REACTION OF CYANIDE WITH CYTOCHROME *d* IN RESPIRATORY PARTICLES FROM EXPONENTIAL PHASE *ESCHERICHIA COLI*

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1. Introduction

Membrane particles prepared from *Escherichia coli* grown to the stationary phase contain much higher levels of cytochrome *d* than of cytochrome *o* [1]. Both of these cytochromes can function as the terminal oxidases of the respiratory chain [2]. In a previous paper [1] we were able to correlate the rate of cyanocytochrome *d* formation with the kinetics of inhibition of NADH oxidase by cyanide. The rate of formation of cyanocytochrome *d* was directly proportional to the rate of electron flux through cytochrome *d*.

In exponential phase cells cytochromes *d* and *o* are present in about equal amounts. In these cells NADH oxidase activity was more sensitive to inhibition by cyanide than in the stationary phase cells [1]. Thus, the increased sensitivity to cyanide appeared to correlate with the presence of cytochrome *o*.

However, the total amount of the cytochrome oxidases present in membrane particles from stationary phase cells is about five times greater than exponential phase cells although the NADH oxidase activities are similar. Thus, a more rapid electron flux must occur through the smaller pool of terminal oxidases in cells grown to the exponential phase. Since cyanide does not react with the oxidized or the reduced forms of cytochrome *d* [1] but with an intermediate form generated in the oxidation–reduction cycle, the increased sensitivity of exponential phase cells to cyanide need not be entirely due to the possession of cytochrome *o* but could also result from the higher aerobic steady state level of this intermediate. The reaction of cyanide with cytochrome *d* in exponential phase cells is discussed in this paper.

2. Experimental

2.1. *Preparation of respiratory particles*

Escherichia coli NRC-482 was grown on 0.8% disodium succinate as previously described [1]. The cells were harvested in the exponential phase of growth Membrane particles were obtained as before [1] and were suspended in 0.1 M phosphate buffer, pH 7.0, containing 0.01 M $MgCl_2$, to a concentration of 3–10 mg particle protein per ml.

2.2. *Measurement of spectra*

Difference spectra were taken as previously described using a Perkin-Elmer Hitachi model 356 spectrophotometer. Cytochromes were quantitated using the extinction coefficients given by Jones and Redfearn [3] and Baillie et al. [4].

2.3. *Determination of kinetics of formation of cyanocytochrome d*

The effect of KCN on the disappearance of the oxidized form of cytochrome *d* under turnover conditions was followed as previously described [1] by measuring the change in absorption at 648 nm (absorption peak of oxidized cytochrome *d*) relative to 607 nm. Since cells grown to the exponential phase contain relatively low levels of cytochrome *d* a full scale deflection of 0.01 absorbance units was used. To initiate the reaction NADH (final concentration, 2 mM) or succinate (final concentration, 1.67 mM) was added to an aerobic suspension of respiratory particles containing varying concentrations of KCN (0–10 mM) in a total volume of 1.5 ml. Oxidation rates for NADH and succinate were determined from the time required

for the system to become anaerobic as measured by the appearance of the absorption peak of reduced cytochrome *d*. All assays were carried out at 22°C.

Protein was estimated by the method of Lowry et al. [5].

3. Results and discussion

The logarithm of the absorbancy change due to the disappearance of oxidized cytochrome *d* plotted vs. time after the addition of various concentrations of cyanide to membrane particles from exponential phase cells in the presence of 1.67 mM succinate is shown in fig.1. The pseudo-first order rate constants determined from the slope of these lines was plotted vs. the concentration of cyanide (fig.2). From the slope of this line a second order rate constant of 0.22 $M^{-1}s^{-1}$ was calculated for the reaction of cyanide with cytochrome *d* in the presence of 1.67 mM succinate. The rate of succinate oxidation with this concentration of succinate was 0.41 ng atom O s^{-1} mg $protein^{-1}$.

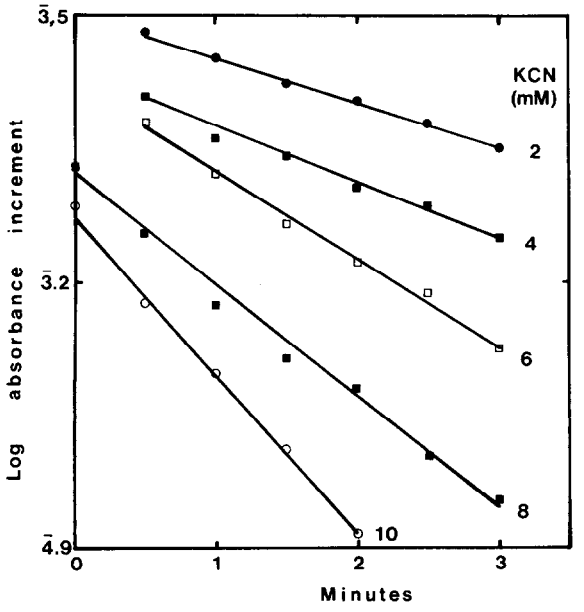

Fig.1. Reaction of cyanide with cytochrome *d* of membrane particles from exponential phase cells in the presence of 1.67 mM succinate. The absorbance at 648 nm relative to 607 nm was measured to follow the disappearance of oxidized cytochrome *d*. Log (absorbance increment at 648 nm relative to 607 nm) is plotted vs. time after addition of succinate. Membrane particles, 8.3 mg protein per ml.

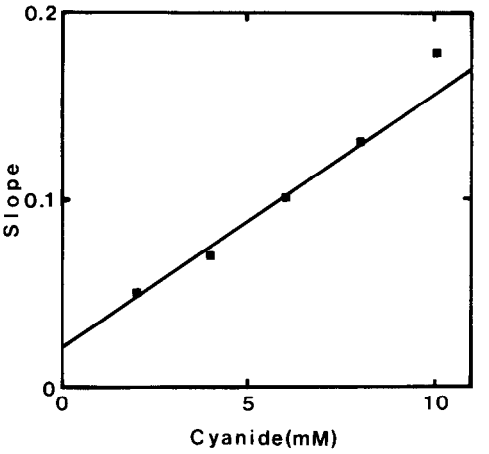

Fig.2. Reaction of cyanide with cytochrome *d* in the presence of 1.67 mM succinate. The slopes of the lines shown in fig.1 are plotted vs. concentration of cyanide present.

In a similar way using 2 mM NADH as substrate the second order rate constant for the formation of cyanocytochrome *d* was found to be 4.0 $M^{-1}s^{-1}$ and the oxidation rate was 7.1 ng atom O s^{-1} mg $protein^{-1}$.

Comparing these data it is seen that in membrane particles from exponential phase cells the rate of formation of cyanocytochrome *d* is directly proportional to the rate of electron flux through this cytochrome as was previously shown with membrane particles from stationary phase cells [1]. Moreover, it is evident that electrons from both NADH and succinate must pass through the same cytochrome *d* pool.

From the data previously presented for stationary phase cells [1] the rate of formation of cyanocytochrome *d* relative to the rate of flux through the cytochrome *d* pool was calculated by dividing the second order rate constant for the formation of cyanocytochrome *d* by the rate of electron flux through cytochrome *d* expressed as g atom O reduced per second per mole cytochrome *d*. A value of 0.096 mole cytochrome *d* M $cyanide^{-1}$ g $atom\ O^{-1}$ was obtained for these cells which contain cytochrome *d* as the only terminal oxidase. A similar calculation from the results in the present paper for exponential phase cells containing 0.066 and 0.076 nmoles of cytochromes *o* and *d* per mg protein, respectively, gave values of

0.043 and 0.042 mole cytochrome *d* M cyanide^{-1} g atom O^{-1} for the inhibition by cyanide of NADH and succinate oxidase activities. This calculation was made assuming that the electrons passed through cytochrome *d* only. However, if the electrons are assumed to be partitioned equally between the almost equal pools of cytochromes *o* and *d* then these values become about 0.085 mole cytochrome *d* M cyanide^{-1} g atom O^{-1}. This value is of the same order of magnitude as that found with the stationary phase cells and suggests that cytochrome *d* and cytochrome *o* are both functional cytochrome oxidases for NADH and succinate oxidation in exponential phase cells of *E. coli*. This conclusion differs from that of Castor and Chance [2] who using photodissociation spectroscopy concluded that cytochrome *d* was functional only in the stationary phase of growth. Moreover the similarity of the constants calculated for the rate of formation of cyano-cytochrome *d* in respiratory particles of exponential and stationary phase cells relative to the electron flux through cytochrome *d* suggests that the difference in the sensitivity of substrate oxidation to cyanide in the two types of cells could be due to the difference in the size of the pool of cytochrome *d* and in the rate of electron flux through it.

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