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ON THE FEASIBILITY OF ELECTRON TRANSFER TO SINGLET OXYGEN FROM MITOCHONDRIAL COMPONENTS

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SUMMARY

It has been demonstrated that $O_2^*(^1\Delta_g)$ is capable of causing oxidation of mitochondrial components via a one electron process. Kinetic data have been obtained for $O_2^*(^1\Delta_g)$ quenching by NADH, NAD+, and reduced cytochrome c. Preliminary mechanistic investigations have been undertaken.

INTRODUCTION

The excitation of molecular oxygen to produce $O_2^*(^1\Delta_g)$ (ΔE = 1eV) should result in a concomitant increase in the one electron reduction potential from -0.33V (vs. NHE at 1 atm. O_2) to $\sim$ +0.67V. for the excited species.[1] The biological ramifications of these arguments are made apparent by considering the one electron redox potentials of the various components of the mitochondrial electron transport chain[2] presented in figure 1. In principle, mitochondrial components could be susceptible to one electron oxidation reactions with $O_2^*(^1\Delta_g)$, both in-vivo and isolated forms, while remaining unaffected by ground state molecular oxygen.

Frenkel et al.[3] have recently proposed that $O_2^*(^1\Delta_g)$, formed via energy transfer from hematoporphyrin sensitizer, can oxidise NADH and NADPH. Concomitant formation of $O_2^{\cdot-}$ was observed as evidenced by reduction of added ferricytochrome c which was inhibited by catalytic concentrations of superoxide dismutase but enhanced by a factor of four to five times in D_2O compared to H_2O. The hematoporphyrin sensitized oxidation of NADPH was also accelerat-

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ed by a factor of five in D_2O, thus implicating $O_2^*(^1\Delta_g)$ in the oxidation process. ($\tau_{1/2}O_2^*(^1\Delta_g)$ = 3 μsec. in H_2O and 53 ± 5 μsec in D_2O[4]). Previous investigations by Bodaness and Chan[5] similarly favored $O_2^*(^1\Delta_g)$ as the oxidizing species in the photo-oxidation of NADPH. These authors however favored a two electron transfer process consistent with the detection of H_2O_2 as a reaction product. Bodaness and Chan were unable to observe any measurable reduction of ferricytochrome C or nitroblue tetrazolium thus apparently ruling out the formation of O_2^- via a one electron transfer process. Frenkel[3] attributed this anomaly to the purity of the hematoporphyrins. We note, however, that the interaction between photo-excited hematoporphyrin and oxygen results in production of both $O_2^*(^1\Delta_g)$ and O_2^- directly.[6] This could contribute to confusion in steady state experiments.

The present communication constitutes a report of preliminary investigations into the feasibility of one electron transfer reactions from $O_2^*(^1\Delta_g)$ to mitochondrial species.

MATERIALS AND METHODS

The following chemicals were used as supplied: Reduced (NADPH), and oxidized (NAD+), forms of β-nicotinamide adenine dinucleotide (Sigma 98%). Reduced cytochrome-c (Fluka A.G. Busch S.G.). Deuterium oxide (Bio Rad. 99.8 atom %). Sodium dodecyl sulfate (BDH, specially pure). 2-Acetonaphthone (Aldrich 99%). 1,3-Diphenylisobenzofuran (Aldrich). Acetonitrile (Burdick and Jackson, spectroquality) and methanol (MCB spectroquality).

Time resolved laser flash photolysis studies employing a N_2 laser (337 nm) have yielded bimolecular rate constants for $O_2^*(^1\Delta_g)$ quenching by NADH and NAD+ in aqueous (D_2O) micellar, sodium dodecyl sulfate (0.1 mol l^{-1}) systems. Additionally, similar investigations were undertaken for the reduced form of cytochrome c in homogeneous (acetonitrile: D_2O 4:1) solutions.

The experimental technique, described earlier,[4] involved generating $O_2^*(^1\Delta_g)$ via 2-acetonaphthone (ACN) sensitizer and monitoring the subsequent removal of 1,3-

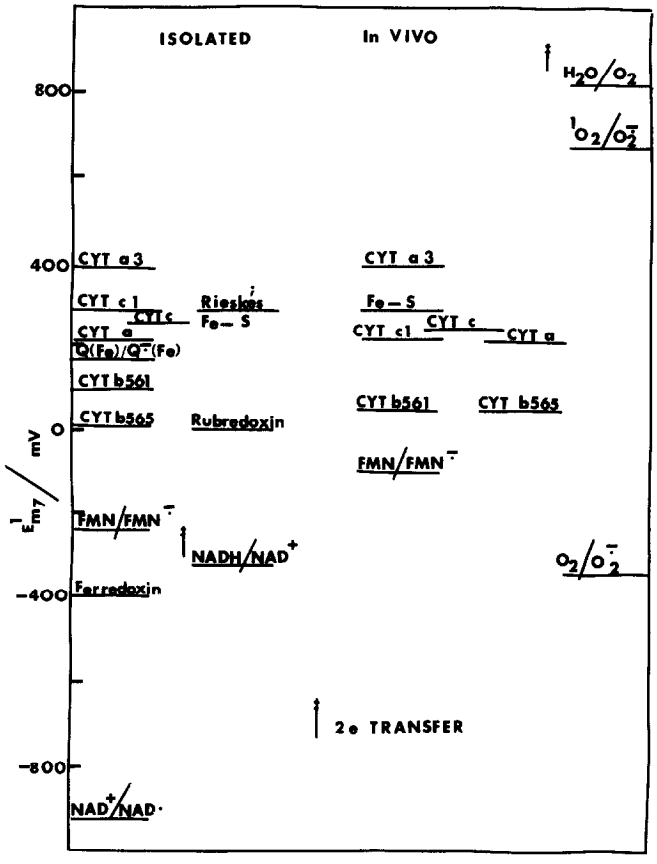

FIGURE 1 ONE ELECTRON REDOX POTENTIALS OF MITOCHONDRIAL COMPONENTS AT p.H7 (VS. NHE)

diphenylisobenzofuran (DPBF) spectroscopically at 415 nm in the presence of varying concentrations of quencher. Experimental conditions for each system are summarized in table 1.

Under the prevailing reaction conditions $O_2^*(^1\Delta_g)$ decay is governed by three competing first order processes:

$$O_2^*(^1\Delta_g) \xrightarrow[k_r]{k_d} O_2(^3\Sigma_g)$$

$$DPBF + O_2^*(^1\Delta_g) \longrightarrow \text{-DPBF}$$

$$QUENCHER + O_2^*(^1\Delta_g) \xrightarrow{k_q} \text{products}$$

and $$k(observed) = k_d + k_r[DPBF] + k_q[QUENCHER]$$

Table 1. SUMMARY OF EXPERIMENTAL CONDITIONS EMPLOYED

[QUENCHER] x 10^5/mol l^{-1}	[ACN] x 10^3/mol l^{-1}	[DPBF] x 10^5/mol l^{-1}
NADH 0-80	1.02	2.50
NAD+ 0-40	1.03	3.15
CYT.C 0-6	0.94	7.78

RESULTS

The resulting kinetic data are presented in figures 2 and 3 from which values of k_q (table 2) were extracted directly from experimental slopes by means of least squares analyses. Each experimental point represents a mean of at least six individual measurements and quoted errors correspond to standard deviations from the mean. A similar determination of k_q for NADH in homogeneous solution (acetonitrile: methanol, 3:1) yielded a value of k_q = 7.47 x 10^7 l $mol^{-1}s^{-1}$.

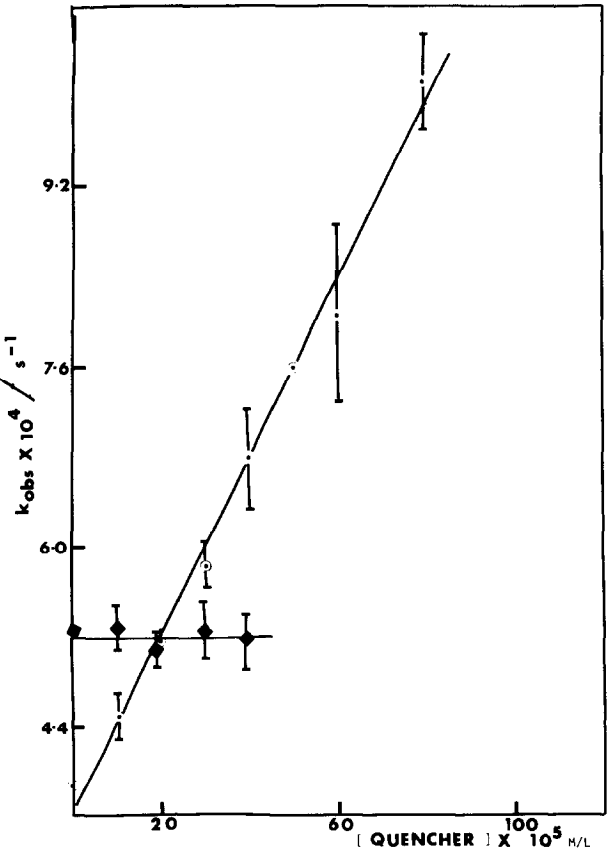

FIGURE 2 DEPENDENCE OF $k_{obs.}$ ON QUENCHER CONCENTRATION FOR NADH, θ, AND NAD+ ◆

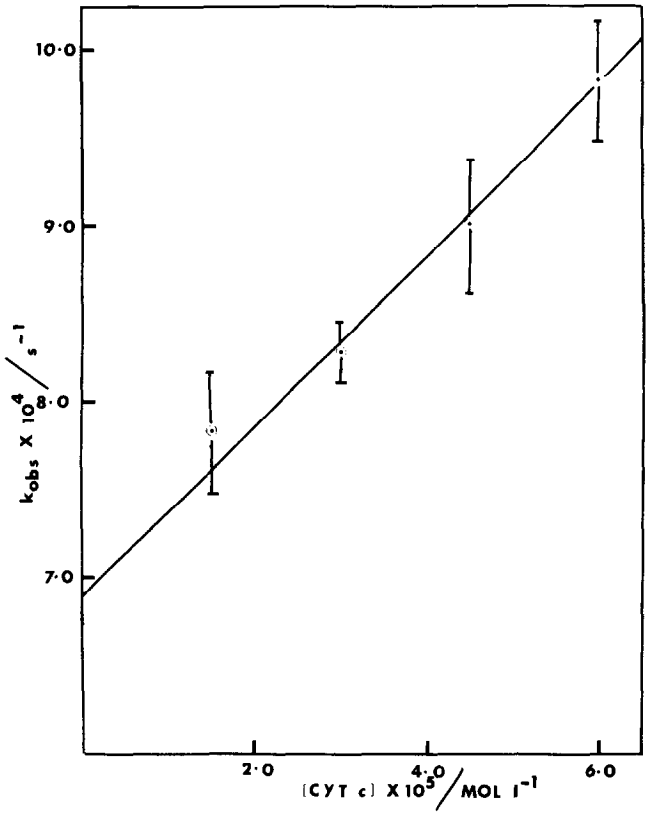

FIGURE 3 DEPENDENCE OF $k_{obs.}$ ON CONCENTRATION OF CYTOCHROME c.

DISCUSSION

The presence of $O_2^*(^1\Delta_g)$ in a mitochondrial environment could, by diverting electrons from the metabolic process, be severely damaging. Indeed photodynamic action could well proceed by such a mechanism. For this reason a detailed understanding of the reactions of $O_2^*(^1\Delta_g)$ with mitochondrial components is of considerable importance and forms the basis of the present researches.

Table 2 QUENCHING RATE CONSTANTS

QUENCHER	k_q x 10^{-7}/l $mol^{-1}s^{-1}$
NAD+	<0.5
NADH	7.92
CYT. c	48.7

The observation that the oxidized form of the nicotinamide, NAD+, was not an effective quencher of $O_2^*(^1\Delta_g)$ is consistent with the operation of an electron transfer mechanism for quenching by NADH. Physical quenching would surely be of comparable efficiency with either substrate. Also experiments at the highest NADH concentration employed demonstrate that, while NADH absorbed 337 nm radiation, no photochemistry resulted in the absence of sensitizer.

Unfortunately we were unable to substantiate the work of Frenkel et al.[3] since kinetic spectrophotometric techniques employed here were unsuited to the detection of $O_2^{\cdot-}$ due to high absorbances of experimental solutions near 250 nm (λ_{max} of $O_2^{\cdot-}$). Nonetheless, single electron transfer from NADH to $O_2^*(^1\Delta_g)$ at pH.7 should result in the formation of NAD· radicals which are known to absorb near 400 nm in aqueous solution.[7] Although observations were made at high [NADH] to ensure efficient $O_2^*(^1\Delta_g)$ capture we were unable to unambiguously demonstrate any absorption due to NAD· radicals. This is attributable to the efficiency with which O_2 reacts with NAD·[7] under the experimental conditions necessary

(1) $NADH + O_2^*(^1\Delta_g) \xrightarrow{7.92 \times 10^7 mol^{-1}s^{-1}} NAD\cdot + O_2^{\cdot-} + H^+$

(2) $NAD\cdot + O_2 \xrightarrow{2 \times 10^9 1\ mol^{-1}s^{-1}} NAD^+ + O_2^{\cdot-}$

Thus even with [NADH] = 10^{-3}M and [O_2] = 2.7×10^{-4}M (air saturated D_2O) the rate of removal of NAD· would be some seven times faster than its rate of formation from singlet oxygen.

Regarding the feasibility of electron transfer, it is notewhorthy that Foote and Thomas[8] have suggested the formation of a partial charge transfer complex involving 2,4,6 -triphenylphenol and $O_2^*(^1\Delta_g)$ which decomposes to $O_2^{\cdot-}$ and the 2,4,6-triphenylphenoxyl radical, the latter species being detected by kinetic spectroscopy. Also preliminary studies of the self-sensitized photo-oxidation of phenothiazine[9] (PTH) have produced evidence consistent with one electron transfer from the ground state substrate to $O_2^*(^1\Delta_g)$.

$$PTH + O_2^*(^1\Delta_g) \longrightarrow PTH^+ + O_2^{\cdot-}$$

In conclusion the present communication reports tentative evidence consistent with the concept of one electron transfer to $O_2^*(^1\Delta_g)$. Current researches are being directed to an extension of kinetic and mechanistic data for model compounds such as phenothiazine and for other components of the mitochondrial electron transport chain.

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