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SINGLE-ELECTRON TRANSFER FROM NADH ANALOGUES TO SINGLET OXYGEN

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Laser flash photolysis techniques have yielded rate constants for physical and reactive quenching modes of $O_2(^1\Delta_g)$ by nicotine, nicotinamide adenine dinucleotide (oxidized and reduced forms) and the reduced forms of nicotinamide mononucleotide, nicotinamide adenine dinucleotide phosphate and nicotinamide hypoxanthine dinucleotide. In the case of the last four named compounds, kinetic spectroscopy furnished evidence for one-electron transfers to $O_2(^1\Delta_g)$. Specifically, production of O_2^- was demonstrated unequivocally by reaction with 1,4-benzoquinone. Quantitative determinations revealed the extent of reactive quenching to be near 60% in each case.

Introduction

Singlet oxygen, $O_2(^1\Delta_g)$, formed by the action of light on photosensitive molecules in biological systems [1–3] is widely regarded as an initiating agent in photodynamic action. It has been implicated in such deleterious phenomena as cell death [4], photodynamic diseases such as porphyria [5–7] and inactivation of enzymes, e.g., alcohol dehydrogenase and trypsin [8].

The possibility has been examined [9] that $O_2(^1\Delta_g)$ could oxidize mitochondrial components via one-electron redox reactions with concomitant formation of superoxide ions (O_2^-). Since the cumulative energy released at each step along the electron-transport chain is utilized in the conversion of ADP into ATP, maximum interference with the metabolic process would arise if $O_2(^1\Delta_g)$ oxidized the first component of the chain, namely NADH. In such a scheme, not only would mitochondrial energy release be prevented, but also the potential cytotoxin O_2^- would be generated at a locus where the cell may be unable to deal with it.

Investigations of $O_2(^1\Delta_g)$ quenching by NADH and its various analogues form the substance of the present study:

$$O_2(^1\Delta_g) + \text{NADH} \rightarrow O_2^- + \text{NAD}^\cdot + \text{H}^+ \quad (1)$$

That the above process is energetically feasible was demonstrated by Koppenol [10] who showed that $E^1(O_2(^1\Delta_g)/O_2^-) = +0.67$ V (versus normal hydrogen electrode at 1 atm O_2) compared with $E^1_7(\text{NAD}^\cdot/\text{NADH}) = +0.30$ V [11,12]. Thus, in principle, NADH is susceptible to one-electron oxidation through reaction with $O_2(^1\Delta_g)$ while remaining unaffected by ground-state molecular oxygen ($E^1_7(O_2/O_2^-) = -0.33$ V at 1 atm O_2).

The general concept of single-electron transfer to $O_2(^1\Delta_g)$ has been investigated by Thomas and Foote [13]. An initial charge-transfer complex involving 2,4,6-triphenylphenol and $O_2(^1\Delta_g)$ was postulated to decompose yielding O_2^- and the 2,4,6-triphenyl-

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Abbreviations: HypNADH, the reduced form of nicotinamide hypoxanthine dinucleotide; BQ, 1,4-benzoquinone; ACN, 2-acetonaphthone; DPBF, 1,3-diphenylisobenzofuran; SDS, sodium dodecyl sulfate.

phenoxy radical, the latter being detected by kinetic spectrophotometric techniques. In the case of NADH and NADPH, however, conflicting mechanisms have been prosposed. Frenkel et al. [14] favor one-electron oxidation of NAD(P)H with concomitant formation of $O_2^{\overline{\cdot}}$ which was detected by the reduction of added ferricytochrome *c*. It should be noted, however, that the hematoporphyrin sensitizer used has been shown to produce $O_2^{\overline{\cdot}}$ directly on photolysis [15]. Conversely, Bodaness and Chan [16], while agreeing that $O_2(^1\Delta_g)$ was the reactive species, were unable to detect $O_2^{\overline{\cdot}}$ and favored a two-electron transfer consistent with the detection of H_2O_2 as a reaction product in steady-state experiments.

Measurements of total quenching rate constants k_q (physical + reactive quenching) of $O_2(^1\Delta_g)$ by NADH and NAD⁺ formed the basis of a preliminary report from these laboratories [9]. However, we were unable to demonstrate that electron transfer occurred, since the kinetic spectrophotometric techniques employed were unsuited to the detection of $O_2^{\overline{\cdot}}$ on account of optical absorbances of experimental solutions near 250 nm (λ_{max} of $O_2^{\overline{\cdot}}$). It was also pointed out that the other anticipated product of one-electron transfer, namely NAD˙, ($\lambda_{max} = 400$ nm [17]) would be rapidly removed by reaction with ground-state molecular oxygen under the experimental conditions [9]. Thus, although the evidence was convincing that $O_2(^1\Delta_g)$ was quenched by NADH, the conclusive proof that the process could act as an electron shunt was lacking.

Subsequent studies elaborated herein have extended measurements of k_q to include nicotine, NMNH, NADPH and HypNADH. Further mechanistic evidence demonstrating the occurrence of a one-electron transfer process has been acquired through the use of BQ to trap $O_2^{\overline{\cdot}}$ [18,19]. These studies have also enabled quantitative partitioning of total quenching rate constants into physical and reactive contributions.

Experimental Procedure

The following materials were used as supplied: NADH (98%), NAD⁺ (98%), NADPH (97%), NMNH (90%), HypNADH (95%) and nicotine (at least 98%) (Sigma), ACN (99%) and DPBF (Aldrich), ^2H_2O (99.8 atom%) and CH_3CN (spectroquality). BQ was purified by vacuum sublimation before use.

TABLE I

SYMMARY OF EXPERIMENTAL CONDITIONS AN KINETIC DATA FOR $O_2(^1\Delta_g)$ QUENCHING BY A SERIE OF NICOTINAMIDES IN $CH_3CN/^2H_2O$ (4 : 1, v/v)

[Nicotinamide]	(M)(x10⁵)	[ACN] (M) (x10³)	[DPBF] (M) (x10⁵)	k_q (l/mol per s) (x10⁻⁷)
Nicotine	0–70	1.0	5.9	5.9
NAD⁺	0–40	1.0	3.2	<0.5
NADH [a]	0–80	1.0	2.5	7.9
NADH [b]	0–50	1.0	5.6	7.5
NMNH	0–20	1.0	5.6	8.0
NADPH	0–20	1.0	5.9	13.4
HypNADH	0–20	1.0	5.3	15.7

[a] 0.1 M SDS micellar solution in ^2H_2O.
[b] CH_3CN/CH_3OH (3 : 1, v/v).

Experimental investigations fall into two categories:

Measurements of k_q values for total quenching o $O_2(^1\Delta_g)$ by nicotinamides

Kinetic data were obtained from laser flash photolysis studies employing the nitrogen gas laser (33 nm) and computer-controlled kinetic spectrophotometer which have been previously described [25] The pulse (8 ns) of ultraviolet light from the laser i absorbed by ACN forming the lowest triplet state This, by energy transfer to O_2, generated $O_2(^1\Delta_g$ which was monitored via its subsequent reaction wit DPBF. The removal of DPBF absorption at 415 n was followed spectrophotometrically [20]. Experiments were undertaken in aerated solutions at varyin concentrations of nitotinamides up to a maximu value limited by absorption of these compounds a 337 nm. Experimental conditions for each system ar summarized in Table I.

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

$$O_2(^1\Delta_g) \xrightarrow{k_d} O_2(^3\Sigma_g)$$

$$\text{DPBF} + O_2(^1\Delta_g) \xrightarrow{k_a} -\text{DPBF}$$

$$\text{nicotinamide} + O_2(^1\Delta_g) \xrightarrow{k_q} \text{quenching/products}$$

Under conditions where the initial concentration of $O_2(^1\Delta_g)$ is much lower than that of the reactive substrates and provided the extent of DPBF removal is small, it can be shown that the first-order rate constant (k_{obs}) for the decay of DPBF is:

$$k_{obs} = k_d + k_a[\text{DPBF}] + k_q[\text{nicotinamide}]$$

A typical bleaching curve is shown in Fig. 1. At constant DPBF concentration, a plot of k_{obs} vs. [nicotinamide] is linear with a slope equal to k_q, the bimolecular rate constant for singlet oxygen quenching. Direct photolysis of DPBF by light from the monitoring source was minimized by interposing an ultraviolet cut-off filter ($\lambda < 400$ nm). A neutral-density filter was employed to attenuate the laser beam, thus ensuring that less than 10% of the DPBF was removed by $O_2(^1\Delta_g)$.

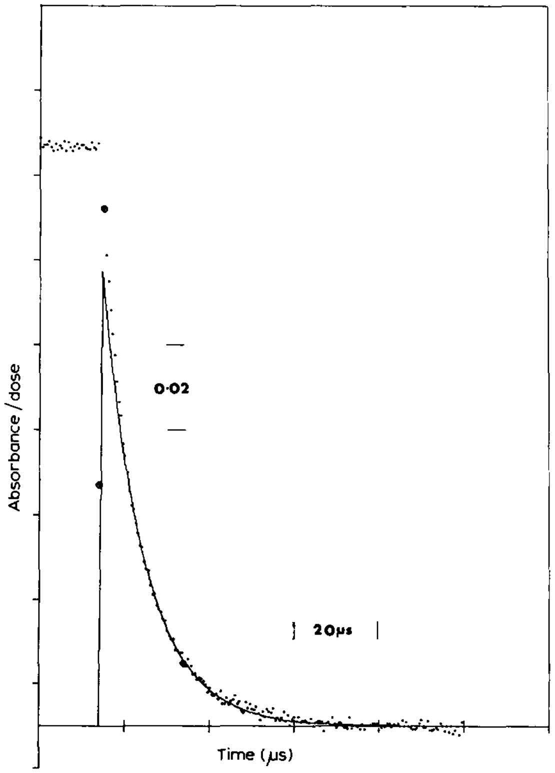


Fig. 1. Typical decay curve for DPBF bleaching at 415 nm in aerated solution comprising ACN ($1 \cdot 10^{-3}$ M), DPBF ($5.33 \cdot 10^{-5}$ M) and HypNADH ($14.6 \cdot 10^{-5}$ M). Dots correspond to experimental points and the solid curve constitutes the computer-generated best least-squares fit.

Initial investigations of NADH quenching of $O_2(^1\Delta_g)$ were undertaken in aqueous micellar systems (0.1 M SDS in ^2H_2O) thought to be more akin to biological milieux than homogeneous organic media. Further determinations in CH_3CN/CH_3OH (3 : 1, v/v), however, revealed no significant variation in k_q. Subsequent studies of the other named nicotinamides were undertaken in $CH_3CN/^2H_2O$ (4 : 1, v/v). These conditions maximized $O_2(^1\Delta_g)$ lifetime, compatible with solubility restrictions, and negated the micellar requirement.

Mechanistic studies of NADH, NADPH, NMNH and HypNADH oxidation

Experimental solutions for mechanistic investigations comprised ACN ($1.65 \cdot 10^{-3}$ M), nicotinamide (10^{-4} M) and BQ (2–$20 \cdot 10^{-5}$ M) in $CH_3CN/^2H_2O$ (1 : 4, v/v). This solvent mixture was chosen to maximize the $O_2(^1\Delta_g)$ natural lifetime and thereby allow smaller quencher concentrations to be used. Additionally, NAD^+ ($5.6 \cdot 10^{-4}$ M) and nicotine ($5.5 \cdot 10^{-3}$ M) were investigated in otherwise identical systems.

Results

Total rate constants for $O_2(^1\Delta_g)$ quenching

Aerated solutions of sensitizer, DPBF and nicotinamide derivative at concentrations shown in Table I were subjected to single pulses of 337 nm radiation. A minimum of six data points were obtained from freshly replenished samples at each nicotinamide concentration. The first-order rate constants for DPBF bleaching were averaged and representative plots of k_{obs} as a function of nicotinamide concentration are presented in Fig. 2. Error bars correspond to ±1 S.D. from the mean values determined for k_{obs}. A complete summary of the resulting kinetic data is contained in Table I. These values of k_q represent the sum of the parameters for physical and chemical reaction (see later).

Electron transfer to $O_2(^1\Delta_g)$ from NADH, NADPH, NMNH and HypNADH

The feasibility of one-electron transfer from nicotinamide derivatives to $O_2(^1\Delta_g)$ was investigated by employing BQ to remove in turn an electron from $O_2^{\bar{\cdot}}$.

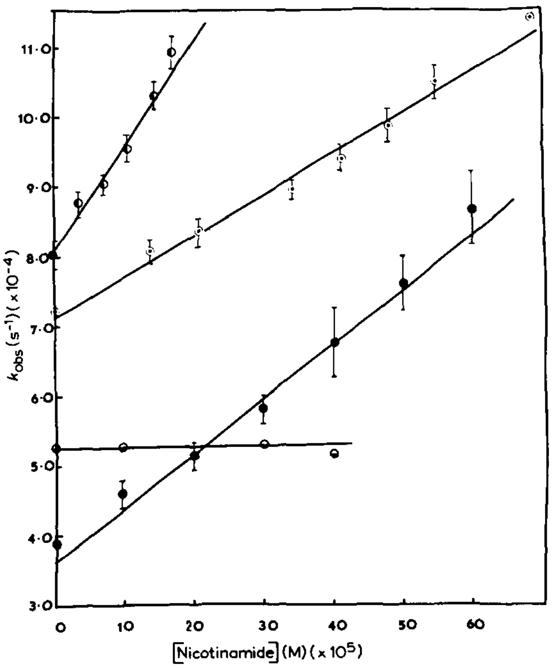


Fig. 2. Dependence of k_{obs} on nicotinamide concentration for: ●, NADH; ⊙, nicotine; ●, HypNADH; ●, NAD$^+$.

The reaction:

$$BQ + O_2^{\overline{\cdot}} \rightarrow BQ^{\overline{\cdot}} + O_2 \qquad (2)$$

has been shown [18,19] to proceed with a bimolecular rate constant of $k_2 = 9.6 \cdot 10^8$ l/mol per s. Solutions (air or O_2 saturated) of ACN ($1.65 \cdot 10^{-3}$ M), BQ ($3.2 \cdot 10^{-5}$ M) and nicotinamide (10^{-4} M) were subjected to laser pulses. Transient absorption spectra obtained over an elapsed time of several hundred microseconds were similar for each nicotinamide system investigated and are exemplified in Fig. 3. The spectra obtained coincide with that of $BQ^{\overline{\cdot}}$ as produced by addition of hydrated electrons to BQ [18].

The rate of formation of the absorption at 430 nm was shown to be first order with respect to BQ. A plot of the rate constants of $BQ^{\overline{\cdot}}$ formation as a function of BQ concentration (2–$20 \cdot 10^{-5}$ M) under O_2 saturation was linear with a slope of $k_2 = 3.2 \pm 0.5 \cdot 10^8$ l/mol per s independent of the particular nicotinamide. In addition to monitoring $BQ^{\overline{\cdot}}$ production from 0 to 300 μs in O_2-saturated solutions, the initial absorption of $^3ACN^*$ was measured under N_2 at 440 nm. Representative data are contained in Fig. 4. The value of k_2 found here was in good agreement with $k_2 = 4 \cdot 10^8$ l/mol per s when we generated $O_2^{\overline{\cdot}}$ directly in the presence of BQ alone in an electron pulse radiolysis experiment. It is suggested that our values for k_2 differ from the literature value [18] of $9.6 \cdot 10^8$ l/mol per s determined in water because of a solvent effect deriving from the $CH_3CN/^2H_2O$ (1 : 4, v/v) employed in the present work. We confirmed the neat water value using electron pulse radiolysis of aqueous BQ solutions.

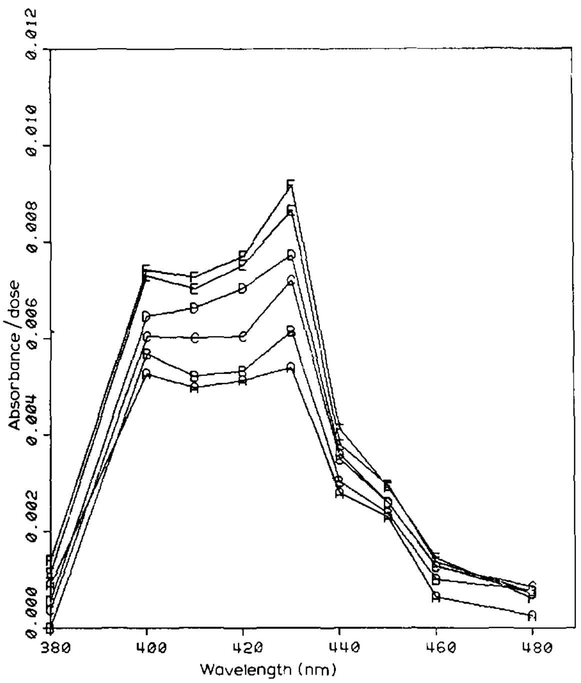


Fig. 3. Delayed formation of the absorption spectrum of $BQ^{\overline{\cdot}}$ produced in aerated solution comprising ACN ($1.63 \cdot 10^{-3}$ M), NMNH ($1.01 \cdot 10^{-4}$ M) and BQ ($3.21 \cdot 10^{-5}$ M) Times after flash: A, 14 μs; B, 34 μs; C, 64 μs; D, 109 μs E, 169 μs; F, 229 μs.

To make quantitative measurements of the fraction of singlet oxygen being converted to $O_2^{\overline{\cdot}}$, separate control experiments in N_2-saturated solutions comprising ACN ($1.65 \cdot 10^{-3}$ M) with (a) BQ (2–$18 \cdot 10^{-5}$ M) and (b) NADH (0–$21.3 \cdot 10^{-5}$ M) in $CH_3CN/^2H_2O$ (1 : 4, v/v) revealed that $^3ACN^*$ undergoes reactive quenching with BQ to produce $BQ^{\overline{\cdot}}$ and with NADH yielding $NAD^{\cdot}$.

$$^3ACN^* + BQ \rightarrow BQ^{\overline{\cdot}} + ACN^+ \qquad (3)$$

$$^3ACN^* + NADH \rightarrow ACNH + NAD^{\cdot} \qquad (4)$$

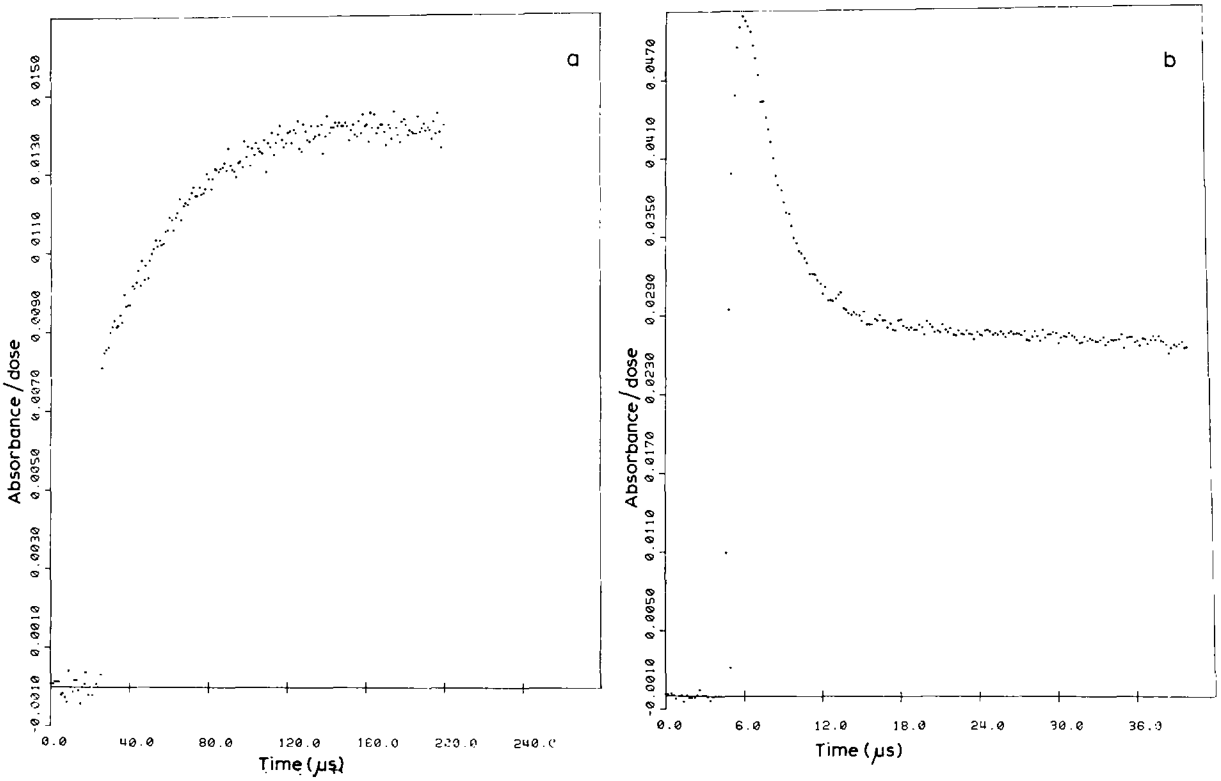


Fig. 4. (a) $BQ^{\cdot-}$ production monitored at 430 nm in an O_2-saturated solution comprising ACN ($1.64 \cdot 10^{-3}$ M), NADH ($1.04 \cdot 10^{-4}$ M) and BQ ($7.81 \cdot 10^{-5}$ M). (b) Absorption of the same solution under N_2 at 440 nm showing initial intensity and decay of ACN triplet.

From measurements of the first-order rate constants for the decay of $^3ACN^*$ under conditions of varying [NADH] and [BQ], we were able to determine $k_3 = 2.8 \cdot 10^9$ l/mol per s and $k_4 = 1.6 \cdot 10^9$ l/mol per s. The occurrence of reaction 4 as a photoreduction process is evidenced, as Fig. 5 demonstrates, by the residual absorption attributable to $NAD^{\cdot}$ [17] remaining after $^3ACN^*$ has decayed. Similar techniques revealed the production of $BQ^{\cdot-}$ via reaction 3.

Fortunately, in O_2-saturated solutions, the reaction of $^3ACN^*$ to form $O_2(^1\Delta_g)$ is complete in approx. 1 μs; thus, the direct formation of $BQ^{\cdot-}$ from $^3ACN^*$ is prompt and readily distinguishable from $BQ^{\cdot-}$ which is formed from $O_2^{\cdot-}$ over about 300 μs. Conversely, the direct formation of $NAD^{\cdot}$ by reaction 4 affords an additional nonsinglet-oxygen-derived source of $O_2^{\cdot-}$ [17] viz:

$$NAD^{\cdot} + O_2 \rightarrow NAD^+ + O_2^{\cdot-} \quad (k_5 = 2.0 \cdot 10^9 \text{ l/mol per s}) \quad (5)$$

Nevertheless, data contained in Fig. 6a demonstrate that direct production of $NAD^{\cdot}$ from $^3ACN^*$ by reaction 4 could be eliminated in O_2-saturated solutions at sufficiently low [NADH]. In this case, the experimental medium comprised a 1 : 4 (v/v) mixture of CH_3CN with H_2O. However, as is evident from Fig. 6b, long-term production of $BQ^{\cdot-}$ reappeared when H_2O was replaced by ^2H_2O as co-solvent, all other experimental conditions being unchanged.

Additional investigations of NAD^+ and nicotine in ^2H_2O-based systems revealed no $BQ^{\cdot-}$ production in the 0–300 μs time domain. In the case of nicotine in particular, the high concentration employed ensured efficient $O_2(^1\Delta_g)$ capture.

Discussion

It is contended that the data contained in Fig. 6 constitute convincing evidence for the involvement

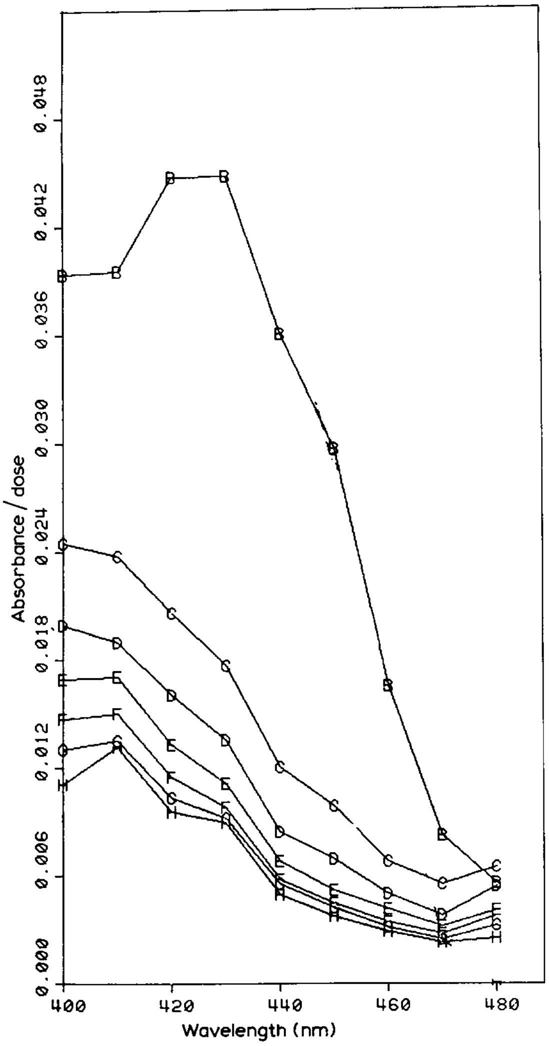
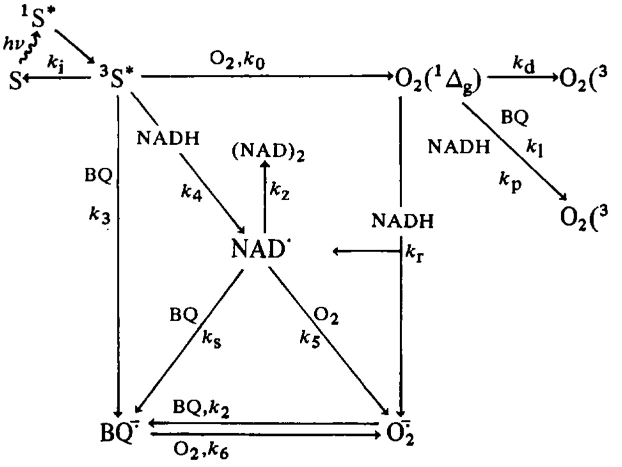


Fig. 5. Absorption spectrum, as a function of time, of an N_2-saturated solution comprising ACN (1.65 · 10^{-3} M) and NADH (1.01 · 10^{-4} M). Spectrum B shows $^3ACN^*$ and the remaining spectra correlate with NAD˙. Times after the flash: B, 2.0 μs; C, 7.4 μs; D, 10.9 μs; E, 14.9 μs; F, 18.9 μs; G, 22.9 μs, H, 26.9 μs.

of $O_2(^1\Delta_g)$ in $BQ^{\cdot-}$ (and therefore $O_2^{\cdot-}$) production. It is apparent, from Fig. 6a, that no significant $BQ^{\cdot-}$ production is occurring after approx. 1 μs in the CH_3CN/H_2O system. This lack of delayed $BQ^{\cdot-}$ formation demonstrates that (i) the involvement of NAD˙ and $O_2^{\cdot-}$ through reactions 4 and 5 is not important at the NADH and O_2 concentrations employed and (ii) when ^2H_2O is replaced by H_2O as co-solvent, singlet oxygen does not form $O_2^{\cdot-}$. The latter conclusion can be understood in terms of the much reduced lifetime of $O_2(^1\Delta_g)$ in the H_2O-containing medium. It has been demonstrated [23] that electronic-vibrational energy transfer is enhanced when O-^{2}H oscillators are replaced by O-^{1}H. Thus, the natural lifetimes of singlet oxygen in ^2H_2O and H_2O are 53 and 4 μs [20], respectively, and in binary $H_2O/^2H_2O$ mixtures the lifetime of singlet oxygen follows a linear relationship [20]. Assuming similar behavior in CH_3CN-based mixtures with H_2O or ^2H_2O, we can write:

$$k_d^{mix} = \Sigma_i x_i \cdot k_d^i$$

where k_d^{mix}, k_d^i are the first-order decay constants of $O_2(^1\Delta_g)$ in the mixture and in the ith component (of mole fraction x_i), respectively. Also $k_d^i = 1/\tau_\Delta^i$ where τ_Δ^i is the natural lifetime of $O_2(^1\Delta_g)$ in component i. In 1 : 4 (v/v) CH_3CN/H_2O $x_{CH_3CN} = 0.08$, $x_{H_2O} = 0.92$, $k_d^{CH_3CN} = 3.33 \cdot 10^4\ s^{-1}$ [23] and $k_d^{H_2O} = 25 \cdot 10^4\ s^{-1}$ [20], whence $k_d^{CH_3CN/H_2O} = 23.3 \cdot 10^4\ s^{-1}$.



Scheme I

Scheme I. Reaction scheme for photosensitized formation of $BQ^{\cdot-}$ via singlet oxygen and $O_2^{\cdot-}$. The rate constants for the main and side reactions are: $k_i = 1 \cdot 10^5\ s^{-1}$ (this work), $k_0 = 2 \cdot 10^9$ l/mol per s [20], $k_d = 2 \cdot 10^4\ s^{-1}$ (this work), $k_l = 3.4 \cdot 10^7$ l/mol per s [21], $k_4 = 1.6 \cdot 10^9$ l/mol per s (this work), $k_3 = 2.8 \cdot 10^9$ l/mol per s (this work), $k_z = 5.6 \cdot 10^7$ l/mol per s [17], $k_5 = 2 \cdot 10^9$ l/mol per s [17], $k_s = 3.6 \cdot 10^9$ l/mol per s [18], $k_6 < 10^7$ l/mol per s [18], $k_2 = 3.2 \cdot 10^8$ l/mol per s (this work), $k_q = k_r + k_p = 7.9 \cdot 10^7$ l/mol per s (this work).

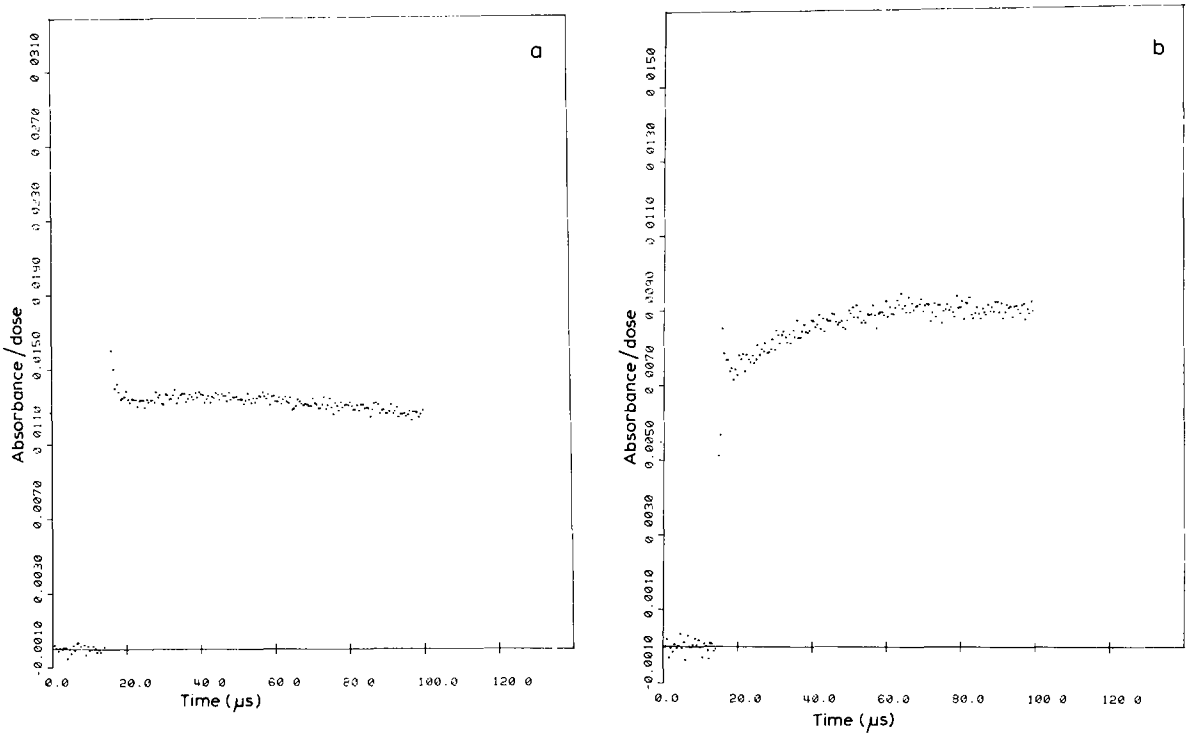


Fig. 6. Yield of BQ$^{\overline{\cdot}}$ in (a) H_2O/CH_3CN (4 : 1, v/v) and (b) $^2H_2O/CH_3CN$ (4 : 1, v/v) systems comprising ACN ($1.6 \cdot 10^{-3}$ M), BQ ($10.5 \cdot 10^{-5}$ M) and NADH ($2.4 \cdot 10^{-5}$ M).

Similarly, in 1 : 4 (v/v) $CH_3CN/^2H_2O$, with $k_d^{^2H_2O} = 1.9 \cdot 10^4\ s^{-1}$ [20], it is found that $k_d^{CH_3CH/^2H_2O} = 2.0 \cdot 10^4\ s^{-1}$. Thus, the natural lifetimes of $O_2(^1\Delta_g)$ in the two mixtures are $\tau_\Delta^{^2H_2O_{mix}} = 50\ \mu s$, $\tau_\Delta^{H_2O_{mix}} = 4.3\ \mu s$. Therefore, only in the ^2H_2O-based system can $O_2(^1\Delta_g)$ survive long enough to react with NADH to produce $O_2^{\overline{\cdot}}$ for subsequent generation of BQ$^{\overline{\cdot}}$. These arguments rely on the reasonable assumption that the rate of $^3ACN^*$ quenching by NADH is independent of the presence of H_2O or ^2H_2O in the solvent system. The lack of reaction exhibited by NAD$^+$ and nicotine is also in good accord with the present interpretation, since NAD$^+$ is fully oxidized and nicotine possesses no readily extractable electrons. We contend that these experiments constitute more definite evidence for one-electron transfer to $O_2(^1\Delta_g)$ than obtained hereto.

The data presented enable quantitative determinations of the rate constants for physical and reactive quenching from the k_q values contained in Table I. The reaction scheme together with the relevant rate parameters is shown diagrammatically in Scheme I. Disproportionation and dimerization reactions of $O_2^{\overline{\cdot}}$ and NAD$^{\cdot}$ are slow under the experimental conditions and have been ignored in the ensuing analysis.

Rate of removal of $^3ACN^*$:

$$\frac{-d}{dt}[S^*] = k_i + k_4[NADH] + k_3[BQ] + k_0[O_2] \qquad (6)$$

Rate of removal of $O_2(^1\Delta_g)$:

$$\frac{-d}{dt}[O_2(^1\Delta_g)] = k_d + k_q[NADH] + k_l[BQ] \qquad (7)$$

Taking $\epsilon_{max}(^3ACN^*) = 1.25 \cdot 10^4\ M^{-1}$ [a], then $[^3ACN^*]_0$, the initial concentration of $^3ACN^*$, was

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[a] A value of $\epsilon_{max}(^3ACN^*) = 1.05 \cdot 10^4\ M^{-1} \cdot cm^{-1}$ was obtained in benzene [22]. Comparison of $^3ACN^*$ absorption at 440 nm in benzene and CH_3CN/H_2O (1 : 4, v/v) showed a 19% increase in the mixed solvent system. Thus, the literature value was multiplied by 1.19 to obtain the present value.

TABLE II

COMPARISON OF CALCULATED AND EXPERIMENTALLY DETERMINED VALUES OF $\Delta[BQ^{\cdot-}]$ IN O_2-SATURATED SYSTEMS AT SEVERAL BQ CONCENTRATIONS

$[NADH] = 1.04 \cdot 10^{-4}$ mol. %R, percent reactive quencing.

[BQ] (M)($\times10^5$)	$[^3ACN^*]_0$ (M)($\times10^6$)	F_{NADH} (%)	F_{O_2} (%)	$[O_2^*]_0$ (M) ($\times10^6$)	$[O_2^*]_q$ (M) ($\times10^6$)	$[NAD^\cdot]_T$ (M)($\times10^6$)	$\Delta[BQ^{\cdot-}]_{calc}$ (M)($\times10^6$)	$\Delta[BQ^{\cdot-}]_{exp}$ (M)($\times10^6$)	%R
3.91	8.7	4	93	4.05	1.10	0.3	2.5	2.0	77
5.86	9.2	4	89	4.10	1.10	0.4	2.6	1.1	32
7.81	8.0	4	88	3.50	0.95	0.3	2.2	1.4	60
9.77	7.6	4	87	3.30	0.85	0.3	2.0	1.4	65
11.72	8.2	4	86	3.55	0.90	0.3	2.1	1.0	39
13.68	8.2	4	85	3.50	0.85	0.3	2.0	1.1	47
15.63	8.1	4	84	3.40	0.85	0.3	2.0	1.5	71
17.59	8.9	4	83	3.70	0.90	0.4	2.2	1.2	45

obtained from absorption data such as those shown in Fig. 4b. Also, the fraction (F_x) of $^3ACN^*$ decaying via each individual channel (x) is readily calculable since all rate constants and concentrations contained in equation 6 are known [a], e.g.:

$$F_{O_2} = \frac{k_0[O_2]}{k_i + k_4[NADH] + k_3[BQ] + k_0[O_2]}$$

$$F_{NADH} = \frac{k_4[NADH]}{k_i + k_4[NADH] + k_3[BQ] + k_0[O_2]}$$

These considerations allow determination of absolute values for $[O_2^*]_0$, the concentration of $O_2(^1\Delta_g)$ prior to reaction, and $[NAD^\cdot]_T$, the concentration of $NAD^\cdot$ generated directly from $^3ACN^*$. We are grateful to a referee for pointing out that the yield of $O_2(^1\Delta_g)$ from quenching of $^3ACN^*$ by $O_2(^3\Sigma_g)$ is not unity. A value of 0.5, determined in benzene [3], was used in the calculation of $[O_2^*]_0$. Also, the calculation of $[NAD^\cdot]_T$ involves the assumption that quenching of $^3ACN^*$ by NADH is totally reactive.

The rate of loss of $O_2(^1\Delta_g)$ is given by Eqn. 7. Again, all rate constants and concentrations are known thus allowing calculation of $[O_2^*]_q$, the concentration of $O_2(^1\Delta_g)$ that is quenched by NADH. Assuming for the moment that k_q constitutes a totally reactive rate constant, reference to the scheme reveals that each quenching act results in the overall formation of two molecules of $O_2^{\cdot}$, one from electron transfer and one from oxidation of $NAD^\cdot$. A further $O_2^{\cdot}$ (and ultimately $BQ^{\cdot}$) is anticipated for each $NAD^\cdot$ produced directly from $^3ACN^*$. (Possible direct reaction of $NAD^\cdot$ with BQ rather than O_2 is a minor process accounting for less than 20% of $NAD^\cdot$ species under the prevailing conditions of O_2 saturation.) These considerations lead to a value of $\Delta[BQ^{\cdot}]_{calc}$, the calculated concentration of $BQ^{\cdot}$ produced from $O_2^{\cdot}$ via reaction 2.

$$\Delta[BQ^{\cdot}]_{calc} = 2[O_2^*]_q + [NAD^\cdot]_T \tag{8}$$

Experimental yields, $\Delta[BQ^{\cdot}]_{exp}$, were measured from absorption data typified by Fig. 4a using $\epsilon_{430}(BQ^{\cdot}) = 7 \cdot 10^3\ M^{-1} \cdot cm^{-1}$ in H_2O [18]. Measurement of only the delayed portion corrects for that $BQ^{\cdot}$ concentration formed promptly from the sensitizer.

The following arguments lead to the conclusion that the differences between measured and calculated values of $\Delta[BQ^{\cdot}]$ reflect partioning of k_q between physical and reactive quenching:

$$[O_2^*]_q = [O_2^*]_r + [O_2^*]_p \tag{9}$$

where $[O_2^*]_r$ and $[O_2^*]_p$ are the absolute concentrations of $O_2(^1\Delta_g)$ which undergo reactive and physical

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[a] A value of $[O_2] = 1.8 \cdot 10^{-3}$ M was calculated from mole fractions of an O_2-saturated $CH_3CN/^2H_2O$ (1 : 4, v/v) mixture. Values of $[O_2] = 1.35 \cdot 10^{-3}$ M and $7 \cdot 10^{-3}$ M in O_2-saturated ^2H_2O [17] and CH_3CN [24] were employed in this calculation.

TABLE III

SUMMARY OF TOTAL, PHYSICAL AND REACTIVE QUENCHING RATE CONSTANTS FOR A SERIES OF NICOTINAMIDE DERIVATIVES WITH $O_2(^1\Delta_g)$

Values of k_q, k_r and k_p are expressed as l/mol per s ($\times 10^{-7}$).

Compound	%$\bar{R}$ [a]	k_q	k_r	k_p
NADH	54.5 ± 15	7.9	4.3	3.6
NADPH	63 ± 9	13.4	8.4	5.0
HypNADH	56 ± 11	15.7	8.8	7.9
Nicotine	0	5.9	–	5.9

[a] Average percent reactive quenching derived from several BQ concentrations ($2-20 \cdot 10^{-5}$ M). Errors correspond to ±1 S.D.

quenching, respectively. The latter concentration can be evaluated from the calculated and measured values of $\Delta[BQ^{\bar{\cdot}}]$ as follows:

$$\begin{aligned}\Delta[BQ^{\bar{\cdot}}]_{calc} - \Delta[BQ^{\bar{\cdot}}]_{exp} \\ &= 2[O_2^*]_q + [NAD^{\cdot}]_T - (2[O_2^*]_r + [NAD^{\cdot}]_T) \\ &= 2([O_2^*]_q - [O_2^*]_r) \qquad (10)\end{aligned}$$

it follows from Eqns. 9 and 10 that:

$$\Delta[BQ^{\bar{\cdot}}]_{calc} - \Delta[BQ^{\bar{\cdot}}]_{exp} = 2[O_2^*]_p \qquad (11)$$

whence the percentages of physical and reactive quenching (%P and %R, respectively) are given by:

$$\%P = 100\,\frac{[O_2^*]_p}{[O_2^*]_q} \quad \text{and :}$$

$$\%P = 100\left(\frac{\frac{1}{2}\{\Delta[BQ^{\bar{\cdot}}]_{calc} - \Delta[BQ^{\bar{\cdot}}]_{exp}\}}{[O_2^*]_q}\right)$$

further:

$$\%R = 100 - \%P$$

Values of $\Delta[BQ^{\bar{\cdot}}]_{calc}$, $\Delta[BQ^{\bar{\cdot}}]_{exp}$, $[O_2^*]_o$, $[O_2^*]_q$, $[NAD^{\cdot}]_T$ and %R are collected in Table II for the O_2-saturated NADH system at several BQ concentrations. Resulting values of k_p and k_r, the rate constants for physical quenching and single-electron transfer, respectively, are contained in Table III for NADH, NADPH, HypNADH and nicotine. The possibility cannot be ruled out that k_p values contained in Table III could incorporate some form of reactive quenching other than electron transfer, although no reaction products were detected.

The total lack of quenching by NAD^+ and the higher rates of overall quenching by NADPH and HypNADH compared to NADH remain open questions. Measurements of one-electron redox potentials for NADPH and HypNADH may aid in the elucidation of this aspect. Nevertheless, the present research confirms the thermodynamic arguments [9] arising from the work of Koppenol [10], Anderson [12] and Farrington et al. [11] and demonstrate experimentally the feasibility of single-electron transfer from NADH analogues to $O_2(^1\Delta_g)$.

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