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Molecular Basis For Inhibition of Mitochondrial Respiration by Naphthalene

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Naphthalenes, abundant in coal tar, synthetic, and natural crude oils, have been detected in industrial and sewage plant effluents, and in drinking waters (U.S. E.P.A. 1980, PUCKNAT 1981). Naphthalene inhibits oxygen consumption by isolated cardiac mitochondria (NADH-O_2) with an EC_{50} value of 78 μM. It also inhibits oxygen consumption by three types of tissue culture cells (monkey kidney, human larynx carcinoma, and primary turkey fibroblasts) with a similar EC_{50} and is toxic to the cells at concentrations below 15 ppm; toxicity parallels the inhibition of oxygen consumption (HARMON & SANBORN 1982). Naphthalene is lethal to fresh and salt water organisms, the EC_{50} in Daphnia magna is 8.75 ppm and in the fathead minnow is 6 ppm (EPA 1980). Naphthalene has also been observed to inhibit oxygen consumption of Daphnia magna (CRIDER et al. 1982).

Harmon and Sanborn (1982) demonstrated that naphthalene affects ubiquinone (UQ) reduction by NADH as well as UQH_2 oxidation by O_2 with no effect on duroquinol (tetramethylbenzoquinol) oxidation. This is consistent with naphthalene inhibiting specifically at the level of ubiquinone (coenzyme Q), an essential component of the mitochondrial electron chain. Inhibition of ubiquinone reduction could occur via effects on the coenzyme itself or by alteration of its binding to other elements of the electron transport system, such as Q-binding protein (YU et al. 1977) either by alteration of Q, the Q-binding protein, or both. This paper will demonstrate an interaction of naphthalene with Q_{10}, the most common form of ubiquinone in mammalian heart mitochondria (TZAGOLOFF 1982), that may be responsible for naphthalene's observed inhibitory effect.

MATERIALS AND METHODS

Duroquinone (tetramethlybenzoquinone) and Q_{10} (ubiquinone-50) were dissolved in absolute ethanol. Reagent grade naphthalene was dissolved in absolute ethanol daily and aliquots from this stock solution were diluted to the desired concentration with absolute ethanol. UV difference spectra (naphthalene and quinone minus naphthalene) and absolute spectra of the quinones in ethanol were recorded using a Cary 14 spectrophotometer.

RESULTS AND DISCUSSIONS

As shown in Fig. 1A trace 3, Q_{10} exhibits a single absorbance maximum at 274 nm. In the presence of 50 ppm (317 μM) naphthalene (sufficient to yield a molar ratio of 260 napthhthalene/Q) two absorbance maxima are observed at 273 nm and 279 nm (Fig. 1A trace 4). The alteration of the spectrum is not prominent at ratios below 175. A dilution artifact would produce changes at wavelengths corresponding to the 265 nm, 275 nm, and 286 nm absorbance maxima of naphthalene (Fig 1B trace 2); this is not observed. The resolution of the spectrophotometer (slits at 1.6

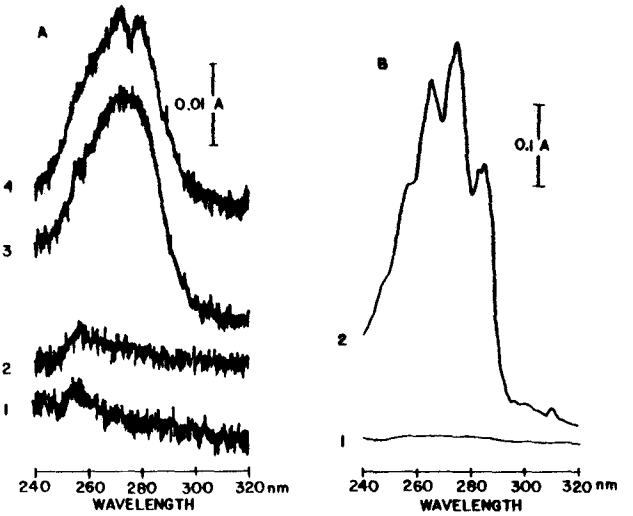

Figure 1. A. Ultraviolet absorbance spectrum of ubiquinone in the presence and absence of naphthalene.
1. ethanol vs. ethanol.
2. naphthalene vs. 50 ppm naphthalene.
3. Absolute spectrum of 1.5×10^{-6}M ubiquinone in ethanol.
4. Difference spectrum of 1.5×10^{-6}M ubiquinone in 50 ppm naphthalene minus 50 ppm naphthalene ($\frac{\text{Naphthalene}}{Q} = 260$).

B. Ultraviolet absorbance of naphthalene. 1. Ethanol vs. ethanol. 2. Absolute spectrum of 120 uM naphthalene in ethanol.

mm) is 3.75 nm, well within the separation of the two maxima. The 279 nm peak is not due to possible light scattering since a sharp absorbance peak would not be produced; a general increase in absorbance at shorter wavelengths would be expected (scattering is proportional to λ^{-4}. Thus the changes in the absorbance spectrum of Q_{10} are due to the interaction of ubiquinone with naphthalene and not artifacts resulting from dilution or light scattering.

The spectrum of duroquinone (Fig. 2 trace 3) was unaffected by the presence of naphthalene (Fig. 2 trace 4) at a molar ratio of up to 20 times as great as that which produced the effect on the spectrum of ubiquinone.

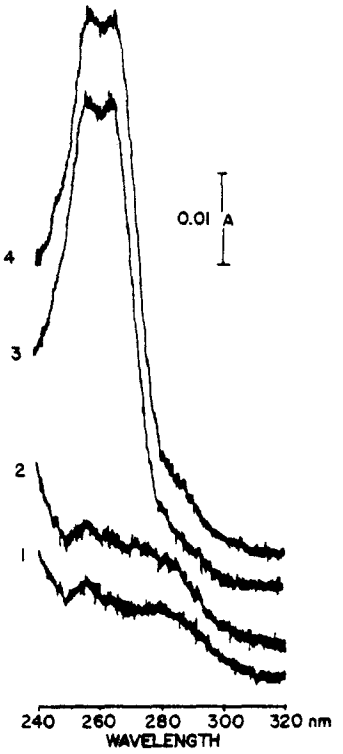

Figure 2. Ultraviolet absorbance spectra of duroquinone in the presence and absence of naphthalene. 1. ethanol vs. ethanol. 2. naphthalene vs. 30 ppm naphthalene. 3. absolute spectrum of 3.0×10^{-7} M duroquinone in ethanol. 4. difference spectrum of 3.0×10^{-7} M duroquinone in 30 ppm naphthalene minus 30 ppm napthalne ($\frac{\text{Napthalene}}{Q}$ = 780).

Naphthalene induces a shift in λ_{max} in the absorbance spectrum of ubiquinone when present in a molar ratio (naphthalene/quinone) of 175 or greater; the alteration of the spectrum of duroquinone is not observed even at a ratio 20 times as great. Results with isolated beef heart mitochondria (HARMON & SANBORN 1982) show that naphthalene does not inhibit duroquinol oxidation but does inhibit NADH oxidation as well as NADH-Q_{10} reductase and $QH_2 \rightarrow O_2$ activities. The concentration of ubiquinone in beef heart mitochondria is 2.5 mg/g protein (CRANE et al. 1957) to 3.5 nmoles/mg protein (HUANG & LEE 1975); at 78 uM naphthalene

(the LC_{50} value reported for mitochondria by Harmon and Sanborn) the naphthalene/ubiquinone ratio in inhibited intact mitochondria would correspondingly be 379 to 460, more than twice that required to cause an observable alteration of the spectrum of ubiquinone.

Other researchers have reported alteration in the spectra of compounds such as porphyrins and quinones induced by changes in the environment of the compound. SCHLEYER et al.(1971a & 1971b) have reported "small but measurable" optical and electron paramagnetic resonance absorbance changes in the spectrum of cytochrome P-450 by the interactions of the cytochrome with various sterols. These differences in spectra were not due to the exchange of ligand (SCHLEYER et al. 1971a) but to the perturbation of the environment of the heme group (SCHLEYER et al. 1971b). Some of these interactions altered the specificity of the hydroxylase system with steroid as substrate.

Dimerization of quinones, (phenanthrenequinone anions) with no evidence of covalent bond formation, have been reported (STAPLES & SZWARC 1970). The dimerization, due to $\pi - \pi$ electron interaction, is accompanied by a bathochromic shift in λ_{max}. The absorbance spectra of quinones are also different in different solvents. The change in the environment of 2,6-dimethylbenzoquinone by replacement of ethanol as solvent with methanol shifts the absorbance maxima approximately three nanometers toward the blue (PHILLIPS et al. 1969). Addition of KOH to either p-toluquinone or phenyl p-benzoquinone in methanol induces a 14 nm or larger shift (SIMMONS 1979).

The bathochromic shift of the spectrum of ubiquinone induced by naphthalene is small but within the resolution of the spectrophotometer. It could indicate either changes in the π-electron cloud or other perturbations of the quinone by its environment. These changes in the quinone may disrupt its ability to bind to Q-binding proteins (YU et al. 1977), or alter the midpotential of Q/QH_2, $Q/QH\cdot$ and $QH\cdot/QH_2$ redox pairs, altering the ability of Q to accept or donate electrons to the rest of the mitochondrial electron transport system. As ubiquinone is essential to electron transport (TZAGOLOFF 1982), this may inhibit respiration.

That naphthalene induces inhibition of $NADH \rightarrow Q_{10}$ and $QH_2 \rightarrow O_2$ while not affecting duroquinol$\rightarrow O_2$ activity, and alters the spectrum of ubiquinone while not affecting that of tetramethylbenzoquinone suggests an interaction of naphthalene specifically with coenzyme Q. The data support a molecular basis for the rather specific effect of naphthalene on respiration of isolated cardiac mitochondria, intact cells, and whole organisms which may suggest a possible mode of action of other polyaromatic hydrocarbons.

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