Coulometric Study of the Ubiquinone,0 Interaction with Nucleic Acid Bases

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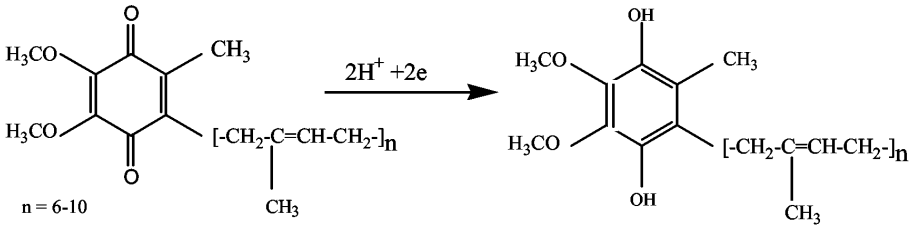
The change in the number of reduction electrons of ubiquinone,0 ($7.5 \cdot 10^{-6}$ mol/l) due to the presence of nucleosides or nucleic acid bases is measured coulometrically. The number of the reduction electrons varies from 2 – 1.72. The results are explained in terms of molecular interactions present between the two components of the mixture even at low concentrations.

Key words: Coulometry; Ubiquinone,0; Nucleosides; Interaction.

1. Introduction

Ubiquinone is a vital component of the respiratory chain in the mitochondria of living cells [1]. As a coenzyme it acts as an electron shuttle between the different components of the chain [2]:



Oxidized Form Reduced Form

In former papers we have shown that both ubiquinone,10 (Ub,*n*, $n = 10$), normally called coenzyme Q-10, and ubiquinone,0 exhibit peculiar behavior in ethanol and in ethanol/water solutions [3]. Both molecules show strong tendencies towards self association [4]. It was shown that they exist in the monomer form at concentrations $< 10^{-5}$ mol/l [5]. Both molecules show considerable tendencies towards complex formation with other molecules, such as antimalarials [6] or quinoline derivatives [7]. It was also found that such complexation causes an increase in the polarographically measured reduction potential ($E_{1/2}$) values of the ubiquinone [8]. Furthermore, the reduction potentials ($E_{1/2}$) of both ubiquinone molecules were found concentration-dependant [9]. For these reasons, the study of the ubiquinone complexation in solution should be done at concentrations $< 10^{-5}$ mol/l of the ubiquinone in order to avoid complication of the study, which might be induced by the self-association of the molecules. Both polarographic [10] and UV-spectrophotometric [11] studies showed that the complex formation of ubiquinone with other molecules is a time consuming process. In all cases of the studied reactions a measurable time interval was required to reach the corresponding equilibrium state.

2. Experimental

A Princeton Applied Research potentiostat/galvanostat, model 273 (PAR 273), supplied with 270 software system was used for the coulometric measurements. A saturated calomel electrode (SCE) was used as reference electrode. The auxiliary electrode was a helical platinum wire. A mercury pool was used as the work-

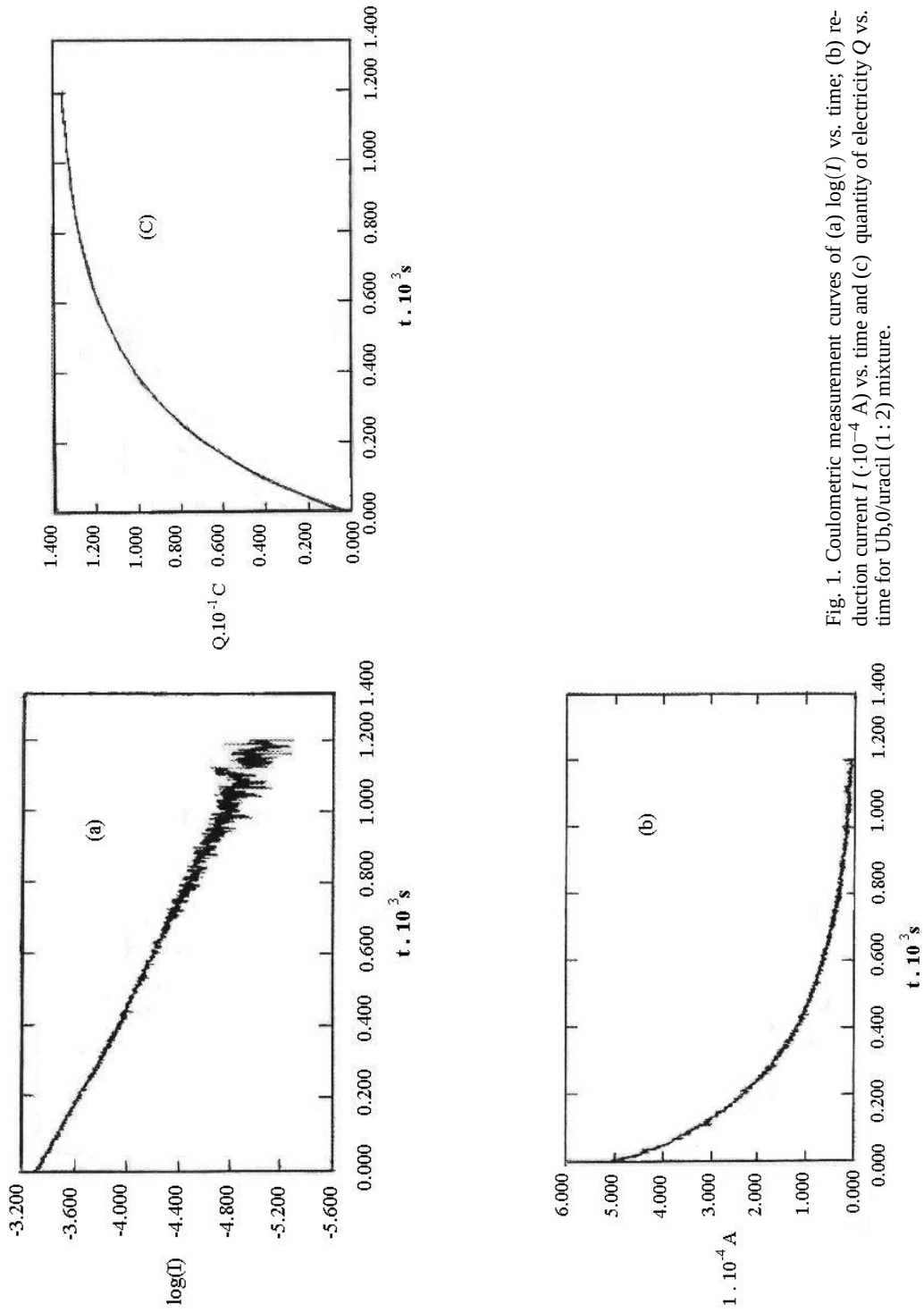


Fig. 1. Coulometric measurement curves of (a) log(I) vs. time; (b) reduction current I ($\cdot 10^{-4}$ A) vs. time and (c) quantity of electricity Q vs. time for Ub,0/uracil (1 : 2) mixture.

ing electrode. The pH measurements were done with Phillips PW94/8 electronic pH meter. Pure deionized water was generated using an LV-08 ultra pure water device. All measurements were done under a con-

Table 1. Number of electrons measured for $7.5 \cdot 10^{-6}$ mol/l solution of Ub,0 (pH 7.4), at different time intervals.

Time (h)	Q (C)	No. of reduction electrons/mol
2	0.145	2.00
4	0.144	1.99
6	0.145	2.00
24	0.144	1.99
48	0.144	1.99
72	0.145	2.00
264	0.145	2.00
360	0.145	2.00

trolled potential at the following conditions: concentration of ubiquinone,0 $7.5 \cdot 10^{-6}$ mol/l; working electrode potential -0.265 V vs. SCE; run time 1400 s.

Aqueous phosphate buffers were prepared by mixing of different volumes of 0.06 mol/l Na_2HPO_4 and 0.06 mol/l KH_2PO_4 to yield the required pH value [12]. Aqueous stock solutions of Ub,0 and nucleic acid bases ($1 \cdot 10^{-3}$ mol/l) in phosphate buffer were prepared and kept in the dark at 4 °C to minimize decomposition. The measurement solution (pH 7.4) was degassed through passing of N_2 gas that was purified and equilibrated by bubbling in acidic V(II) solution over heavily amalgamated Zn, and then distilled water. Mercury was purified by distillation under vacuum. As a test, the measurement was done for a 0.1 N $CuSO_4$ solution in NH_4Cl buffer. The obtained charge-time curve for the measurement under such conditions yielded exactly 2.0 electrons/mol for the reduction of Cu^{2+}.

3. Results and Discussion

The coulometric measurement was done for Ub,0 in aqueous solution of $7.5 \cdot 10^{-6}$ mol/l. The results are shown in Table 1.

It is seen that the number of electrons, 2, applies for all the measurements done at different time intervals, extending from 2 to 360 h. The even number of electrons found for all measurements suggests a reversible reduction process. As for the ubiquinone/nucleic acid bases mixtures, the measurements showed normal reduction behavior, i. e. exponential decline of the current (I) and linear relation of $\log(I)$ versus time. This can be seen in the curves of Fig. 1 that were measured for the Ub,0/uracil (1 : 2) mixture. The results for the (1 : 1), (1 : 2) and (1 : 3) mixtures are listed in Table 2.

It is seen that in all three solutions the number of reduction electrons (n) starts deviating from 2 after 2 h. The decrease in n continues till the values 1.83 e for the (1 : 1) and (1 : 2) mixtures and 1.78 e for the (1 : 3) mixture are reached [after 48 h for the (1 : 1) and 72 h for the (1 : 2) and (1 : 3) mixtures]. Similar changes in the number of reduction electrons are noticed for the Ub,0 mixtures with adenosine, adenine, cytosine and cytidine (Table 3 and Fig. 2).

Table 2. Coulometric measurement results for the (1 : 1), (1 : 2) and (1 : 3) mixtures of Ub,0/uracil, taken at different times.

	(1 : 1) Mixture		(1 : 2) Mixture		(1 : 3) Mixture	
Time (h)	Q (C)	No. of electrons	Q (C)	No. of electrons	Q (C)	No. of electrons
2	0.145	2.0	0.136	1.88	0.134	1.85
4	0.140	1.94	0.136	1.90	0.137	1.89
6	0.140	1.94	0.136	1.88	0.134	1.85
24	0.133	1.83	0.136	1.88	0.129	1.78
48	0.133	1.83	0.136	1.88	0.132	1.82
72	0.133	1.83	0.136	1.83	0.129	1.78
144	0.133	1.83	0.136	1.83	0.129	1.78

Table 3. Coulometrically measured number of coulombs (Q) and reduction electrons (n) of Ub,0 mixtures with adenosine, adenine, cytosine and cytidine with different ratios and different time intervals.

	(1 : 1) Mixture		(1 : 2) Mixture		(1 : 3) Mixture	
Time (h)	Q (C)	No. of electrons	Q (C)	No. of electrons	Q (C)	No. of electrons
Adenosine						
2	0.139	1.92	0.140	1.90	0.143	1.98
4	0.139	1.92	0.145	2.00	0.140	1.93
6	0.140	1.93	0.138	1.91	0.140	1.93
24	0.147	(2.03)	0.138	1.91	0.145	2.00
48	0.140	1.93	0.138	1.91	0.144	1.99
72	0.139	1.92	0.127	1.75	0.134	1.85
144	0.121	1.66	0.110	1.52	0.133	1.84
Adenine						
2	0.143	1.93	0.138	1.90	0.145	2.00
4	0.138	1.90	0.138	1.90	0.145	2.00
6	0.138	1.90	0.138	1.90	0.145	2.00
24	0.138	1.90	0.138	1.90	0.146	2.00
48	0.138	1.90	0.136	1.89	0.138	1.90
72	0.162	1.62	0.138	1.89	0.137	1.89
144	0.161	1.61	0.125	1.72	0.137	1.89
Cytosine						
2	0.144	1.99	0.137	1.89	0.142	1.96
4	0.137	1.89	(0.149)	(2.06)	0.142	1.96
6	0.137	1.89	0.137	1.89		0.142
24	0.133	1.83	0.137	1.89	0.138	1.90
48	0.133	1.83	0.130	1.79	0.132	1.83
72	0.133	1.83	0.130	1.79	0.130	1.79
144	0.133	1.83	0.130	1.79	0.130	1.79
Cytidine						
2	0.144	1.99	0.145	2.00	0.144	1.99
4	0.134	1.97	0.144	1.99	0.143	1.97
6	0.134	1.97	0.145	2.00	0.143	1.97
24	0.134	1.97	0.143	1.97	0.132	1.82
48	0.133	1.83	0.143	1.97	0.131	1.81
72	0.125	1.72	0.130	1.79	0.126	1.74
144	0.129	1.78	0.129	1.78	0.125	1.72

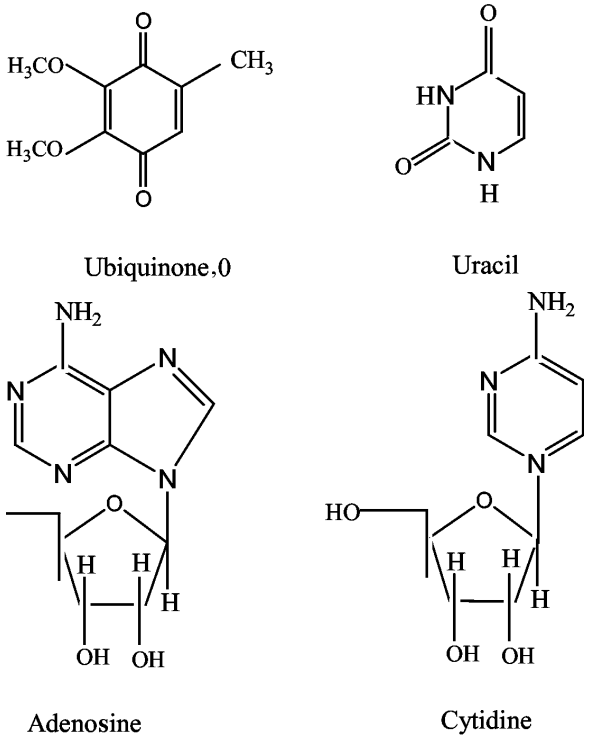


Fig. 2. Structures of ubiquinone,0, uracil, adenosine, and cytidine.

It is seen that the rate of decrease in the *n* values changes from one mixture to the other. Inspection of the structures (Fig. 2) shows the following interesting facts:

– for adenosine the final *n* values, reached after 144 h, were: 1.66 [(1 : 1) mixture], 1.52 [(1 : 2) mixture] and 1.84 [(1 : 3) mixture];
– for adenine, the final *n* values, reached after 144 h, were: 1.61 [(1 : 1) mixture], 1.72 [(1 : 2) mixture] and 1.89 [(1 : 3) mixture];
– for cytosine, the final *n* values, reached after 144 h, were: 1.83 [(1 : 1) mixture], 1.79 [(1 : 2) mixture] and 1.79 [(1 : 3) mixture];
– for cytidine, the final *n* values, reached after 144 h, were: 1.78 [(1 : 1) mixture], 1.78 [(1 : 2) mixture] and 1.72 [(1 : 3) mixture].

Table 4. Measured number of reduction electrons for the Ub,0/nucleic acid bases mixtures with different ratios taken after 48 h.

Nucleic acid base	(1 : 1) Mixture	(1 : 2) Mixture	(1 : 3) Mixture
Adenosine	1.83	1.88	1.82
Adenine	1.93	1.91	1.99
Cytosine	1.83	1.79	1.83
Cytidine	1.83	1.97	1.81

It is to be emphasized that each reported number of coulombs (*Q*) and reduction electrons (*n*) represents the average of different results obtained for the same solution.

Both the number of reduction electrons and rate of reduction seem to depend on the relative concentration of the base. An increase in the reduction rate and decrease in the final *n* value is noticed on increasing the relative nucleic acid base concentration. However some exceptions are to be mentioned, and a systematic dependence of the results on the mixing ratio might be excluded. In Table 4 this is obvious when considering the *n* values taken after 48 h. No systematic change of the *n* values can be detected.

4. Conclusion

The measured change in the number of reduction electrons of ubiquinone,0 due to the presence of nucleosides or nucleic acid bases indicates that a molecular interaction is present between the two components of the mixture even at the low concentrations, $7.5 \cdot 10^{-6}$ mol/l. This finding is of biological importance in view of the known fact that free ubiquinone is found in the mitochondrial matrix of living cells. It is also important on considering the possibility that drug molecules of nucleoside structure might block the function of ubiquinone as electron shuttle in the respiratory chain of the mitochondrion. Although no definite mechanism for the nonreversible reduction process might be concluded directly from the results, one may suggest that a donor-acceptor complex is formed, which seems to undergo a nonreversible reduction.

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