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Light-Stimulated Generation of Free Radicals by Quinones-Chelators

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Abstract: The role of metal ions in the mechanism of light-stimulated redox activity of potential anticancer agent 2-phenyl-4-(butylamino)naphtha[2,3-h]quinoline-7,12-dione (Qc) has been studied by CIDNP (chemically induced dynamic nuclear polarization) and EPR methods. The photo-induced oxidation of NADH and its synthetic analog – substituted dihydropyridine (DHP) – by quinone Qc was used as a model. The Qc capability of producing chelating complexes with divalent metal ions of Fe, Zn and Ca was studied quantitatively by optical absorption spectroscopy. A significant decrease of electrochemical reduction potential of Qc ($\Delta E = 0.4 - 0.6$ eV for ACN and ACN/PBS solutions) in chelating complexes and in protonated form of Qc was observed. A pronounced increase in efficiency of DHP oxidation in chelating complexes with Zn²⁺ and Ca²⁺ ions compared with free Qc was demonstrated. The yields of free radicals, including reactive oxygen species (ROS) and reaction products, were a few times higher than those in the absence of metal ions. Application of such chelating compounds to enhance ROS generation looks very promising for anti-cancer therapy, including the photodynamic therapy.

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Dedicated to: Kev Salikhov on the occasion of his 80th birthday.

1 Introduction

A significant role of metal ions, especially iron, in human body is well described in a number of review articles [1, 2]. Recent advances in understanding the role of iron and ROS in cell death suggest new therapeutic approaches to treat cancer and other diseases [1]. In addition, metal ions can affect chemical properties (e.g. antioxidant or pro-oxidant activity) of various biomolecules, drugs and vitamins [2–5]. According to recent findings the antitumor activity of antracycline antibiotics, such as adriamycin, daunomycin and others, is associated with the production of reactive oxygen species (ROS) [6–9]. The processes of ROS generation stimulated by quinones are presented in Scheme 1 derived from the results obtained by EPR spin trapping, optical and electrochemical methods [6, 10–14].

The quinones capable of chelating iron ions (adriamycin, for example) demonstrate higher activity in the enzymatic generation of toxic hydroxyl radicals [10, 11]. However, the mechanism of their chelation impact on ROS production is still debated [10, 15–17]. The increase of ROS generation by quinone-chelator in the reaction with NADPH-dependent enzyme is due to the intramolecular electron transfer inside the molecular complex of quinone with Fe³⁺ [10]. The intra molecular electron transfer from semiquinone radical to Fe³⁺ in the complex might be an the source of redox-active Fe²⁺ ions (see Scheme 1), which is considered to be a source of hydroxyl radicals killing the cancer cell [18].

Quinones-chelators have shown high "killing" activity in various types of cells (in dark conditions) [11–13]. Testing of a possible light stimulated redox

 $\begin{array}{l} Q + e \rightarrow Q^{\star-} \\ Q^{\star-} + Q_2 \Leftrightarrow Q + Q_2^{\star-} \\ O_2^{\star-} + Q_2^{-} & \xrightarrow{2H^{\star}} H_2O_2 + O_2 \\ O_2^{\star-} + H_2O_2 \rightarrow O_2 + HO^{-} + HO^{\star} \\ Q^{\star-} + Fe(\mathrm{III}) \rightarrow Q + Fe(\mathrm{II}) \\ H_2O_2 + Fe(\mathrm{III}) \rightarrow HO^{-} + HO^{\star} + Fe(\mathrm{III}) \end{array}$

Scheme 1: The scheme of ROS generation during the interaction of quinones (Q) with electron donors in the presence of iron in the system.

activity of quinone-chelator and possible influence of metals on this activity was the purpose of the present work. To answer this question, we traced individual stages of NADH photo oxidation by quinone-chelator and its metal complexes. Significant opportunities are provided by chemically induced dynamics nuclear polarization (CIDNP) technique. It allows detection of short-lived paramagnetic intermediates resulting from photo-oxidation [19]. This is important because the process under study may involve the electron or "electron + proton" transfer. CIDNP intensity is generally proportional to the concentration of radical species. Thus, the impacts of metal ions on the yield of radical ions or free radicals generated by quinones can be compared quantitatively [19]. We used model processes of photoinduced oxidation of NADH and its synthetic analog 1,4-DHP by quinones Qc and Qn (as a control, Figure 1). The reaction mechanism proposed on the basis of CIDNP data was confirmed by EPR, including spin trapping, studies.

Results obtained in the study of photoinduced processes may also be used to describe the dark oxidation reactions of quinone-chelator because photoirradiation is frequently used to simulate the electron transfer and radicals generation processes [20–22]. It is based on two assumptions: (1) the reactivity of active intermediates would not depend on their generation pathway; (2) if the activation barrier in the drug-receptor or drug–enzyme complexes is decreased through the charge–transfer complex formation, a similar effect in model photoinduced reaction can be achieved by increasing the oxidation potential of the drug in its excited state. Moreover, substitution of photo-generation for enzymatic reduction leads to a significant increase in concentration of radical intermediates.

Note that the photoinduced electron and energy transfer, involving quinones is of independent interest because some quinones are also known as



Fig. 1: The structure of quinone-chelator 2-phenyl-4-(butylamino)naphtho[2,3-h] quinoline-7,12-dione (Qc) and quinone 2-phenyl-5-nitronaphtho[2,3-g]indole-6,11-dione (Qn) without a chelating group.

agents for photodynamic therapy [6]. Most likely, their cytotoxicity occurs through photo-redox reactions, via two major reaction pathways (see Scheme 2) [6, 23–26].

The mechanism of radical particles generation involves a direct reaction of quinone (Q^{*}) in excited triplet state with a substrate via electron transfer or hydrogen transfer from the electron donor to the quinone (see Scheme 2). These pathways are oxygen independent and could occur in hypoxic environments. In the presence of oxygen, the resulting semiquinone radicals will lead to ROS generation, as in dark reactions (see Scheme 1). Thus, knowledge of a detailed mechanism of impact of metal ions on semiquinone formation might be important for Qc application in photodynamic therapy as well.

Since the CIDNP method is based on the NMR phenomenon, the chemical nature of the impact of chelation with metal ions on the ability of Qc to generate the paramagnetic particles was elucidated using the non-paramagnetic ions of calcium and zinc (Zn²⁺ and Ca²⁺). Formation of chelating complexes with Fe³⁺, Fe²⁺, Zn²⁺ and Ca²⁺ ions was investigated by optical absorption spectroscopy. Electrochemical redox potentials were measured by the standard CV method. The formation of chelating complexes of Qc with Zn²⁺ and Ca²⁺ was also confirmed by ¹H NMR technique that allows detection of so-called "weak interactions", namely, non-covalent binding of quinone with metal ions.

2 Experimental

2.1 Reagents

Quinone chelators, 2-phenyl-4-(butylamino)naphtho[2,3-h]quinoline-7,12-dione (Qc), and a quinone 2-phenyl-5-nitronaphtho[2,3-g]indole-6,11-dione (Qn) without chelating group (Figure 1) were synthesized according to the procedure described in [10]. 2,2,6,6-Tetramethyl piperidine (TEMP), 5,5-dimethylpyrroline N-oxide (DMPO), alpha-(4-pyridyl-1-oxide)-N-tert-butylnitrone (4-POBN) and

 $\begin{aligned} Q + hv &\to Q^* \\ Q^* + D &\to Q^{*-} + D^{*+} \\ Q^* + D &\to QH^* + (D-H)^* \\ Q^* + O_2 &\to Q+^1O_2 \end{aligned}$

Scheme 2: The scheme of free radicals and singlet oxygen generation in the presence of electron donor (D) and molecular oxygen under photoexcitation of quinones (Q).

nicotinamide adenine dinucleotide (NADH) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). The colored impurity present in the commercial DMPO was removed by treatment with neutral decolorizing charcoal [27]. 2,6-Dimethyl-3,5-dicarbomethoxy-1,4-dihydropyridine (DHP) was received from Prof. G. Duburs, Institute of Organic Syntesis, Riga, Latvia. Deuterated solvents for NMR, CD₃OD, CD₃CN and D₂O (Aldrich) were used as supplied.

2.2 Complex preparation

Chelating complexes were prepared using FeSO_4 , $\text{Fe}(\text{ClO}_4)_3$, CaCl_2 , $\text{Zn}(\text{NO}_3)_2$ and ZnCl_2 (98%, Aldrich). Optical spectra of Qc and its complexes were measured in ethanol solution using SF-2000 (Spectrum, Russia) spectrophotometer in 1 cm quartz cuvette.

2.3 NMR and CIDNP measurements

The mechanism of phototransformation of Qc and its complexes with Zn²⁺ and Ca²⁺ was elucidated by CIDNP and NMR techniques. CIDNP and NMR spectra were detected using NMR spectrometer DPX-200 (Bruker). For CIDNP experiments, samples in standard 5 mm Pyrex NMR tubes were irradiated directly in the probe of an NMR spectrometer at room temperature. An EMG 101 MSC Lambda Physik excimer laser was used as a light source for CIDNP experiments (λ =308 nm, pulse duration 15 ns, average pulse energy 100 mJ). Quasi Steady State (QSS) CIDNP experiments were performed using the special presaturation technique: saturation – 180° pulse – a number of laser pulses – evolution time – detection pulse – free induction decay. Since the background (equilibrium) NMR signals in the pulse CIDNP experiments were suppressed, only the signals of the products demonstrating nuclear polarization could be observed [28].

2.4 EPR measurements

All EPR experiments were performed with a Bruker electron paramagnetic resonance ELEXSYS-500 spectrometer at 25 °C. For EPR studies, the irradiation source was a KL 1500 electronic projector lamp (Schott, Germany). Irradiations were carried out with appropriate filters according UV/visible spectra at band-pass of 320–510 nm with λ_{max} = 400 nm or cutoff filters of 355 nm. Samples were irradiated directly inside the EPR cavity with an optical fiber while the EPR spectra

were recorded. For spin trapping studies, a mixture of DMPO (0.1 M) or POBN or TEMP (0.1 M) and quinone (0.05–0.1 mM) was irradiated in a EPR flat cell. NADH was used as potential donor in phosphate buffered saline (PBS) solutions. For *g* factor measurements EPR signal of DPPH (g=2.0037) was used.

2.5 The complex stability and stoichiometry calculation

Calculation of the complex formation parameters was based on the model of stepped complexation:

$$L + M \rightleftharpoons LM$$
$$LM + L \rightleftharpoons L_2M$$

where L is light absorbing ligand, M is metal. Stability constants in this case are equal to

$$K_1 = \frac{[LM]}{[L][M]},\tag{1}$$

for complex 1:1 and

$$K_{2} = \frac{[L_{2}M]}{[LM][L]},$$
(2)

for complex 2:1.

If C_{M} and C_{L} are initial concentrations of metal and ligand in solution then:

$$C_{M} = [M] + [LM] + [L_{2}M], \qquad (3)$$

$$C_{L} = [L] + [LM] + 2[L_{2}M]$$
(4)

Reformulation of equations (3) and (4) using equations (1) and (2) results in:

$$C_{M} = [M] + K_{1}[L][M] + K_{1}K_{2}[L]^{2}[M],$$
(5)

$$C_{L} = [L] + K_{1}[L][M] + 2K_{1}K_{2}[L]^{2}[M]$$
(6)

According to equation (5) $[M] = \frac{C_M}{K_1 K_2 [L]^2 + K_1 [L] + 1}$. Substitution of this

expression in equation (6) results in the following equation:

$$K_1 K_2 [L]^3 + (2K_1 K_2 C_M - K_1 K_2 C_L) [L]^2 + (K_1 C_M - K_1 C_L + 1) [L] - C_L = 0$$
(7)

The relative change of the ligand absorption is equal to

$$\Delta D = \varepsilon_0[L] + \varepsilon_1 K_1[L][M] + \varepsilon_2 K_1 K_2[L]^2[M] - \varepsilon_0 C_L, \tag{8}$$

where ε_0 , ε_1 and ε_2 are extinction coefficients of the ligand, the complex 1:1 and the complex 2:1, respectively. Substituting the solution of (7) in the expression (8), one can obtain the dependence of ΔD on the metal concentration in solution. To determine the parameters of complex formation from the experimental dependence of ΔD on the metal concentration, the script was written in Python. It performs a numerical solution of the equation (7) for each experimental point C_M . Thus the coefficients in (8) were obtained. Then the method of least squares was used to obtain the best fitting parameters ε_1 , ε_2 , K_1 and K_2 , and the covariation matrix was calculated.

2.6 Electrochemical (CV) measurements

Electrochemical (CV) measurements were carried out in dry acetonitrile or ACN/Phosphate buffer with 0.1 M tetraethylammonium perchlorate (TEAP) as described previously [29].

3 Results and discussion

Since parameters of NMR and optical spectra of chelating quinones are very sensitive to the binding with metal ions, these techniques were applied to prove that chelating complexes could form complexes with Fe³⁺, Fe²⁺, Zn²⁺ and Ca²⁺ ions. Stoichiometry, extinction coefficients and stability constants of the resulting complexes were calculated.

3.1 Detection of chelating complexes formation by ¹H NMR technique

A high sensitivity of NMR spectra of organic molecules with chelating ability to the presence of divalent metal ions was demonstrated earlier [3]. Since NMR spectra of Qc in aqueous and methanol solutions show very broad signals of quinone protons, the impact of Zn ion on ¹H NMR spectra of quinone was measured in CDCl₃ solution. Figure 2 shows the changes of chemical shifts and line widths of Qc aromatic protons in the presence of zinc acetate. It should be emphasized that



Fig. 2: Fragment of ¹H NMR spectra (aromatic region) of Qc in the absence and in the presence of zinc acetate in chloroform solution.

these changes do not occur in the NMR spectrum of quinone Qn in the presence of metal ions. The dependence of chemical shifts of Qc and its line widths on the metal concentration indicates the complex formation characterized by fast equilibrium (on the NMR time scale) with free Qc molecules.

However, for the quantitative characterization of the complex we will use optical spectroscopy due to the complexity of the analysis of NMR spectra.

3.2 Optical absorption study of chelating complexes

The complex formation of Qc with Fe³⁺, Fe²⁺, Zn²⁺ and Ca²⁺ ions has been proved using optical absorption method. Optical absorption spectra of the quinone were measured for divalent ion concentrations ranging from 0.01 to 0.1 mM. As an example, one can see the changes of optical density of Qc in the presence of Zn ions. Figure 3 shows that optical density at 433 nm increased with increasing metal concentration up to 0.05 mM and then saturated. This indicates that the equilibrium is completely shifted towards the complex. The absorption spectrum of quinone Qn without chelating group (which was applied as a control) does not show any changes with addition of metal ions (data not shown).

Taking into account the concentration of Qc = 0.05 mM and the presence of isobestic points, we can conclude that the chelating complex with Zn ions have 1:1 stoichiometry. The same measurements were carried out for other metals, and similar changes in absorption spectra were observed. These complexes enhanced



Fig. 3: The absorption spectra of 0.05 mM Qc solution for different concentrations of $Zn(NO_3)_2$ (0–0.1 mM) in ethanol.

absorption below 475 nm and significantly reduced absorption at λ > 475 nm. To determine the stoichiometry, extinction coefficients and stability constants of all complexes, the experimental dependences of ΔD were compared with a theoretical one using the specifically developed program based on a step-by-step model of complex formation (see Experimental part). The examples of simulation are presented in Figure 4, and all results are summarized in Table 1.



Fig. 4: The experimental and calculated dependences of ΔD of quinone Qc (0.05 mM) as a function of metal ion concentration, [Me], in ethanol solution.

	Complex stoichiometry	Extinction coefficients at 433 nm, (M ⁻¹ ·cm ⁻¹)	Stability constants, (M ⁻¹)
Q + Fe(II)	2:1	$\varepsilon_1 = (1.1 \pm 0.1) \cdot 10^4$	$K_1 = (4.4 \pm 1.0) \cdot 10^5$
		$\varepsilon_{2} = (17.9 \pm 0.1) \cdot 10^{3}$	$K_{2} = (6.2 \pm 2.1) \cdot 10^{5}$
Q+Ca(II)	2:1	$\varepsilon_{1} = (8.0 \pm 0.1) \cdot 10^{3}$	$K_1 = (1.7 \pm 0.5) \cdot 10^5$
		$\varepsilon_{2} = (10.7 \pm 0.2) \cdot 10^{3}$	$K_{2} = (2.5 \pm 1.6) \cdot 10^{4}$
Q+Zn(II)	1:1	$\varepsilon_1 = (9.2 \pm 0.1) \cdot 10^3$	$K_1 = (3.3 \pm 1.5) \cdot 10^6$
Q + Fe(III)	1:1	$\varepsilon_1 = (8.8 \pm 0.1) \cdot 10^3$	$K_1 > 3.10^7$

Tab. 1: Calculated parameters of Qc chelating complexes in ethanol solution.

Note that the presence of an isobestic point on a graph at a concentration ratio of 1:1 does not mean the absence of 2:1 complex with Zn^{2+} similar to other divalent ions under study. This complex can be "invisible" for $\Delta\varepsilon_{1:1}(Q) = 2\Delta\varepsilon_{1:2}(Q)$. The same behavior was observed for the complex with Fe³⁺: we cannot exclude 3:1 complex formation at lower iron concentrations, as it was detected by Dikalov et al. for CDCl₃ solution of Qc [10]. From Table 1 it follows that the stability constants of Qc complexes with zinc and calcium ions are inferior to those of the iron. The proposed structure of the chelating complex with divalent metal ions is shown in Figure 5.

3.3 CIDNP and EPR study of free radicals formation during photoinduced interaction of the quinone-chelator with NADH

Nowadays CIDNP and EPR methods are widely used to study the mechanisms of practically important photoinduced radical processes [30, 31]. The possibility



Fig. 5: The structure of Qc chelating complex with divalent metal ions.

of electron transfer in the photoinduced process is usually estimated using the Rehm–Weller equation [19, 32].

$$\Delta G_{\rm et} = E_{\rm ox} - E_{\rm red} - E_T + \frac{2.6 \,\mathrm{eV}}{\varepsilon} - 0.13 \,\mathrm{eV}$$

Using E_{ox} (DHP, NADH) = 0.5–0.7 V [33], E_{red} (anthraquinones) = – 0.8 – 0.9 V and E_T (AQs) = 2.3 – 2.5 eV [34–36], we can estimate that in our systems $\Delta G_{et} < 0$. Thus, the photo-induced electron transfer between the triplet excited state of quinone and NADH (or DHP) in the ground state is thermodynamically possible.

Figure 6 shows EPR evidence of influence NADH and light on generation semiquinone and ROS in the presence of Qc in solution. The observed singlet [Figure 6a (1–4)] has characteristic line width and g-value typical for organic semiquinones. From Figure 6a also it is clear that influence of light is much stronger, than effect of a reducing agent. The highest concentration of semiquinone was observed at simultaneous effects of NADH and light.

Figure 6b (1) shows emergence, characteristic for the spin adduct of DMPO with OH radical $a_N = a_H = 14.95$ G (the quartet 1:2:2:1 is shown by asterisks). The data obtained with DMPO have allowed to use 4-POBN as a spin trap. Spin adduct of 4-POBN with hydroxyl radical increased at addition in system of iron ions [Figure 6b (4)]. EPR data are well described by Schemes 1 and 2. When using TEMP (a spin trap for a singlet oxygen) at radiation we have not found emergence of a characteristic EPR triplet. It means, for the studied system more effective is photo electron transfer than energy transfer.

In the CIDNP experiment, the UV irradiation of oxygen-free Qc solution produces a weak polarization (emission) of $N-CH_2$ and 1-CH protons in the CIDNP spectrum (Figure 7).

Note that the CIDNP effect occurred only in the presence of a metal ion. An analysis using Kaptein rules [19] shows that the effects might be associated with electron transfer between the carbonyl group of the quinone molecule in a triplet excited state and amine nitrogen of another Qc molecule in the ground state (see Scheme 3, *marks the polarized product).

In this case, negative polarization corresponds to the product of back electron transfer in a singlet spin state of the radical ion pair (RIP) with triplet precursor, where *g*-factor of Qc radical anion is higher than that of the corresponding radical cation, and hfi constant of 14-CH, protons is positive.

The addition of NADH to the reaction mixture causes the appearance of polarized 4-H protons of NADH and reaction product NAD+ in CIDNP



Fig. 6: Photogeneration of semiquinone radical (a) and ROS (b) by Qc (50 mM) in phosphate buffer/acetonitrile (10%) at pH = 7.3 (aerobic condition). (a) EPR spectra of the Qc: 1 – dark; 2 – dark in the presence of 150 mM of NADH; 3 – under irradiation in an EPR cavity (3 min); 4 – under irradiation in the presence of NADH (3 min). (b) EPR spectra of spin adducts of spin traps under irradiation of Qc (0.1 mM) in the presence of NADH (0.3 mM): 1 – in the presence of DMPO (50 mM), time of irradiation 1.5 min; 2 – in the presence of POBN (50 mM), time of irradiation 3 min; 4 – in the presence of Fe³⁺ (0.2 mM) and POBN (50 mM), time of irradiation 3 min.

spectrum (Figure 8) in accordance with the scheme of NADH oxidation (see Scheme 4) [37].

Evidence for CIDNP formation in the RIP consisted of quinone radical anion and NADH radical cation comes from the observation of a relatively high polarization intensity on 4-H protons of NADH and reaction products NAD+. This result is in agreement with the spin density distribution in the corresponding radical cations (a(4-H) = 46 G). This value is significantly higher than hfi constants for



Fig. 7: CIDNP spectra detected during photolysis of 1 mM Qc in the absence and presence of 4 mM Zn(NO₃)₂ in methanol solution. Top: NMR spectra of Qc in the presence of Zn(NO₃)₂ in methanol solution.



Scheme 3: The scheme of electron transfer between two quinone molecules, and the structure of corresponding radical ion pair.

other protons in the radical pair (1-6 G) [36–41]. According to the Kaptein rules of CIDNP analysis [19, 37], the negative polarization of NADH protons means that the polarized NADH results from the back electron transfer in singlet RIP with triplet precursor, a4 > 0, $\Delta g < 0$. Note, light in this system is largely absorbed by quinone (ε (Qc) $\approx 10^4$ M⁻¹ cm⁻¹, ε (NADH) = 6.2×10^3 M⁻¹ cm⁻¹ at 340 nm. Positive polarization of NAD+ 4-H protons means that NAD+ is an "escape" product of the same RIP in accordance with Scheme 4. CIDNP of quinone protons has not been detected due to relatively low hfi constants. So, the analysis of the EPR and the CIDNP data shows that the quinone interacts with the NADH through the electron transfer step (see Scheme 4).



Fig. 8: Fragments of CIDNP spectrum (red) and NMR spectra detected before and after the photolysis of 1 mM Q in the presence of 1 mM NADH in $1:1 \text{ phosphate buffer/CD}_3\text{CN}$ solution at pH 6.8.



Processes in solution:



Scheme 4: The mechanism of NADH photo-oxidation by quinones [37].

3.4 CIDNP study of the role of metal binding in photoinduced interaction of the quinone-chelator with NADH analogs

To study the influence of metal binding on the efficiency of the free radicals generation by quinone, CIDNP technique was also applied. Taking into account low solubility of quinone and its chelating complexes in water and acetonitrile aqueous solutions, the role of metal ions was studied in methanol and acetonitrile solutions of Qc with synthetic analog of NADH – 2,6-dimethyl-3,5-dicarbomethoxy-1,4-dihydropyridine (DHP) in the presence of Zn and Ca ions. The photoinduced electron transfer from the electron donor DHP to quinones was examined in detail previously and the mechanism of DHP oxidation was similar to that for NADH oxidation (Scheme 4, [37]). In both cases, the first step of photoreaction is electron transfer from donor to quinone with RIP formation accompanied by CIDNP effects. Similar to NADH oxidation, evidence for the CIDNP formation in RIP consisted of quinone radical anion and DHP radical cation comes from the observation of high polarization intensity on 4-H protons of DHP and 2,6-dimethyl-3,5-dicarbomethoxy-pyridine in accordance with spin density distribution in DHP radical cations [36–41].

Figure 8 shows CIDNP spectra registered during quinone irradiation with DHP in the presence and absence of an excess of ZnCl_2 . The main polarized lines in CIDNP spectra are 4-H and 2-CH₃ protons of initial DHP and reaction product – corresponding pyridine (Pyr, Figure 9). One can see a significant increase of polarization intensity in the presence of metal ions. The same changes were observed in NMR spectra of the reaction products after photolysis (Figure 10). A three-fold increase of the yield of reaction product was detected in the presence of Zn ions.

It is important that both CIDNP intensity and the yield of reaction product increase with Zn^{2+} concentration up to 0.5 mM ([Zn]/[Qc] = 0.5, Figure 11). It means that the complex with stoichiometry 1:2 contributes most to the increase of quinone reactivity.



Fig. 9: QSS CIDNP spectra detected during photolysis of 1 mM Q solution with 4 mM DHP in the absence and presence of ZnCl, (10 mM) in CD₃OD. Irradiation – 256 laser pulses at 308 nm.



Fig. 10: NMR spectra detected after the photolysis of 1 mM Qc solution with 4 mM DHP in the absence and presence of ZnCl, (10 mM) in CD₃OD.



Fig. 11: Dependences of CIDNP intensity of 4-H protons of DHP and the yield of reaction product on Zn^{2+} concentration detected after the photolysis of 1 mM Qc solution with 4 mM DHP in CD₃OD.

This observation disagrees with optical measurements that show only 1:1 complex formation. We can suggest that, like for other divalent ions, 2:1 complex with Zn^{2+} is also formed at low Zn^{2+} concentration; however, as it was mentioned above, this complex can be "invisible" for $\Delta \varepsilon_{1:1}(Q) = 2\Delta \varepsilon_{1:2}(Q)$. For this reason we have also measured the dependence of the product yield and CIDNP intensity in the photoinduced interaction of DHP with Qc on the concentration of Ca²⁺ ions (Figure 12).



Fig. 12: Dependences of the reaction product yield (Pyr) and its CIDNP intensity (4-H protons) on Ca^{2+} concentration detected after the photolysis of 1 mM Q solution with 4 mM DHP in CD₃OD.

Both complexes (with Ca^{2+} and Zn^{2+} ions) show similar dependences on salt concentration. This confirms our previous suggestion about the structure of reactive chelating complexes.

Thus, the growth of the CIDNP intensity and the yield of reaction product suggests that the presence of metal ions increases the reactivity of quinone Qc. This result is entirely consistent with the published observations on the impact of chelation on the enzymatic NADH oxidation effectiveness [10]. Earlier, the quinone Qc capable of producing hydroxyl radicals was compared with another quinone (Qn, Figure 1) without chelating ability [10]. The quinone Qc in the Fe³⁺ complex stimulated the formation of OH radicals in the enzymatic system NADPH and NADPH-cytochrome P-450 reductase more efficiently than Qn. We have confirmed that Qn does not form chelating complexes with Ca²⁺ and Zn²⁺ ions. Neither we have observed any increase of Qn reactivity in the presence of metal ions.

3.5 CV study of chelating complexes

Earlier a slight decrease in polarographic half-wave potential of Qc was found in the presence of iron in DMSO solution [10]. To test the hypothesis that the increased activity of quinones-chelators may be associated with the influence of metal ions on their oxidation capacity, electrochemical potentials of Qc were measured in acetonitrile and acetonitrile/phosphate buffer (pH=7.4) in the absence and pres-

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ence of metal ions (Zn, Ca, Fe), Table 2. The reaction was carried out in a buffer for the control, in order to eliminate the influence of quinone protonation, which according to our measurements, also leads to a significant change of quinone reduction potentials (Figure 13).

Svstem	Solvent	E . (V)	E . (V)
		p1 • •	p2
Q	Dry ACN	-0.85 ± 0.01	-1.10 ± 0.01
Q:CaCl ₂	Dry ACN	-0.30 ± 0.02	-0.67 ± 0.02
Q:ZnCl ₂	Dry ACN	-0.27 ± 0.02	-0.67 ± 0.02
$Q:Zn(OAc)_{2}$	Dry ACN	-0.32 ± 0.02	-0.64 ± 0.02
$Q: Fe(ClO_4)_3$	Dry ACN	-0.20 ± 0.04	-0.66 ± 0.01
		(Double peak :	
		-0.16 and -0.24)	
Q	ACN/buffer (pH = 7.4)	-0.78 ± 0.01	_a
Q:ZnCl ₂	ACN/buffer (pH = 7.4)	-0.33 ± 0.02	_a

Tab. 2: Reduction potentials of quinone-chelator in the presence of metal ions (1:1).

^aOne broad peak.



Fig. 13: CV of Qc in CH₃CN – 0.1 M TEAP in the absence (left) and in the presence (right) of HCl ([Q] : [HCl] = 2 : 1). $E_{p}^{c1} = -0.28$ V, $E_{p}^{c2} = -0.67$ V, $E_{p}^{1C} = -0.87$ V, $E_{p}^{2C} = -1.10$ V.

From Table 2 it follows that binding with positive metal ions reduces the energy barrier for electron transfer. It might be important for initiating both the dark and photo-induced reactions of Qc. The examples of strong influence of metal ions on redox potentials of chelating ligands are well known [42].

Figure 13 also demonstrates significant changes of the reduction potential of the Qc protonated form. (The calculated $P_{\rm ka}$ (Qc)=4.8). This finding might be responsible for enhanced selectivity of Qc antitumor activity since the difference in acidity of tumor and healthy cells is well established [43].

4 Conclusion

The formation of chelating complexes of quinone Qc with metal ions, Zn^{2+} , Ca^{2+} , Fe^{2+} and Fe^{3+} , which are characterized by high stability ($K = 10^5 - 10^7 \text{ M}^{-1}$) has been demonstrated by NMR and optical spectroscopy. As found, chelation leads to a significant decrease of electrochemical potentials of Qc. The study of the model processes of photo-induced oxidation of NADH and its synthetic analog by Qc chelating complexes has demonstrated that chelation has a strong effect on generation of semiquinone radicals and ROS. The yields of free radicals and reaction products were a few times higher than when compared with reaction in the absence of an electron donor the semiquinone radicals can be generated by Qc itself and by its chelating complex with zinc under UV irradiation. According to the CIDNP and EPR data, in the absence of an external donor the electron transfer occurs between the carbonyl and amino groups of Qc.

We suppose that the influence of chelation on the electrochemical halfwave potential of Qc is the important factor of the impact of chelation on the photo-induced oxidation of donors. Considering the similarity between the enzymatic and photoinduced mechanisms of free radical generation by quinones, the proposed mechanism of the impact of chelation on the efficiency of NADH oxidation will be useful for understanding the nature of anti-tumor activity of quinones – chelators.

Another possible mechanism of enhanced cytotoxicity of the quinones – chelators is the possibility of electron transfer from semiquinone radical anion to Fe^{3+} in the chelating complex [6, 10]. This reaction results in the formation of steady-state concentration of redox active Fe^{2+} to be responsible for production of hydroxyl radicals in cancer cells via Fenton-like processes [18, 44, 45].

We suppose that these results might be also important for designing new anticancer compounds, which will be active not only in the 'dark' conditions but also for photodynamic therapy, which is now regarded as one of the most promising therapies of selected cancer diseases [6, 46].

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