_2$ potentials in three different aqueous alcoholic background solutions at a concentration of $1 \cdot 10^{-4}$ M of UQ-n. This potential is 57 mV more negative than for UQ-0, containing no isoprenoid chain, this obviously being connected with the electron-donating effect of the chain, which is characteristic for alkyl substituents [3] (Fig. 1). Since the redox potentials of the ubiquinones depend on the pH ($\partial E_1/_2/\partial$ pH $\approx$ 60 mV) [3], on comparing the measured values of $E_1/_2$ with those known from the literature the pH of the aqueous alcoholic solution must be taken into account. The use of an organic solvent leads to a change in the dissociation constant of the components of the buffer system which, in the case of a Britton–Robinson buffer, considerably increases the pH [6] (from 7.25 in aqueous solution to 8.8-9.0 in methanol:water (4:1) solution).

The pH of a Tris-HCl buffer undergoes considerably lower changes (from 8.0 in aqueous solution to 7.9 in a methanol:water (4:1) solution and to 7.6-7.7 in an ethanol:water (9:1) solution). The measured values of $E_1/_2$ for UQ-n (Table 1) with the application of a pH correction agree well with the values reported previously in the literature [3, 4]. The limiting currents of the polarographic waves for all the ubiquinones were proportional to the concentration from $5 \cdot 10^{-6}$ M to $4 \cdot 10^{-4}$ M (for UQ-10, this was the case only in the ethanol:water (9:1) solution, where the solubility of the higher ubiquinones was better).

A linear relationship was found between the limiting current and the square root of the height of the mercury column, which permits the limiting currents to be regarded as diffusion-limited [7]. Logarithmic analysis of the polarographic curves in accordance with the Heyrovsky-Ilkovic equation showed the participation of two electrons in the electrode reaction, which corresponds to the reduction in aqueous solutions with the addition of $2e^-/2H^+$ that is characteristic for all quinones [8]. At electrode potentials of from 0.0 to −0.3 V, when the electrode in the supporting solvent is charged positively, a rise of the polarograms of ubiquinones above the background curve is observed with an increase in the lengths of the isoprenoid chain (see

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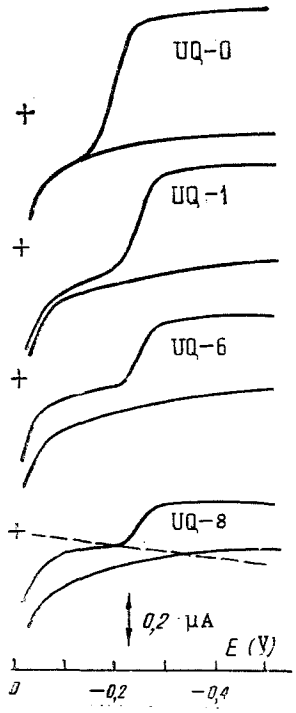

Fig. 1

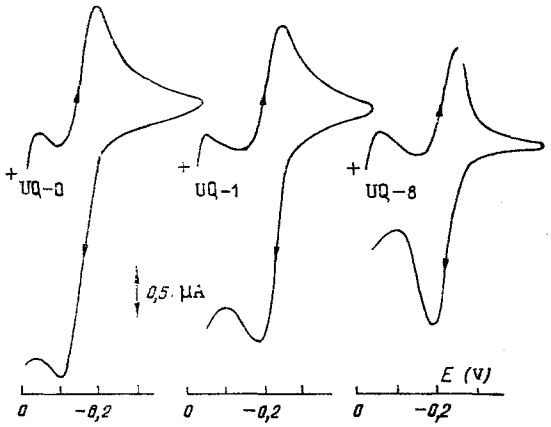

Fig. 2

Fig. 1. Polarograms of the reduction of $1 \cdot 10^{-4}$ M solutions of ubiquinones with isoprenoid chains of different lengths in a methanol: aqueous 0.1 M Tris-HCl buffer, pH 8.0 (4:1). The dashed line shows the galvanic zero of the instrument with compensation for the capacity current.

Fig. 2. Cyclic volt-ampere curves for $1 \cdot 10^{-4}$ M solutions of ubiquinones with isoprenoid chains of different lengths on a methanol: aqueous 0.1 M Tris-HCl buffer, pH 8.0 (4:1) support. Rate of scanning of the potential, 10 V/min.

TABLE 1. Potentials of Polarographic Reduction, $E_1/_2$ for Ubiquinones with Isoprenoid Chains of Different Lengths (V, relative to the normal calomel electrode)

Solutions of background electrolytes	Methanol:water (4:1), Britton-Robinson buffer pH ≈ 8.8	Methanol:water (4:1), Tris-HCl buffer, pH ≈ 7.9	Ethanol:water (9:1), Tris-HCl buffer, pH ≈ 7.7
Ubiquinones containing isoprenoid chains (UQ-n; n > 0).	−0,280	−0,235	−0,224
Ubiquinone without an isoprenoid chain (UQ-0).	−0,223	−0,178	−0,167

Fig. 1). This is apparently connected with a depression of the capacity of the electrode through the adsorption of the ubiquinones in this region of potentials, which, however, had no effect on a diffusional nature of the polarographic wave itself.

The cyclic volt-ampere curves obtained for $1 \cdot 10^{-4}$ M solutions of the ubiquinones (Fig. 2) show quasireversible reduction (difference in the potentials of the cathodic and anodic peaks, $\Delta E_p \approx$ 70-90 mV at a rate of scanning of the potential of 10 V/min). In sum, the values of $E_1/_2$ obtained for $1 \cdot 10^{-4}$ M solutions can be regarded as close to the thermodynamically reversible value E^0 not distorted by adsorption and aggregation processes.

An increase in the concentration of ubiquinones above $1 \cdot 10^{-4}$ M led to a gradual decrease in the electrochemical reversibility of the redox process which was apparently due to the absorption of the quinones on the electrode [9]. This led to a decrease in the slope of the

polarographic waves and to some shift of $E_1/_2$ in the negative direction. This effect was least appreciable for the reduction of UQ-0, the adsorption of which is minimal as the result of the absence of an isoprenoid chain. The value of $E_1/_2$ for the reduction of UQ-0 remained practically unchanged over a wide range of concentrations. In aqueous phosphate buffer, pH 6.86, it amounted to −0.105 V in the range of concentrations of from $1 \cdot 10^{-5}$ M to $1 \cdot 10^{-4}$ M. With a further increase in the concentration of UQ-0 to $3 \cdot 10^{-3}$ M, the $E_1/_2$ potential became −0.120 V. The observed slight shift in potential (by 15 mV in the negative direction) is connected with a decrease in the reversibility of the electrode process (the values of ΔE_p were 30 and 75 mV for $1 \cdot 10^{-5}$ and $1 \cdot 10^{-3}$ M solutions, respectively, at S = 10 V/min). These results do not confirm statements of a substantial shift in the $E_1/_2$ potential for UQ-0 in the positive direction with a rise in the concentration of its aqueous solution [10], which is the basis for the hypothesis of the influence of the aggregation of ubiquinones on their redox potential [5, 10].

For the other ubiquinone the effect of a decrease in electrochemical reversibility and a shift in the $E_1/_2$ potential in the negative direction was greater, becoming appreciable at concentrations above $3 \cdot 10^{-4}$ M. However, here as well, there were no fundamental differences in the $E_1/_2$ potentials for UQ-n with isoprenoid chains of different lengths.

EXPERIMENTAL

Electrochemical measurements were performed in a thermostated cell (25 ± 0.5°C) on a GWP-673 polarograph (GDR) by a three-electrode scheme relative to the normal calomel electrode under the regime of tast (current-sampled) polarography (controlled dropping period 0.6 sec, imposition of the potential in 1-mV steps, rate of flow of mercury m = 1.5 mg/s at a height of the mercury column of 50 cm). The dependence of the limiting current of the polarographic wave on the height of the mercury column was recorded by DC polarography for a freshly dropping capillary (S = 0.01 V/min). The accuracy of measurement of the $E_1/_2$ potential was ±2 mV. The pH values of aqueous alcoholic buffer solutions were measured with an OP-211/1 pH-meter (Hungary) with the aid of a glass electrode of the 91-03 type (USA) calibrated with aqueous buffer solutions.

Alcoholic buffer solutions were used for the electrochemical measurements: methanol–aqueous 0.08 M Britton-Robinson buffer, pH 7.25 (4:1); methanol–aqueous 0.1 M Tris-HCl buffer, pH 8.0 (9:1); to these were added ethanolic solutions of ubiquinones (<10% by vol.). UQ-0 was also investigated in aqueous 0.5 M phosphate buffer solution with pH 6.86 (a weighed amount of UQ-0 was dissolved directly in the aqueous buffer). Oxygen was eliminated from the electrochemical cell by flushing with argon.

Ubiquinones with isoprenoid chains of different lengths – UQ-0 (Switzerland), UQ-1 and UQ-2 (West Berlin), UQ-6 (USA), and UQ-10 (FRG) – were used without additional purification. UQ-8 was isolated from freeze-dried cells of the purple sulfur bacteria Thiocapsa roseopersicina with preliminary saponification of the lipids [11] and subsequent thin-layer rechromatography on Silufol plates (Czechoslovakia) in the petroleum ether–diethyl ether–acetic acid (90:30:1) system). Ethanolic solutions with a concentration of $5 \cdot 10^{-3}$ M were prepared by dissolving known weights of the ubiquinones. The concentrations of these solutions determined spectrally [11]. (Specord M-30) agreed to within an accuracy of 5%. The spectral characteristics of all the ubiquinones used corresponded to those published [11].

SUMMARY

1. It has been shown that in aqueous alcoholic solutions the polarographic potentials $E_1/_2$ for ubiquinones having isoprenoid chains of different lengths are the same, −0.235 V (relative to the normal calomel electrode) at pH 7.9, and correspond to quasireversible reduction with the addition of $2e^-/2H^+$.

2. It has been found that the potential $E_1/_2$ for the ubiquinone UQ-0, having no isoprenoid chain, is 57 mV more positive that the potential of the other ubiquinones.

3. It has been shown that some changes in $E_1/_2$ for concentrations greater than $1 \cdot 10^{-4}$ M are connected with a fall in the reversibility of the electrode process for the ubiquinones.

4. Results according to which the polarographic potential $E_1/_2$ of the ubiquinones depends on the length of their isoprenoid chains [5, 10] have not been confirmed experimentally.

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LITERATURE CITED

1. G. Lenaz, in: Coenzyme Q. Biochemistry, Bioenergetics and Clinical Applications of Ubiquinone, (ed. G. Lenaz), Wiley-Interscience, New York (1985), p. 435.
2. E. Yu. Katz [Kats], A. Ya. Shkuropatov, O. I. Vagabova, and V. A. Shuvalov, Abstracts of an International Conference on the Chemical Physics of Enzyme Catalysis, Tallin (1987), p. 108
3. L. E. Morrison, J. E. Schelhorm, T. M. Cotton, C. L. Bering, and P. A. Loach, in: Function of Quinones in Energy Conserving Systems, (ed. B. L. Trumpower), Academic Press, New York 1982, p. 35.
4. V. Moret, S. Pinamonti, and E. Fornasari, Biochim. Biophys. Acta, 54, 381 (1961).
5. H. A. Wahab, S. M. Khall, and M. Shanshal, Stud. Biophys., 118, 75 (1987).
6. B. A. Kiselev, Yu. N. Kozlov, and V. B. Evstigneev, Biofizika, 19, 430 (1974).
7. A. M. Bond, Polarographic Methods in Analytical Chemistry, Marcel Dekker, New York (1980).
8. J. Q. Chambers, in: The Chemistry of the Quinonoid Compounds, (ed. S. Patai), Interscience, New York, Part 2 (1974), p. 739.
9. F. L. O'Brien and J. W. Olver, An. Chem., 41, 1810 (1969).
10. M. Shanskal and K. H. Hassan, Stud. Biophys., 105, 59 (1985).
11. F. L. Crane and R. Barr, Method. Enzymol., 18, 137 (1971).

FORMATION OF GOSSYPURPURIN FROM ANHYDROGOSSYPOL

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It has been established that gossypurpurin is formed from anhydrogossypol and not from gossypol itself during column chromatography on Chemapol silica gel. This is explained by the high reactivity of anhydrogossypol and the catalytic role of the silica gel. The results obtained permit it to be considered that gossypurpurin is a compound of molecules of monoanhydrogossypol and of diaminomonoanhydrogossypol through a nitrogen bridge.

Anhydrogossypol, formed by the splitting out of two water molecules from gossypol, is distinguished by high reactivity. It interacts with butadiene, dienic fatty acids, acylglycerols, aniline, and anthranilic acid [1].

Anhydrogossypol is usually obtained by heating crystalline gossypol to 190-200°C or by treating it with pyridine hydrochloride in toluene solution [2]. A hypothesis has been put forward of the dehydration of gossypol on silica gel and in alcoholic solutions [3, 4].

We have established that in methanolic solution gossypol undergoes changes accompanied by a decrease in the proportion of aldehyde groups from 96.3% for gossypol to 10.6% in the final conversion product through the formation mainly of dianhydrogossypol (DAG) and a very small amount of monoanhydrogossypol (MAG) [5].

For the separation of a mixture of anhydroderivatives of gossypol into individual components we made use of column chromatographs (CC) on silica gel. Six colored fractions were obtained. It was established with the aid of analytical TLC that fraction (I) contained mainly MAG (R_f 0.36), a small amount of gossypol (R_f 0.60), and traces of DAG (R_f 0.31); fractions II and III consisted of MAG and traces of DAG; and fractions (IV-VI) contained MAG and a more polar violet-purple substance (R_f 0.27) giving a deep blue coloration with $SbCl_3$.

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