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Effect of Naphthalene on Cytochrome Oxidase Activity

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Previous reports have demonstrated that naphthalene inhibits oxygen consumption in Daphnia magna (Crider et al.1982), tissue culture cells (Harmon and Sanborn 1982), and intact mitochondria and submitochondrial particles (Harmon and Sanborn 1982). Struble and Harmon (1985) extended the studies to algal mitochondrial respiration as well as photosynthetic activity. We were able to demonstrate the specific site of apparent respiratory inhibition to be coenzyme Q (ubiquinone, UQ) (Harmon and Sanborn 1982) and later to demonstrate the molecular basis of this inhibition at ubiquinone (Struble and Harmon 1983).

Harmon and Sanborn (1982) could not demonstrate an effect of naphthalene on cytochrome oxidase activity. The observation by Struble and Harmon (1985) that naphthalene can stimulate respiration in algae prompted the reinvestigation of the effect of naphthalene on the kinetics of cytochrome oxidase. Cytochrome oxidase is a multi-subunit membranous protein responsible for the oxidation of cytochrome c and the reduction of molecular oxygen to water. Because of the complicated nature and mechanism of this enzyme, the potential exists for multiple and possibly opposite effects of naphthalene on its function.

MATERIALS AND METHODS

Beef heart mitochondria were isolated by the procedure of Crane et al (1956). Lipid-depleted cytochrome oxidase was purified from beef heart mitochondria by the procedure of Yu, Yu, and King (1975) except that 20% neutralized cholic acid (Aldrich) was added at the levels of 3.75 ml/100 ml and 5.8 ml/100 ml protein suspension in in the first and second detergent fractionations, respectively.

Cytochrome oxidase activity was measured at 25 C in a glass water-jacketed chamber fitted with a Clark oxygen electrode as described previously (Harmon And Crane 1976; Harmon and Sanborn 1982).

CO recombination kinetics were measured at 448 nm with a Gilford Model 252 spectrophotometer. A clear glass dewar with a 2 mm light

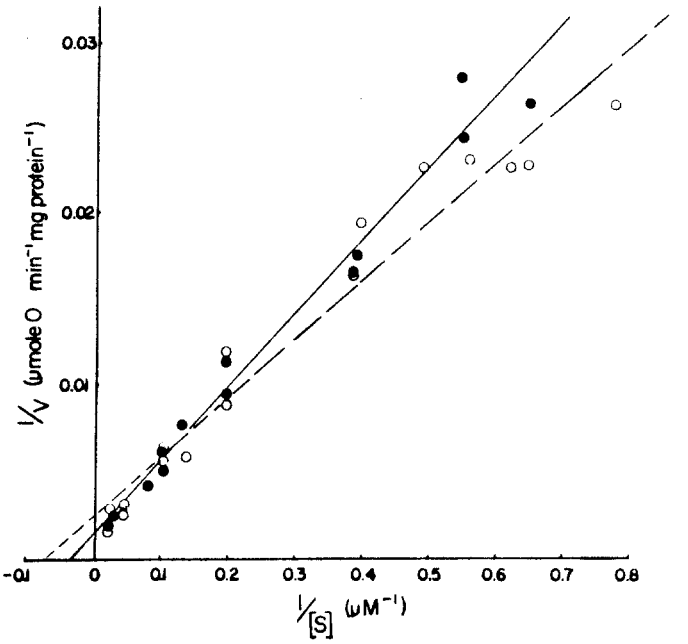

Figure 1. Representative plot of the inverse of substrate (cytochrome c) concentration vs. the inverse of the rate of oxygen consumption in control (open circles) and 15 ppm naphthalene-treated (filled circles) cytochrome oxidase. Lines drawn were calculated by least square linear regression and have a 0.99 coefficient of fit.

path plexiglass sample holder as used previously (Harmon and Sharrock 1978) was fitted in place of the cuvette holder. The sample was suspended above liquid nitrogen level in the dewar; the temperature was measured by a copper-constantan thermocouple in the frozen sample and regulated by a small electric heater coil in the liquid nitrogen. The output of the Gilford spectrophotometer was connected to a 16-bit A/D converter in an IBM-PC computer for data acquisition.

Low temperature kinetics were initiated by a single flash of two 25 Joule xenon flash tubes. The xenon tubes were fitted with Wratten #9 and #15 interference filters to absorb wavelengths of light below 450 nm and a Corning 5113 glass filter was placed in front of the photomultiplier to block the wavelengths of the xenon flash yet transmit 448 nm light. Data acquisition was initiated upon triggerring the flash tubes by the computer. Acquistion software was written by Dr. James T. Blankemeyer of this department.

Fully reduced carboxy-cytochrome oxidase was formed by the addition of 12.5 g N-N-N'-N'-tetramethylphenylenediamine dihydrochloride (TMPD) and 90 mM sodium ascorbate (pH 6.8) to a 100% CO-flushed mixture containing 0.1 mg cytochrome c (Sigma, type VI) and approximately 6 mg of cytochrome oxidase in 0.25 M sucrose-50 mM

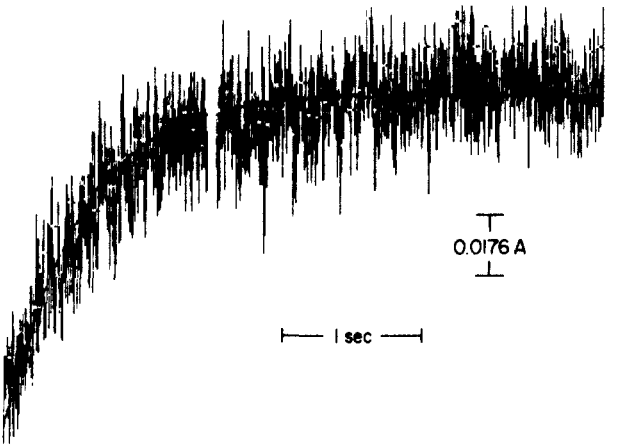

Figure 2. Plot of the change in 448 nm absorbance (increase in amount of cytochrome oxidase-CO complex in upward direction) vs. time. This plot represents typical raw data acquired by the computer. Following flash photolysis of the carboxy-oxidase at time=0, CO rebinds to cytochrome oxidase. The computed least squares fit of the data indicates the time dependence of the curves is exponential and can be described by the equation $f(t) = e^{-kt}$ where f(t) is the fraction of unbound oxidase not recombined with CO at time t after the flash. k is proportional to the rate of recombination of cytochrome oxidase with CO after the dissociating flash.

sodium phosphate butter (pH 7.4). This mixture was bubbled with 100% CO for 20 minutes in the dark and then loaded into the sample holder in the dark prior to freezing in liquid nitrogen.

RESULTS AND DISCUSSION

In the presence of 15 ppm (117 M) naphthalene, the values of K_m and V_{max} as derived from Lineweaver-Burk plots increase almost two-fold relative to the values in control samples (shown in Fig. 1). For the data shown in Fig. 1, the value of K_m increases from 13.1 to 26.6 M and V_{max} increases from 766 to 1304 ng-atom O/min/mg protein. From all experiments, the K_m values for control and naphthalene-treated oxidase were 17.1 $\pm$ 4.8 and 30.8 $\pm$ 7.8 (S.D.) M, respectively.

Since $v/V_{max} = [S] / K_m + [S]$, at a given substrate concentration the v/V_{max} ratio will be smaller in the presence of naphthalene. Since the value of V_{max} is increased by naphthalene, the overall measured velocity of the reaction is essentially unchanged. Thus our previous report of overall apparent lack of effect on cyto-

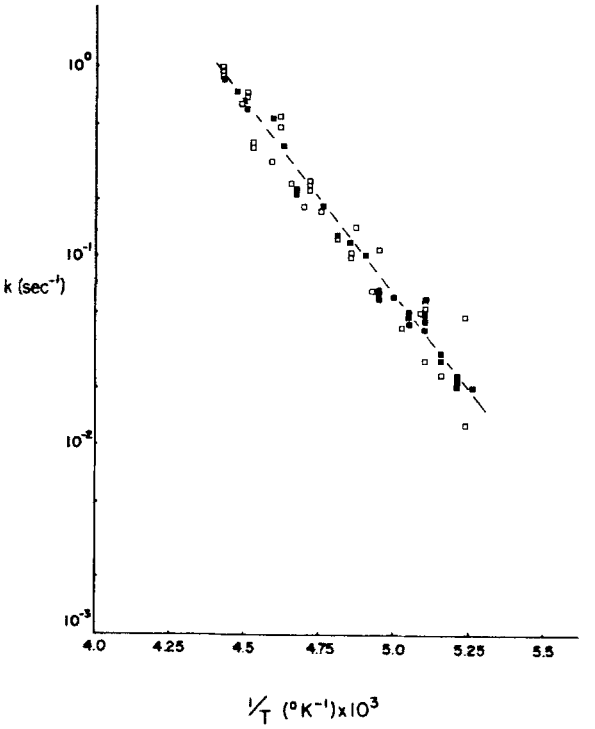

Figure 3. Plot of log k of CO recombination vs. inverse temperature in control (open squares) and 15 ppm naphthalene-treated (filled squares) cytochrome oxidase. The energy of activation of both data sets computed from the least squares fit of the data points is 9.28 kcal/mole.

chrome oxidase activity was correct even though the enzyme is actually altered by naphthalene.
CO recombination following flash photolysis in the control and naphthalene-treated samples proceeds via single monophasic exponential reaction kinetics as shown in Figure 2 and is described by the following reaction:

$$a^{2+}a_3{}^{2+}CO \qquad a^{2+}a_3{}^{2+} + CO$$

The energy of activation for the reaction can be obtained from a plot of log k vs. 1/T. As shown in Figure 3, the energy of activation is approximately 9.3 kcal/mole for both samples; the rates of recombination of CO are not altered by naphthalene.

Cytochrome oxidase contains four electron centers, two coppers and two cytochromes. Cytochrome a is involved in the binding and oxidation of cytochrome c, while cytochrome a_3 is associated with the binding and reduction of molecular oxygen to water. To determine if naphthalene alters the binding of oxygen to cytochrome a_3, the rebinding of CO, an oxygen analog, to the oxidase was measured. Previous studies (Harmon and Sharrock 1978; Harmon and Wikstrom 1978; Chance et al. 1975) have indicated that

the low temperature binding kinetics of oxygen and CO are very similar if not identical. The presence of naphthalene does not alter the binding of ligand to cytochrome a3; we can therefore assume that naphthalene does not effect the nature or activity of cytochrome a3.

This study illustrates two important concepts. First, the lack of apparent enzymatic rate changes by addition of a compound does not mean that the enzyme is totally unaffected. The second concept is a reiteration of the specificity of naphthalene. We previously showed the site and mechanism of action at ubiquinone. In this study we have demonstrated an effect at cytochrome a but not at other cytochrome oxidase centers.

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