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The Effect of Measuring Light Photolysis on the Kinetics of Reaction of Reduced Cytochrome a_3 and Carbon Monoxide

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The kinetics of reaction of reduced cytochrome a_3 and CO are re-investigated by non-linear optimization techniques. When photolysis by the monitoring light is taken into account, the experimental data are best fitted by a single-intermediate mechanism.

The kinetics of the reaction between CO and fully reduced membrane-bound cytochrome oxidase (EC 1.9.3.1) over the 178–203 K range have been analysed by non-linear optimization techniques (Clore & Chance, 1978) by using the computer program FACSIMILE (Curtis, 1976, 1977; Curtis & Kirby, 1977). The kinetic data were obtained by dual-wavelength multichannel spectroscopy at three wavelength pairs (591–630, 608–630 and 445–460 nm). The kinetic model that fitted the data best was a two-intermediate sequential mechanism (model 2 in Scheme 1): flash photolysis of the CO-bound enzyme (II) yields free cytochrome oxidase (E) and CO, which then recombine to form intermediate I. Intermediate I is then converted to complex II.

The present study is a re-investigation of the above reaction and the method of kinetic analysis, and an extension of this work to ferricyanide-pretreated cytochrome oxidase. Some modifications of the optical set-up were necessary to improve the quality of the data and the method of digitization of the kinetic traces.

The photolysis flash which initiates the reaction causes approx. 85% dissociation of the CO–enzyme complex. Since the absorbance change in the 590–610 nm, region, due to the reaction, is small (approx. 0.01 A unit), it is necessary to use a high-intensity beam to monitor the reaction at these wavelengths in order to improve the signal-to-noise ratio and to minimize the photolysis artifact. However, the intensity of the monitoring beam is sufficient to cause photolysis of the CO–enzyme complex, and, when the experiment is performed at 160 K, causing the rate of recombination to be negligible, photodissociation proceeds at approx. 10^{-3} s^{-1} for the particular optical set-up. The data are digitized directly, by using an on-line PDP-11 computer, and fitted to three kinetic models, which are shown in

Abbreviations used: χ^2_{av} , the average value of chi-squared per degree of freedom (in general, the number of degrees of freedom was about 120); $\bar{c}$, mean absolute correlation index; s.d._{in} , standard deviation of the natural logarithm of a parameter.

Scheme 1 together with the initial concentrations of all the reactants. In models 1, 2 and 3, the steps represented by k_{-1} , k_{+3} and k_{-2} respectively correspond to photodissociation of the CO–enzyme complex (II). Since the CO concentration is about three orders of magnitude higher than the concentration of enzyme, step k_{+1} is kinetically pseudo-first-order in enzyme.

The initial concentrations of E, I and II, and the trial values of the parameters that may be optimized, are specified at the start of the computer simulation. The parameters that are optimized by the program are, in general: (a) rate constants (k_{+1} , k_{+2} etc.); (b) scale factors (S591, S608, S445) which normalize the kinetic curves at different wavelengths; (c) relative absorption coefficients of E and I at the three different wavelength pairs [(c) applies only for models 2 and 3].

The values of the optimized rate constants for model 1, together with $\bar{c}$ and χ^2_{av} for four runs, are presented in Table 1. The scale factors are always well determined ($\text{s.d.}_{\text{in}} = 0.005$). It is noted that: (a) $\bar{c}$ is approximately unity for the reaction of ferricyanide-pretreated oxidase, but it is significantly larger for fully reduced oxidase; (b) the rate constants are all well determined (s.d._{in} is 0.1 or less); (c) k_{-1} is approximately temperature-independent, as expected for a photochemical reaction, and is approx. 0.003 s^{-1} .

The statistical fit is considerably improved when the data are fitted to models 2 or 3, which involve an additional intermediate (I); the values of χ^2_{av} and $\bar{c}$ are considerably less than those in Table 1. However, model 2, which was proposed by Clore & Chance (1978), fails, since most of the optimized parameters are very badly determined ($\text{s.d.}_{\text{in}} > 10$). Model 3 also fails because of under-determination, though in this case the standard deviations are smaller (s.d._{in} approx. 1).

Hence, for the ferricyanide-pretreated case, model 1 gives by far the best fit to the experimental data. For fully reduced cytochrome oxidase, model 1 introduces a slight systematic deviation, causing a