Complexation Constants of Ubiquinone,0 and Ubiquinone,10 with Nucleosides and Nucleic Acid Bases

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UV spectrophotometric measurements were done on mixtures of the ubiquinones Ub,0 and Ub,10 in their monomeric form ($c < 10^{-5}$ mol/l) with the nucleosides; adenosine, cytidine, 2′-desoxy-adenosine, 2′-desoxy-quanosine, guanosine and thymidine, as well as the nucleic acid bases; adenine, cytosine, hypoxanthine, thymine and uracil. Applying the Liptay method, it was found that both ubiquinones form 1 : 1 interaction complexes with the nucleic acid components. The complexation constants were found in the order of 10^5 mol^{-1}. The calculated ΔG values were negative (~ -7.0 kcal/mol), suggesting a favoured hydrogen bridge formation. This is confirmed by the positive change of the entropy ΔS. The complexation enthalpies ΔH for all complexes are negative, suggesting exothermal interactions.

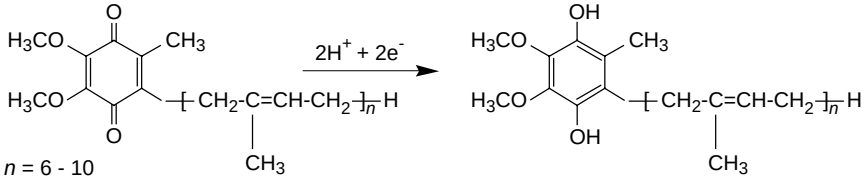
Key words: Ubiquinones; Nucleosides; Complexation; UV Spectra.

1. Introduction

The consumption of oxygen to produce energy required for the biological functions of the living cells [1 – 3] was studied by pioneering chemists such as Wieland [4], Warburg [5 – 7] and Keilin [1], 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 the negative potential and O_2 the positive potential (+0.80 V). Inhibiting any of the electron carriers or a reducing or an oxidizing enzyme in the chain slows down the respiration activity of the cell and leads ultimately to its death. Several such chemical inhibitors had 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



Oxidized form Reduced form

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

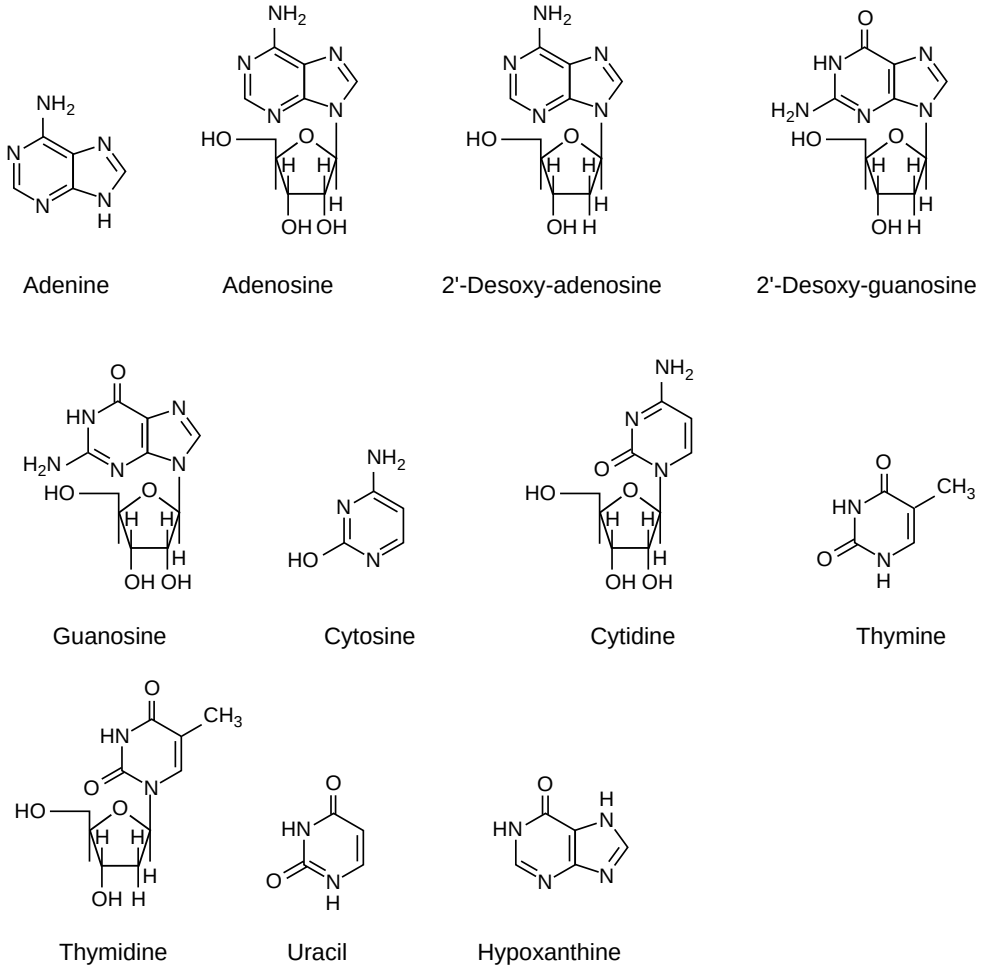


Fig. 2. Structures of examined nucleosides and nucleic acid bases.

electron carriers of the respiratory chain [18]. Due to their mobile property within the mitochondrion, the ubiquinones seem much more susceptible to interact with strange molecules than other electron carriers. They should therefore be the most environmentally sensitive components 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 behaviour of the ubiquinones in solution as well as their modes of interaction with other strange 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.

Recently we have published electrochemical coulometric [28] and polarographic [29] studies of the interaction of the ubiquinones with several nucleic acid bases and nucleosides. It was found that, due to the molecular interactions, significant changes were noticed in the number of reduction electrons, reduction potentials and limited diffusion currents of the ubiquinones.

In the present paper we discuss the molecular complex formation of these nucleic acid bases and nucleosides with the ubiquinones as studied spectrophotometrically. The complexation constants and the related thermodynamic functions are evaluated too. The results should show thermodynamically the extent of complexation affinity which these molecules, or any drug molecules of similar chemical constitu-

tion, possess towards the mitochondrial ubiquinones (CoQ). They should add further support to our recently published results obtained by electrochemical studies [28, 29]. Further, such results may help in the design of new drugs which inhibit the respiration activity of undesired living cells present in a living organism.

2. Experimental

The UV measurements were done applying a Shimadzu 160-UV double beam, computerized spectrophotometer. Constant temperatures were maintained applying a Haake AG thermostat and 1-cm as well as 5-cm matched pairs of quartz cells. The temperature was measured in the cell compartment. 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.

Ubiquinone,0 (Ub,0) was synthesized according to the known procedure [30]. 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 [31]. Aqueous stock solutions of Ub,0 and nucleic acid bases ($1 \cdot 10^{-3}$ 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 (Fig. 2) were kindly supplied by Prof. W. Pfleiderer, Konstanz, Germany.

Due to 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 to avoid complications on analyzing the results. It was necessary to inspect the non-association of each nucleoside, nucleic acid base as well as Ub,0 through UV measurements at concentrations of $2.196 \cdot 10^{-5}$ M and lower, and for Ub,10 at 10^{-5} M and lower. For all solutions constant absorptivity values, ε, were obtained at the wavelengths 240 – 290 nm, indicating the absence of association of the measured species in the solutions, as expected.

To measure the UV spectra of the complexation solutions of Ub,0 with the nucleosides or nucleic acid bases, the following procedure was followed. To a series of 25-ml volumetric flasks containing 2.5 ml of $2.1956 \cdot 10^{-5}$ M Ub,0 2.5 – 10 ml of $1.956 \cdot 10^{-5}$ M nucleoside (or nucleic acid base) were added, and the volume was diluted to the mark with a composite phosphate buffer solution (pH 7.3). The solutions were then placed in a dark water bath at 37 °C for 3 h. The absorbance values were read against the corresponding blanks to establish the composition of the solutions. The same procedure was followed at 27 °C, 20 °C and 13 °C.

To a series of 25-ml volumetric flasks containing 0.1 ml of $5.3175 \cdot 10^{-5}$ M Ub,10 (ethanol solution) 0.1 – 0.4 ml of $5.3175 \cdot 10^{-5}$ M nucleoside (or nucleic acid base) were added, and the volume was diluted to the mark with a composite phosphate buffer solution (pH 7.3). The solutions were then placed in a dark water bath at 37 °C for 3 h. The absorbance values were read against the corresponding blanks to establish the composition of the solutions. The same procedure was followed at 27 °C, 20 °C and 13 °C.

Calculations of the stability constants of the formed molecular complexes were done applying the Benesi-Hilderbrand equation [32]

$$C_A^0 \cdot C_D^0/AD + AD/\varepsilon^2 = 1/\varepsilon K + (C_A^0 + C_D^0)/\varepsilon, \quad (1)$$

where C_A^0 is the initial concentration of the acceptor (Ub,0 or Ub,10), C_D^0 the initial concentration of the donor (nucleosides or nucleic bases), K the stability constant, ε the extinction coefficient (absorptivity) of the formed complex, and AD the observed difference in the absorbance, defined as

$$AD = A_{tot} - \varepsilon_A C_A - \varepsilon_D C_D. \quad (2)$$

ε_A, ε_D and A_{tot} are taken from the UV spectra of the acceptor, the donor and of their mixtures, respectively.

Solutions of (1) for K and ε were obtained using a multiple variable regression program (STAT-21, Hewlett-Packard). The number of complexes in the solution was determined according to Liptay [32] through inspection of the rank of the optical density difference matrix $\mathbf{AD}_{ik}$ as defined using (2). The values of $\mathbf{AD}_{ik}$ were calculated for different concentrations (k) and different wavelengths (i). The applied concentrations are listed in Tables 1 and 2.

The obtained values of K at different temperatures are used to compute the thermodynamic data ΔG, ΔH and ΔS according to

$$\ln K = -\Delta G/RT, \quad (3)$$

$$\Delta G = \Delta H - T\Delta S, \quad (4)$$

or

$$\ln K = \Delta S/R - \Delta H/R \cdot 1/T. \quad (5)$$

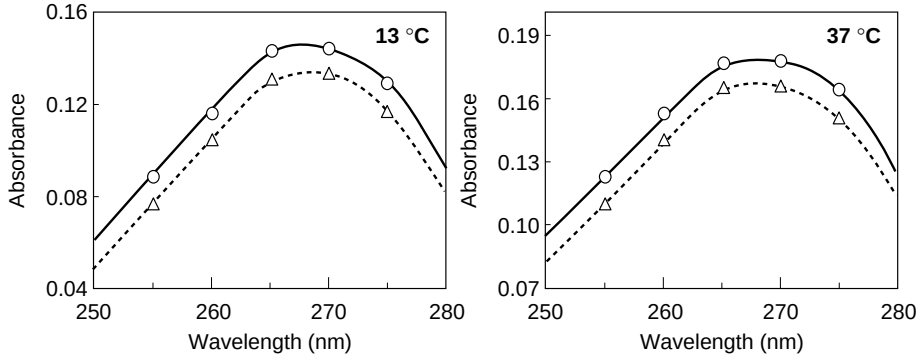


Fig. 3. UV spectra of Ub,0 + adenine ($2.1956 \cdot 10^{-6}$ M) measured at different temperatures.

Table 1. Concentration of all nucleic acid bases and nucleosides applied for the complex formation study with Ub,0.

Sample No.	Concentration, M $\cdot 10^6$	Sample No.	Concentration, M $\cdot 10^6$
1	2.1956	5	6.5868
2	3.2934	6	7.6846
3	4.3912	7	8.7824
4	5.4890		

Table 2. Concentration of all nucleic acid bases and nucleosides applied for the complex formation study with Ub,10.

Sample No.	Concentration, M $\cdot 10^6$	Sample No.	Concentration, M $\cdot 10^6$
1	2.1276	5	6.3828
2	3.1914	6	7.4466
3	4.2552	7	8.5060
4	5.3190		

Plotting $\ln K$ vs. $1/T$, the values of ΔH and ΔS could be obtained from the slope and intercept of the line, respectively.

3. Results and Discussion

As for the complexation of Ub,0 with nucleosides and nucleic acid bases, the UV spectra of the mixtures showed similar bands to that of Ub,0, i. e., no change in λ_{max} was noticed and no new charge transfer band appeared. However, considerable changes in the ε values were noticed. Figure 3 shows, as example, the UV spectra of Ub,0 + adenine ($2.1956 \cdot 10^{-6}$ M) measured at different temperatures.

Table 3 shows the values of the optical density matrix elements according to (2), calculated for the Ub,0-adenine mixture (37 °C). The average value for the $\mathbf{AD}_{ik}$ is 0.011. Division of each matrix element over the corresponding element in the first column of the matrix yields Liptay ratio values ranging from 0.91 – 1.12, i. e., approximate 1.00. The almost unity value of all matrix elements indicates a matrix rank of 1 and a number of complex species in the solution of 1, indicating a 1 : 1 complex formation pattern. A similar treatment shows 1 : 1 complexation for all other bases and nucleosides with both Ub,0 and Ub,10. To compute the K values for each complex, the absorbance values for the mixtures with 7 different concentration ratios taken at 7 different wavelengths were considered. In all measurements the Ub,0 concentration was $2.196 \cdot 10^{-6}$ M, whereas the concentration of the nucleic acid bases and nucleosides varied from $2.196 \cdot$

Table 3. Values of the optical density difference matrix elements (10^{-3}), according to (2), calculated for the Ub,0-adenine mixture (37 °C).

λ, nm	AD^1	AD^2	AD^3	AD^4	AD^5	AD^6	AD^7
250	12	11	12	13	12	13	11
255	12	11	13	11	13	12	14
260	11	12	11	10	13	13	13
265	12	13	12	13	12	11	12
270	11	10	10	10	11	12	12
275	11	11	12	10	12	11	12
280	12	11	13	12	11	14	11

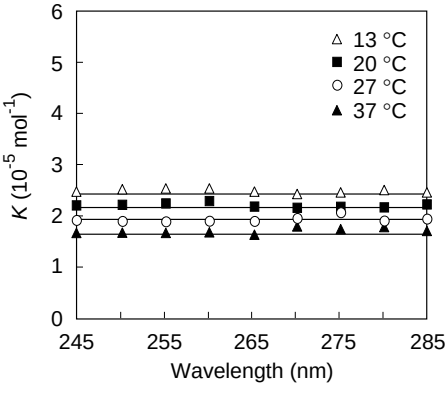


Fig. 4. Variation of the stability constant K of the Ub,0-adenosine complex with different wavelengths.

Table 4. Computed K ($\cdot 10^{-5}$) values for Ub,0-nucleosides (nucleic acid bases) at different temperatures.

Complex	13 °C	20 °C	27 °C	37 °C
Ub,0-adenine	3.317	3.052	2.615	2.364
Ub,0-adenosine	3.786	3.489	3.152	2.837
Ub,0-2′-desoxy-adenosine	3.717	3.445	3.123	2.811
Ub,0-guanosine	4.176	3.868	3.695	3.137
Ub,0-2′-desoxy-guanosine	4.068	3.739	3.583	3.131
Ub,0-hypoxanthine	3.533	3.338	3.031	2.691
Ub,0-uracil	4.074	3.751	3.563	3.127
Ub,0-cytosine	3.746	3.594	3.223	2.933
Ub,0-cytidine	3.924	3.669	3.300	3.071
Ub,0-thymine	4.307	4.053	3.797	3.599
Ub,0-thymidine	4.572	4.308	4.065	3.725

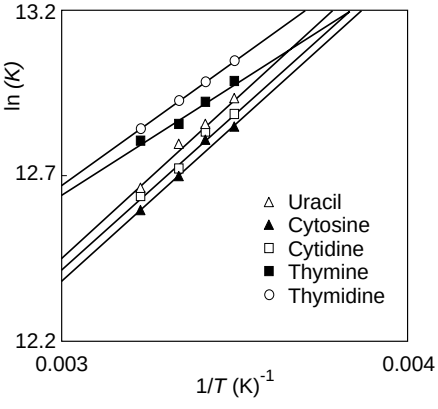


Fig. 5. Plot of the K values of Ub,0 complexes with the nucleosides (nucleic acid bases) vs. $1/T$.

10^{-6} to $8.782 \cdot 10^{-6}$ M in composite phosphate buffer solutions (pH 7.3).

The wavelengths at which the absorbance values were recorded are 280, 275, 270, 265, 260, 255 and 250 nm. The temperatures at which the spectra were recorded are 13, 20, 27, and 37 °C. The computed K values at different wavelengths were almost constant. Figure 4 shows the evaluated K values for the Ub,0-adenosine complex taken at different wavelengths.

The measured K values for all complexes are given in Table 4.

Plotting the K values vs. $1/T$, Fig. 5, it was possible to evaluate the three thermodynamic values for each complex according to (5) (Table 5).

In a similar manner the complexation constants for the Ub,10-nucleosides (nucleic acid bases) complexes were determined. In all measurements, the Ub,10 concentration was $2.127 \cdot 10^{-6}$ M, whereas the concentration of nucleic bases or nucleosides varied

Table 5. Evaluated thermodynamic functions of Ub,0 complexes with nucleosides and nucleic acid bases, measured in aqueous phosphate buffer solutions.

Complex	ΔG, kcal/mol	ΔH, kcal/mol	ΔS, cal/mol·deg
Ub,0-adenine	−7.408	−2.591	16.210
Ub,0-adenosine	−7.502	−2.124	18.086
Ub,0-2′-desoxy-adenosine	−7.495	−2.084	18.203
Ub,0-guanosine	−7.571	−2.043	18.598
Ub,0-2′-desoxy-guanosine	−7.557	−1.860	19.167
Ub,0-hypoxanthine	−7.481	−2.045	18.262
Ub,0-uracil	−7.557	−1.897	19.039
Ub,0-cytosine	−7.513	−1.883	18.942
Ub,0-cytidine	−7.534	−1.861	19.085
Ub,0-thymine	−7.607	−1.336	20.102
Ub,0-thymidine	−7.640	−1.504	20.645

Table 6. Computed K ($\cdot 10^{-5}$) values for Ub,10-nucleosides (nucleic acid bases) at different temperatures, measured in 80% ethanol/20% aqueous phosphate buffer solution.

Complex	13 °C	20 °C	27 °C	37 °C
Ub,10-adenine	2.469	2.240	1.915	1.703
Ub,10-adenosine	3.018	2.717	2.449	2.140
Ub,10-2′-desoxy-adenosine	2.879	2.572	2.311	2.056
Ub,10-guanosine	3.302	2.938	2.637	2.335
Ub,10-2′-desoxy-guanosine	3.160	2.822	2.469	2.173
Ub,10-hypoxanthine	2.709	2.452	2.127	1.880
Ub,10-uracil	2.802	2.513	2.255	1.952
Ub,10-cytosine	3.131	2.814	2.547	2.263
Ub,10-cytidine	3.325	3.010	2.696	2.412
Ub,10-thymine	3.432	3.146	2.843	2.494
Ub,10-thymidine	3.742	3.476	3.258	2.991

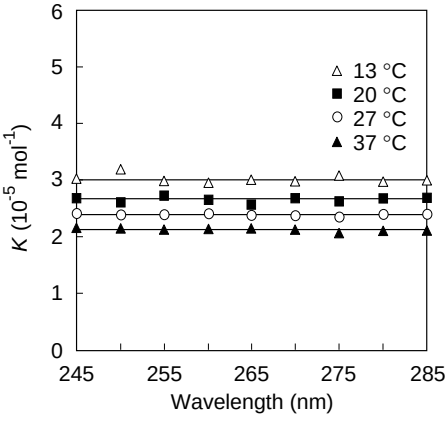


Fig. 6. Variation of the complexation constant of Ub,10-adenosine vs. wavelength, measured at different temperatures.

from $2.127 \cdot 10^{-6}$ to $8.508 \cdot 10^{-6}$ M in the composite solutions. The computed K values at different temperatures (Table 6) showed wavelength independence (Fig. 6).

Table 7. Evaluated thermodynamic functions of Ub,10 complexes with nucleosides and nucleic acid bases, measured in 80% ethanol/20% aqueous phosphate buffer solution.

Complex	ΔG, kcal/mol	ΔH, kcal/mol	ΔS, cal/mol·deg
Ub,10-adenine	−7.227	−2.786	14.942
Ub,10-adenosine	−7.351	−2.529	16.230
Ub,10-2′-desoxy-adenosine	−7.321	−2.480	16.295
Ub,10-guanosine	−7.402	−2.547	16.331
Ub,10-2′-desoxy-guanosine	−7.350	−2.780	15.407
Ub,10-hypoxanthine	−7.290	−2.748	15.254
Ub,10-uracil	−7.305	−2.657	15.636
Ub,10-cytosine	−7.377	−2.386	16.792
Ub,10-cytidine	−7.414	−2.382	16.927
Ub,10-thymine	−7.455	−2.363	17.076
Ub,10-thymidine	−7.514	−1.639	19.765

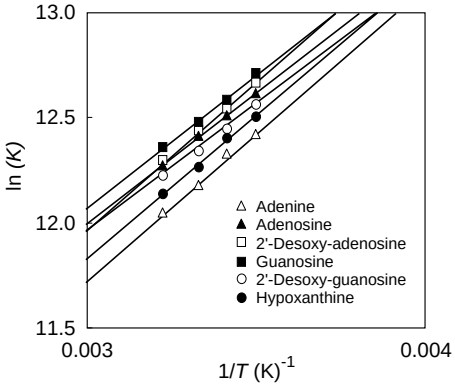


Fig. 7. Plot of the stability constant values of Ub,10 complexes with the nucleic acid bases vs. $1/T$ (K^{-1}).

Plotting the K values vs. $1/T$, Fig. 7, it was possible to evaluate the three thermodynamic values for each complex according to (5) (Table 7).

4. Conclusion

Concluding our results one finds that:

1. The complex formation of Ub,0 and Ub,10 with the nucleosides or nucleic acid bases is an exothermal process, unlike that of the quinoline derivatives and antimalarial [19], which was found to be endothermal. The calculated complexation enthalpy is, however, small (-1.34 to -2.79 kcal·mol^{-1}).

2. The free energy of complexation, ΔG^*, is greater and more negative than the free enthalpy, ΔH, obviously due to the big changes in the entropy for the same process (14.94 – 20.64 cal·mol^{-1}·deg^{-1}).

3. The entropy change is the major factor causing the complex formation. Interesting is that ΔS has approximately similar values for Ub,0 and Ub,10 in spite of the absence of the polyisoprenoid side chain in Ub,0.

4. The entropy change ΔS, for these complexes (14.94 – 20.64 cal·mol^{-1}·deg^{-1}) is smaller than that for the antimalarials and quinoline derivatives with Ub,10 (21.56 – 40.77 cal·mol^{-1}·deg^{-1}) [19].

5. Apparent from the small ΔH values, the exothermal complex formation is due to a face to face (π-π) interaction of the ubiquinone ring with the nucleoside (nucleic acid base) heterocyclic ring.

6. Neglecting the role of the isoprenoid side chain, the change in entropy, ΔS, might then be due to the change in the number of solvent molecules surrounding both interaction partners, i. e., more solvent molecules are liberated on forming the molecular complex.

7. Introduction of a sugar rest increases the complexation affinity of the nucleic acid base with both ubiquinone molecules.

The present results confirm our assumption, based on the recent electrochemical studies [28, 29], that molecular complexes are formed between the ubiquinone molecules and the nucleic acid bases and nucleosides in the solution mixtures. The complex formation causes partial changes in the electrochemical as well as thermodynamic properties of the ubiquinones in the solution. This change should have obvious impacts on the function of the ubiquinones within the respiration activity of the mitochondrion.

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