Electrochemical Study of the Interaction of Ubiquinone,0 and Ubiquinone,10 with Nucleosides and Nucleic Acid Bases

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Z. Naturforsch. **61a,** 569 – 576 (2006); received July 14, 2006

Dedicated to Prof. R. Gleiter on the occasion of his 70th birthday

Electrochemical polarographic measurements done for mixtures of the ubiquinone,0 (Ub,0) and Ubiquinone,10 (Ub,10) in their monomeric form ($c < 10^{-5}$ mol/l) with nucleosides and nucleic acid bases show that their half wave potentials, $E_{1/2}$, as well as limited diffusion currents, i_d, decrease as functions of the concentrations of the added components. The change in the $E_{1/2}$ values is attributed to the change in the lowest unoccupied molecular orbital (LUMO) energy of the ubiquinone, resulting from its interaction with the heterocyclic rings. The decrease in i_d is explained in terms of complex formation and consequently increase in the mass of the reducible species, according to the Ilkovic equation. In all mixtures the number of reduction electrons (n) is smaller than that of free ubiquinone. The changes in all electrochemical variables of the native Ub,10 are more pronounced than those of the synthesized Ub,0, confirming the former accepted assumption that the complex formation ability of Ub,10 is higher than that of Ub,0. The results indicate a possible interaction of such molecules when they migrate into the mitochondria with ubiquinone molecules (coenzyme Q).

Key words: Ubiquinones; Nucleosides; Polarography.

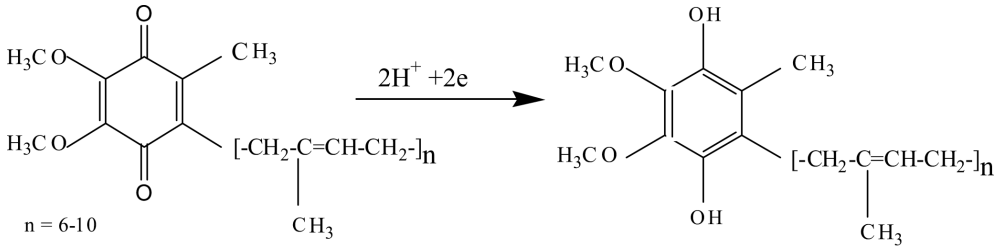
1. Introduction

The consumption of oxygen, to produce energy required for biological functions of living cells [1 – 3], was studied by pioneering chemists such as Wieland [4] and Warburg and Christian [5 – 7], who had shown that this process proceeds via chemical reactions in which organic substrates are oxidized and oxygen is reduced. The redox reaction is mediated through a number of enzyme complexes, "Atmungsfermente", in the mitochondria of the cell. The group of complexes is presently called the respiration chain or "Atmungskette". Each complex is composed of an oxido-reductive organic or organometallic compound which may be reduced or oxidized in the presence of adequate enzymes.

The redox process proceeds through the transfer of electrons from the reducing agent, here the organic substrate, to the oxidizing agent, here O_2. As for the mitochondrial respiration, the electrons are transferred to oxygen from the substrates through different organic and organometallic intermediates (electron carriers). The respiratory chain then resembles an electrochemical system in which the substrates possess a negative potential and O_2 a positive potential (+0.80 V). Inhibiting any of the electron carriers or reducing or oxidizing enzymes in the chain slows the respiration activity of the cell and leads ultimately to its death. Several such chemical inhibitors have been discovered and the site of their action located [2].

Ubiquinone,10 (Ub,10) was isolated by Morton et al. from beef heart mitochondria [8, 9] and identified as 2-methyl-3-polyisoprenyl-5,6-dimethoxy-*p*-benzoquinone (Fig. 1, $n = 10$).

It was isolated almost simultaneously by Crane et al. [10] and later by Wolf et al. [11] from other organisms. Subsequently, different ubiquinones (Ub,n) were isolated from the cells of different living organisms [12 – 17]. Unlike other electron carriers in the respiratory chain, which are known to be fixed in the mitochondrial membrane, the ubiquinone molecules move freely in the mitochondrial matrix and resemble free electron shuttles between the different electron carriers of the respiratory chain. Due to their mobile property within the mitochondrion, ubiquinones seem much more susceptible to interact with molecules other than electron carriers [18]. They should therefore be the most environmentally sensitive component



Oxidized Form Reduced Form

Fig. 1. Ubiquinone,10 (coenzyme Q10).

of the respiratory chain. Interruption of the mitochondrial respiration activity is the natural result of such interactions. Indeed, it was found by Porter and Folkers [16] that antimalarials stop the respiration activity of the plasmodium parasite through the interaction with the mitochondrial ubiquinone. The exact understanding of such interaction requires a detailed study of the electrochemical behavior of the ubiquinones in solution as well as their modes of interaction with other molecules [18 – 27]. It is important then to investigate the interaction of ubiquinones with all possible molecules which might migrate into the mitochondrial fluid [28]. Among these are nucleosides and nucleic acid bases, some of which are studied in the present work.

2. Experimental Section

A Princeton Applied Research polarographic analyzer, model 174 (PAR 174), was used for the polarographic measurements. A dropping mercury electrode was used as working electrode and a saturated calomel electrode (SCE) as reference electrode. The auxiliary electrode was a mercury pool. The pH measurements were done using a Phillips PW94/8 electronic pH meter. Pure deionized water was generated using an LV-08 ultra pure water device. The UV spectra were measured with a Shimadzu UV-106 recording spectrophotometer.

Ub,0 was synthesized according to the known procedure [29]. Aqueous phosphate buffers were prepared through mixing of different volumes of 0.06 M. Na_2HPO_4 and 0.06 M KH_2PO_4 to yield the required pH value [30]. Aqueous stock solutions of Ub,0 and nucleic acid bases (10^{-3} M) in a phosphate buffer were prepared and kept in the dark at 4 °C to minimize decomposition. Ub,10 was supplied by Fluka AG, Buchs, Switzerland. The nucleosides and nucleic acid bases were kindly supplied by Prof. W. Pfleiderer, Konstanz, Germany.

The measurement solution (pH 7.4) was degassed by passing of N_2 gas that was purified and equilibrated through bubbling in acidic V(II) solution over heavily amalgamated Zn and thereafter through distilled water. Mercury was purified by distillation under vacuum.

3. Results

Due to the self-association of the ubiquinone molecules and the dependence of their physical properties on that [20 – 27], it was necessary to carry out measurements at concentrations low enough to secure the monomeric form of Ub,*n* and avoid complications on analyzing the results. Repeating the direct current (DC) polarographic measurements for both Ub,0 and Ub,10 at different concentrations confirmed that their half wave potential ($E_{1/2}$) values shift towards more negative values on increasing the concentration (Fig. 2).

Due to this fact, the concentrations $7.5 \cdot 10^{-6}$ mol/l for Ub,0 and 10^{-5} mol/l for Ub,10 were accepted as the highest, suitable concentrations for the present study. For each polarographic measurement, the Heyrovsky-Ilkovic equation [30] was used to calculate the number of electrons (*n*) required for the reduction:

$$E = E_{1/2} - (R \cdot T/n \cdot F) \cdot \ln(i/i_d - i), \qquad (1)$$

where n is the number of reduction electrons, $E_{1/2}$ the half wave potential, i the diffusion current and i_d the limited diffusion current. The whole number value of n is taken as a sign of a reversible reduction, the broken number as a sign of a nonreversible process [31, 32]. Due to the different time scales of the polarographic measurement, 50 ms for normal pulse polarography and 3 s for direct current polarography, the

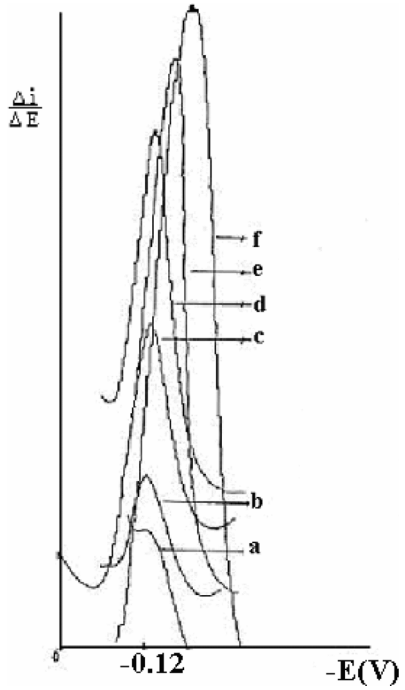


Fig. 2. Differential pulse polarographic (DPP) curves of Ub,10 measured at different concentrations: (a) $5 \cdot 10^{-6}$ M; (b) $7.5 \cdot 10^{-6}$ M; (c) $1 \cdot 10^{-5}$ M; (d) $2 \cdot 10^{-5}$ M; (e) $5 \cdot 10^{-5}$ M; (f) $7.5 \cdot 10^{-5}$ M.

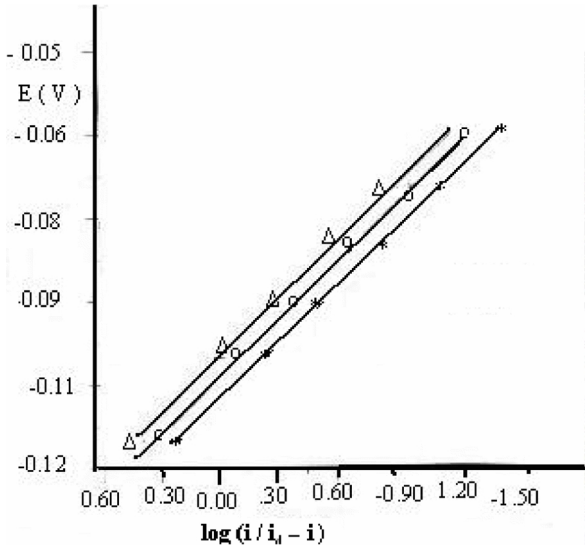


Fig. 3. Heyrovsky-Ilkovic plots for Ub,0 reduction taken at different concentrations. △ $7.5 \cdot 10^{-6}$ M; ○ $2.5 \cdot 10^{-5}$ M; ∗ $5 \cdot 10^{-5}$ M.

reversible reduction processes within the direct current method could change to pseudo-reversible processes,

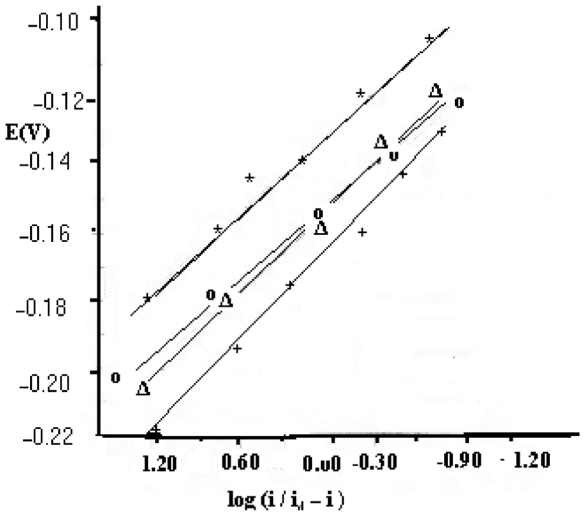


Fig. 4. Heyrovsky-Ilkovic plots for Ub,10 reduction taken at different concentrations. ∗ 10^{-5} M; ○ $5 \cdot 10^{-5}$ M; △ $2.5 \cdot 10^{-5}$ M; + $7.5 \cdot 10^{-5}$ M.

when measured with normal pulse polarography [33].

As for Ub,0 it was found formerly that the polarographic reduction process in solutions shows a whole number of electrons, $n = 2$, resembling a reversible reduction, whereas its reduction in alcoholic media shows a broken number of electrons, indicating a nonreversible reduction process [21, 22]. Figure 3 shows the Heyrovsky-Ilkovic plots for Ub,0 at different concentrations.

As for Ub,10 the measurements done in 80% ethanol + 20% aqueous Britton-Robinson buffer solution at different concentrations yielded n values of 1.52 – 1.57, indicating nonreversible reduction processes [21, 25]. Figure 4 shows similar plots for Ub,10 measured at different concentrations.

The irreversibility of reduction might be due to the recombination of the Ub,10 (Q) semiquinone radicals in solution:

$$\begin{aligned} \mathrm{Q} &\overset{+\mathrm{e},\,\mathrm{H}^*}{\rightleftharpoons} \mathrm{QH}^*, \\ 2\,\mathrm{QH}^* &\rightleftharpoons (\mathrm{QH})_2. \end{aligned}$$

Tables 1 and 2 include the results of the differential pulse polarographic (DPP) measurements for the 1 : 1 and 1 : 3 mixtures of Ub,0 with the corresponding nucleosides and nucleic acid bases (Fig. 5) taken for two different concentrations of Ub,0. All measurements yield a single reduction wave for the ubiquinone. The measurements were taken two hours after mixing

Table 1. DPP (E_p) values of Ub,0 + nucleosides and nucleic acid bases measured at $2.5 \cdot 10^{-6}$ mol/l Ub,0.

	1 : 1 Mixture		1 : 3 Mixture	
Ub,0 +	$E_p \cdot 10^4$	$\Delta E_p \cdot 10^4$	$E_p \cdot 10^4$	$\Delta E_p \cdot 10^4$ (V)
Adenine	−925	−25	−925	−25
Adenosine	−910	−1	−910	−1
2-Desoxy-adenosine	−925	−25	−25	−25
Guanine	−900	000	−900	000
Guanosine	−900	000	−900	000
2-Desoxy-guanosine	−925	−25	−925	−25
Hypoxanthine	−900	000	−900	000
Cytosine	−900	000	−900	000
Cytidine	−900	000	−900	000
2-Desoxy-cytidine · HCl	−900	000	−900	000
Uridine	−910	000	−910	000
Uracil	−900	000	−900	000
Thymine	−900	000	−900	000
Thymidine	−900	000	−900	000

Table 2. DPP (E_p) values of Ub,0 + nucleosides and nucleic acid bases measured at $7.5 \cdot 10^{-6}$ mol/l Ub,0.

	1 : 1 Mixture		1 : 3 Mixture	
Ub,0 +	$E_p \cdot 10^3$	$\Delta E_p \cdot 10^3$	$E_p \cdot 10^3$	$\Delta E_p \cdot 10^3$ (V)
Adenine	−99	−4	−99	−4
Adenosine	−96	−1	−96	−4
2-Desoxy-adenosine	−98	−3	−98	−3
Guanine	−96	−1	−96	−1
Guanosine	−98	−3	−98	−3
2-Desoxy-guanosine	−98	−3	−98	−3
Hypoxanthine	−95	000	−95	000
Cytosine	−95	000	−95	000
Cytidine	−95	−000	−95	−000
2-Desoxy-cytidine · HCl	−96	−1	−96	−1
Uridine	−96	−1	−96	−1
Uracil	−95	000	−95	000
Thymine	−95	000	−95	000
Thymidine	−95	000	−95	000

Table 3. DPP (E_p) values of Ub,10 + nucleosides and nucleic acid bases measured at 10^{-5} mol/l Ub,10.

	1 : 1 Mixture		1 : 3 Mixture	
Ub,10 +	$E_p \cdot 10^3$	$\Delta E_p \cdot 10^3$	$E_p \cdot 10^3$	$\Delta E_p \cdot 10^3$ (V)
Adenine	−138	−18	−180	−60
Adenosine	−142	−22	−169	−49
2-Desoxy-adenosine	−142	−22	−192	−72
Guanine	−133	−13	−164	−44
Guanosine	−136	−16	−161	−41
2-Desoxy-guanosine	−131	−11	−153	−33
Hypoxanthine	−132	−12	−150	−30
Cytosine	−138	−18	−180	−60
Cytidine	−126	−6	−152	−32
2-Desoxy-cytidine · HCl	−133	−13	−164	−44
Uridine	−136	−16	−177	−57
Uracil	−136	−16	−164	−44
Thymine	−133	−13	−171	−51
Thymidine	−139	−19	−152	−32

Table 4. DPP (E_p) values of Ub,10 + nucleosides and nucleic acid bases measured at $7.5 \cdot 10^{-6}$ mol/l Ub,10.

	1 : 1 Mixture		1 : 2 Mixture	
Ub,10 +	$E_p \cdot 10^3$	$\Delta E_p \cdot 10^3$	$E_p \cdot 10^3$	$\Delta E_p \cdot 10^3$ (V)
Adenine	−144	−22	−178	−58
Adenosine	−144	−22	−187	−57
2-Desoxy-adenosine	−137	−17	−168	−48
Guanine	−120	000	−143	−23
Guanosine	−134	−14	−162	−42
2-Desoxy-guanosine	−132	−12	−168	−48
Hypoxanthine	−120	000	−135	−15
Cytosine	−135	−15	−172	−52
Cytidine	−133	−13	−167	−47
2-Desoxy-cytidine · HCl	−124	−4	−155	−35
Uridine	−127	−7	−160	−40
Uracil	−130	−10	−152	−32
Thymine	−124	−4	−137	−17
Thymidine	−120	−000	−135	−15

the components to permit equilibration. Tables 3 and 4 include the differential pulse polarographic results for the mixtures of Ub,10 with the same nucleosides and nucleic acid bases, obtained for two concentrations, in 80% ethanol + 20% Britton-Robinson buffer (pH 7.4) solution. It is seen that for both ubiquinone mixtures the reduction potential (E_p) is shifted to more negative values compared to the E_p values of the pure ubiquinones; see the corresponding ΔE_p values. Similar results were explained in former papers to be due to the increase in the LUMO energy of Ub,*n* caused by complex formation [18, 19]. The change in E_p of Ub,10 is greater than that of Ub,0. Former papers had shown too, that Ub,10 shows a greater tendency for complex formation than Ub,0, due to entropy effects [25]. The increase in the nucleoside base concentrations increases the magnitude of E_p shifts of both uniquinones too:

$$\Delta E_p(1:3) > \Delta E_p(1:2) > \Delta E_p(1:1).$$

The percent shifts in the E_p values for Ub,10, measured at 10^{-5} mol/l, are: 12% (1 : 1), 22% (1 : 2) and 35% (1 : 3); for $7.5 \cdot 10^{-6}$ mol/l: 8% (1 : 1), 21% (1 : 2) and 30% (1 : 3).

The effect with Ub,10 is greater than that with Ub,0. The decrease in E_p for Ub,0 is small and negligible

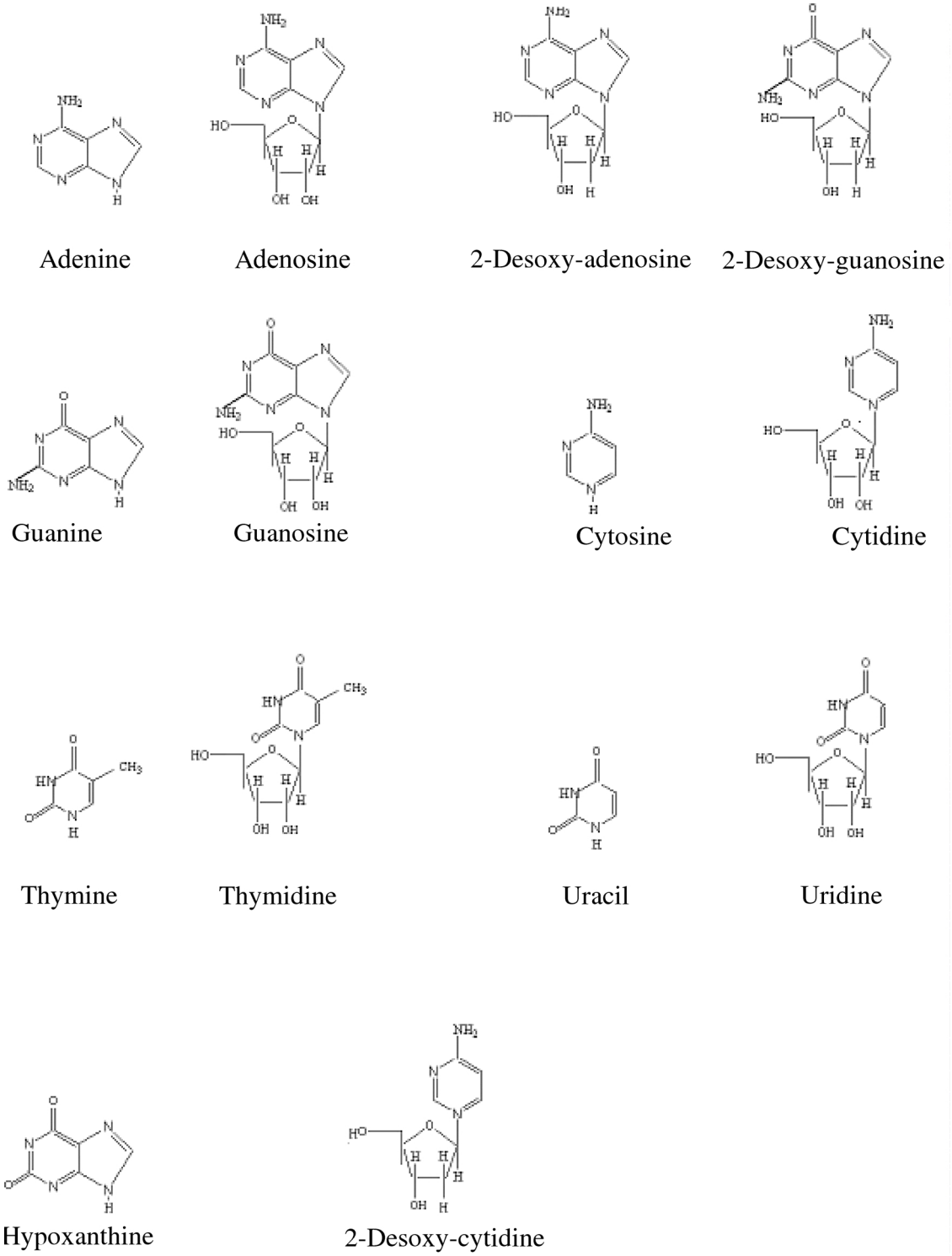


Fig. 5. Structures of examined nucleosides and nucleic acid bases.

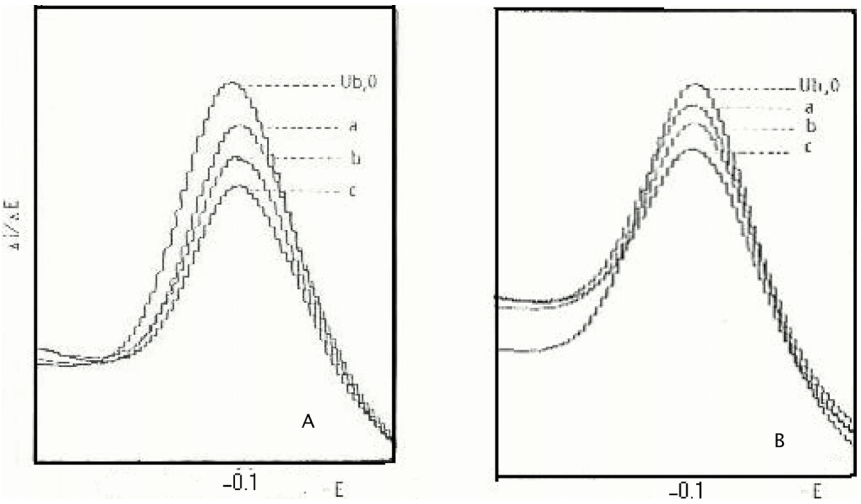


Fig. 6. DPP curves of Ub,0 ($7.5 \cdot 10^{-5}$ mol/l) in (a) 1 : 1; (b) 1 : 2 and (c) 1 : 3 mixtures with (A) adenine and (B) thymidine.

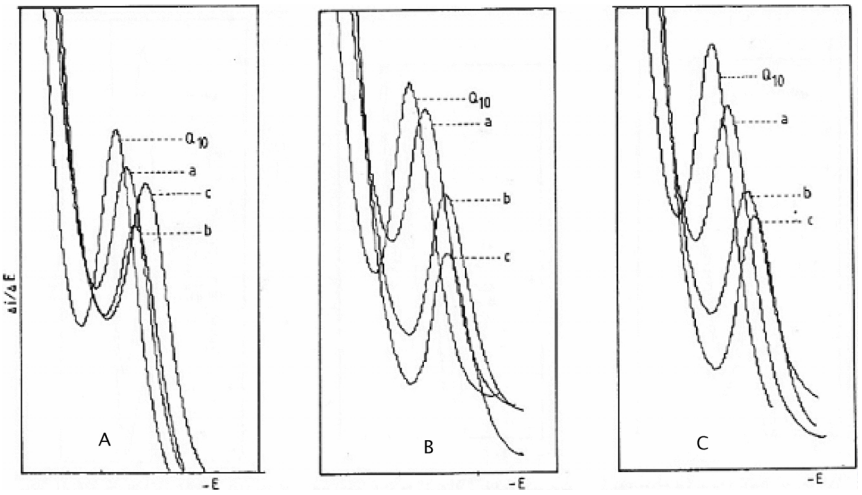


Fig. 7. DPP curves of Ub,10 (10^{-5} mol/l) in (a) 1 : 1; (b) 1 : 2 and (c) 1 : 3 mixtures with (A) guanosine; (B) adenosine and (C) 2-desoxy-adenosine.

in most cases. This might be clearly seen on studying the values for hypoxanthine, cytosine, cytidine, uracil, thymine and thymidine mixtures in the Tables 1 and 2.

Figure 6 shows the change in differential pulse polarographic curves of mixtures of Ub,0 + nucleosides and nucleic acid bases taken at $7.5 \cdot 10^{-6}$ mol/l Ub,0. Figure 7 shows the change in differential pulse polarographic curves of mixtures of Ub,10 + nucleosides and nucleic acid bases taken at $7.5 \cdot 10^{-6}$ mol/l Ub,10.

4. Discussion

Studying the E_p shifts in the tables one finds out that:

(i) The E_p shifts of Ub,0 are smaller than those of Ub,10. This goes parallel to the known stronger complexation affinity of Ub,10 compared to Ub,0 [20 – 22].

(ii) Increasing the concentrations of the nucleic acid bases or nucleosides, i. e. going from 1 : 1 to 1 : 3 mixtures does increase the E_p shifts, obviously due to the equilibrium shift towards the complex formation.

(iii) The presence of sugar moieties decreases the E_p shifts, which seems to be due to steric hinderance of the complex formation.

(iv) The presence of electron donating substituents, e. g. -NH_2 or -CH_3, increases the E_p shift too.

Tables 5 and 6 show the changes in the polarographic limited diffusion current, i_d, measured for the nucleosides and nucleic acid bases with Ub,0 and Ub,10. Apparent from the values in both tables is the decrease in the i_d values for the mixtures of all nucleo-

Table 5. Measured DPP limited diffusion current, i_d, for Ub,0 ($7.5 \cdot 10^{-6}$ mol/l, $i_d = 181 \cdot 10^{-3}$ μA) in mixtures with nucleosides and nucleic acid bases ($\cdot 10^{-3}$ μA).

Ub,0 +	1 : 1 Mixture	1 : 2 Mixture	1 : 3 Mixture
Adenine	172	147	122
Adenosine	158	129	113
2-Desoxy-adenosine	152	123	105
Guanine	162	143	123
Guanosine	156	131	120
2-Desoxy-guanosine	155	133	129
Hypoxanthine	153	135	113
Cytosine	155	134	117
Cytidine	154	127	109
2-Desoxy-cytidine · HCl	138	118	106
Uridine	151	132	115
Uracil	141	135	117
Thymine	151	121	113
Thymidine	158	134	112

Table 6. Measured DPP limited diffusion current, i_d, for Ub,10 ($7.5 \cdot 10^{-6}$ mol/l, $i_d = 62 \cdot 10^{-3}$ μA) in mixtures with nucleosides and nucleic acid bases ($\cdot 10^{-3}$ μA).

Ub,10 +	1 : 1 Mixture	1 : 2 Mixture	1 : 3 Mixture
Adenine	44	49	50
Adenosine	34	42	48
2-Desoxy-adenosine	37	42	50
Guanine	49	56	49
Guanosine	36	29	38
2-Desoxy-guanosine	38	33	25
Hypoxanthine	31	31	31
Cytosine	44	48	51
Cytidine	42	48	55
2-Desoxy-cytidine · HCl	40	41	40
Uridine	28	55	53
Uracil	48	61	61
Thymine	55	59	59
Thymidine	37	32	31

sides and nucleic acid bases with the ubiquinones relative to those of the free ubiquinones. Considering the complex formation that causes the increase in the mass of the reduced molecule, the decrease in i_d may be understood in the light of the Ilkovic equation [32]

$$i_d = 607 n D^{1/2} m^{-2/3} t^{1/c} C.$$

According to this equation the diffusion current is proportional to the inverse of the cube of the mass of the reductant. The decrease in i_d is of similar order for both ubiquinones. The higher concentrations of Ub,10, however, cause the stronger decrease in the i_d value as compared to Ub,0.

As pointed out in the former paragraph, the polarographically measured number of reduction electrons for Ub,0 was 2 electrons in aqueous solution, independent of the concentration, and representing a reversible

Table 7. Number of reduction electrons measured for Ub,0, at $7.5 \cdot 10^{-6}$ mol/l, in mixtures with the corresponding nucleosides and nucleic acid bases.

Ub,0 +	1 : 1 Mixture	1 : 2 Mixture	1 : 3 Mixture
Adenine	1.97	1.91	1.82
Adenosine	1.92	1.90	1.85
2-Desoxy-adenosine	1,99	2.00	1.94
Guanine	1.85	1.89	1.74
Guanosine	2.00	2.00	1.99
2-Desoxy-guanosine	1.93	1.87	2.00
Hypoxanthine	1.82	1.83	1.83
Cytosine	1.99	1.96	1.89
Cytidine	1.92	1.83	1.76
2-Desoxy-cytidine · HCl	1.92	1.77	1.79
Uridine	1.99	1.86	1.76
Uracil	1.96	1.96	1.89
Thymine	1.95	1.88	1.71
Thymidine	1.94	1.79	1.74

Table 8. Number of reduction electrons measured for Ub,10, at 10^{-5} mol/l, in mixtures with the corresponding nucleosides or nucleic acid bases.

Ub,10 +	1 : 1 Mixture	1 : 2 Mixture	1 : 3 Mixture
Adenine	1.54	1.54	1.50
Adenosine	1.20	1.22	1.16
2-Desoxy-adenosine	1,14	1.15	1.20
Guanine	1.55	1.50	1.47
Guanosine	1.12	1.48	1.12
2-Desoxy-guanosine	1.09	1,18	1.17
Hypoxanthine	1.20	1.16	1.13
Cytosine	1.10	1.40	1.43
Cytidine	1.04	1.37	1.30
2-Desoxy-cytidine · HCl	1.30	1.15	1.35
Uridine	1.48	1.57	1.59
Uracil	1.29	1.43	1.43
Thymine	1.56	1.43	1.42
Thymidine	1.56	1.53	1.47

reduction process. For Ub,10 the number n was 1.52 – 1.57 electrons, depending on the Ub,10 concentration and representing an irreversible reduction process. Addition of the nucleosides or nucleic acid bases changes the picture. The reduction process for Ub,0 becomes nonreversible, i. e. n is a broken number, and for Ub,10 the value of n decreases. Obviously, the change in both cases is due to the complex formation of the solution components. Tables 7 and 8 show the values of the reduction electrons measured polarographically.

5. Conclusion

According to the polarographic measurements, both Ub,10 (coenzym Q-10) and Ub,0 interact with nucleic acid bases and nucleosides, most probably via molecular complex formation, even at low concentrations (10^{-5} mol/l). The interaction is noticed through the shift in the $E_{1/2}$ and i_d values, and the changes in

the number of reduction electrons of the ubiquinones. The presence of nucleosides and nucleic acid bases influences the reduction process to be nonreversible for both ubiquinones in the solution.

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