BBA 46393

SOME PECULIARITIES OF THE STEADY-STATE KINETICS OF ELECTRON TRANSFER IN SUBMITOCHONDRIAL PARTICLES. A KINETIC MODEL BASED ON THE IDEA OF ACTIVATION ON THE RESPIRATORY CHAIN INDUCED BY ELECTRON TRANSFER

V. A. SAKS, V. V. KUPRIYANOV AND V. N. LUZIKOV

Laboratory of Bioorganic Chemistry, Moscow State University, Moscow (U.S.S.R.)

(Received April 25th, 1972)

SUMMARY

1. The dependence of the steady-state levels of reduced cytochromes on the rate of electron transfer in submitochondrial particles has been studied.

2. The modified Pring equation (M. Pring, in F. Heinmets and L. D. Cady, *Concepts and Models in Biomathematics*, Dekker, New York, 1969, p. 75) has been used to calculate the rate constants for the interaction of adjacent cytochromes. The constants have been shown to increase linearily with increasing electron transfer rate in the steady state.

3. In order to explain these results a kinetic model has been suggested, based on the assumption that the respiratory chain exists in activated and non-activated forms the relationship of which depends on the rate of electron transfer.

4. The kinetic model gives an equation for the dependence of the steady-state levels of reduced cytochromes upon the rate of electron transfer .

5. The equation allows one to find the rate constants of interaction of the cytochromes (s^{-1}) in the activated and non-activated respiratory chains. The values of the constants agree satisfactorily with the data of Pring and Chance obtained from non-stationary kinetics.

INTRODUCTION

The kinetics of electron transfer *via* the respiratory chain have been extensively studied in recent years[1-9]. Two rather similar theoretical approaches now exist regarding the quantitative description of this process. One of them, put forward by Chance and co-workers[1,3,6,7], was preferentially used to study the steady-state kinetics of electron transfer. It is based on the assumption that electron transfer may be considered as a system of consecutive second-order reactions, operating in the homogeneous phase[1,3] (see also ref. 5). Some restriction of the mobility of the carriers in the membrane was taken into account by introducing a steric coefficient in the rate equation[2].

The second approach to the above problem was suggested recently by Pring, who relied upon the following assumptions: (1) the inner mitochondrial membrane contains respiratory ensembles (oxysomes) whose interaction is rather weak; (2) such

a system may obey the law of mass action; (3) the rate constant for electron transfer between any pair of adjacent carriers does not depend upon the state of any other carrier in the chain; (4) the oxidized and reduced forms of two adjacent carriers are distributed randomly in relation to each other[8]. The following equation for the electron transfer rate was derived from the above assumptions:

$$V = k_i \cdot p_i^{r} \cdot p_{i+1}^{ox} \cdot C_0 \quad (1)$$

where k_i is the rate constant for electron transfer between the ith and (ith $+1$) carrier (s^{-1}); p_i^{r} is the proportion of ith carriers in the system in the reduced state; p_{i+1}^{ox} is the proportion of (ith $+1$) carriers in the system in the oxidized state; C_0 is the quantity of respiratory chains (oxysomes) in the system. Using this equation to evaluate the experimental data obtained when studying the non-stationary kinetics of oxidation and reduction of electron carriers with limited quantities of oxygen, Pring and Chance arrived at the conclusion that interchangeable activated and non-activated forms of the respiratory chain exist[8,9]. The relationship between these two forms is (in accordance with these authors) controlled by the concentration of oxygen in the medium[8,9].

In the present work the idea of the existence of activated and non-activated forms of the respiratory chain is developed. Contrary to the assumptions of Pring and Chance, our assumption is that the relationship between these two forms depends on the rate of electron transfer. An equation is given describing the dependence of the steady-state levels of the reduced cytochromes on the rate of electron transfer. This equation fits the experimental data for a wide range of electron transfer rates. It permitted the rate constants for the interaction between the cytochromes in the non-activated chains to be determined and also the order of these values in the activated chains. The values obtained are in satisfactory agreement with the data of Pring and Chance[8,9]. The approach developed in this work helps to overcome some difficulties in interpreting the experimental results which were indicated by Pring[8].

MATERIALS AND METHODS

Submitochondrial particles ETP_H (Mg^{2+},Mn^{2+}) were isolated as described by Beyer[10]. Alkaline submitochondrial particles, obtained as described by Crane *et al.*[11], were also used in this work.

The steady-state levels of reduced cytochromes *b*, c_1 and *c* were determined by measuring the absorbance differences at 562–577 nm, 554–540 nm, and 550–535 nm, respectively[4,12]. The levels of reduced cytochromes *a* and a_3 were evaluated, both at 605–630 nm, and 444–455 nm[4,13]. The measurements were carried out in a Hitachi-356 two-wavelength double-beam spectrophotometer. The steady-state value of the electron transfer rate was determined by timing the exhaustion of oxygen in the spectrophotometric cuvette. The solubility of oxygen at 30 °C was assumed to be 430 natoms/ml[14]. The assay medium contained 80 mM potassium phosphate (pH 7.5) and 1 mM EDTA (in the case of the alkaline particles). The concentration of the particles was 0.7–2.5 mg protein per ml. The electron transfer rate in the case of the alkaline particles was varied by adding various quantities of amital (0.2–1.4 mM). The concentration of NADH was thus 1.8 mM. With submitochondrial particles

ETP_H the rate was controlled by changing the concentration of alcohol dehydrogenase (from 20 to 800 μg/ml) in an NADH-generating system, which also contained 0.3 mM NAD^+, 30 mM ethanol and 10 mM semicarbazide. When succinate (10 mM) was used as substrate, the medium contained various quantities of malonate (0.2–4.0 mM). The temperature of the assay medium was 30 °C.

It is known that reduced cytochromes *a* and a_3 have absorption bands at 605 nm and at 444 nm[13,15] and cytochromes *b*, *c* and c_1 have overlapping absorption bands[12]. Therefore, the levels of reduced cytochromes in the steady state had to be determined more precisely in each case. The method of Vanneste[12] was used in order to account for the overlapping absorption bands of cytochromes c_1, *c* and *b*, and the system of equations of Yonetani[16] was used in the case of cytochromes *a* and a_3.

In these equations we used the values of the extinction coefficients of cytochromes *a* and a_3 as recently determined by Van Gelder[17]. We obtained therefrom:

$$p_a{}^r = \frac{5p_\alpha{}^r - 2p_\gamma{}^r}{3} \qquad \text{(II)}$$

$$p_{a_3}{}^r = \frac{16p_\gamma{}^r - 10p_\alpha{}^r}{6} \qquad \text{(III)}$$

where $p_\alpha{}^r$ and $p_\gamma{}^r$ correspond to the steady-state levels of reduced cytochromes determined experimentally at 605–630 nm and 444–455 nm, respectively. It is interesting to note that it follows from Eqns II and III (as well as from the equations of Yonetani[16]) that at $p_\alpha{}^r = p_\gamma{}^r$ cytochromes *a* and a_3 are reduced in the steady state to the same degree (Fig. 1).

Calculation of the kinetic constants

The rate constants for the interaction between the cytochromes were calculated using Pring's equation for the steady state:

$$k_i' = \frac{V_{st}}{p_i{}^r \cdot p_{i+1}{}^{ox}} \qquad \text{(IV)}$$

Here, V_{st} is the steady-state value of the specific rate of electron transfer (natoms of reduced oxygen/mg protein per unit of time), $p_i{}^r$ is the steady-state level of the reduced *i*th cytochrome, $p_{i+1}{}^{ox}$ is the steady-state level of the oxidized (*i*th + 1) cytochrome and $k_i' = k_i C_0$ (C_0 is the content of respiratory chains in the preparation per mg protein).

The second-order rate constant, calculated by the method of Chance[2] (without taking the steric coefficient into account), is related to k_i' in the following way:

$$k_i'' = \frac{m \cdot 10^{-6}}{[X_i] \cdot [X_{i+1}]} \cdot k_i' \qquad \text{(V)}$$

where *m* is the concentration of the preparation (mg/l), $[X_i]$ and $[X_{i+1}]$ are the concentrations of the carriers in the assay medium (M) and k_i'' is the second-order rate constant ($M^{-1} \cdot s^{-1}$).

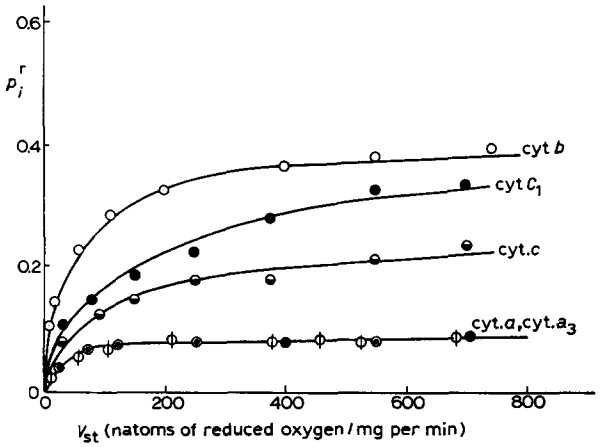

Fig. 1. The change in the steady-state levels of reduced cytochromes (p_i^r) with change in the rate of NADH oxidation by submitochondrial particles ETP_H (Mg^{2+}, Mn^{2+}). Measurements were carried out in 80 mM potassium phosphate (pH 7.5) at the wavelengths indicated in Materials and Methods. The precise steady-state levels of individual cytochromes were calculated as described by Vanneste[12] and Yonetani[16]. The concentration of the particles was 0.75–2.5 mg/ml. The rate of NADH oxidation was controlled by changing the rate of its generation in the system by adding 30 mM ethanol, 0.3 mM NAD^+, 10 mM semicarbazide and from 20 to 800 μg alcohol dehydrogenase per ml. Temperature, 30 °C.

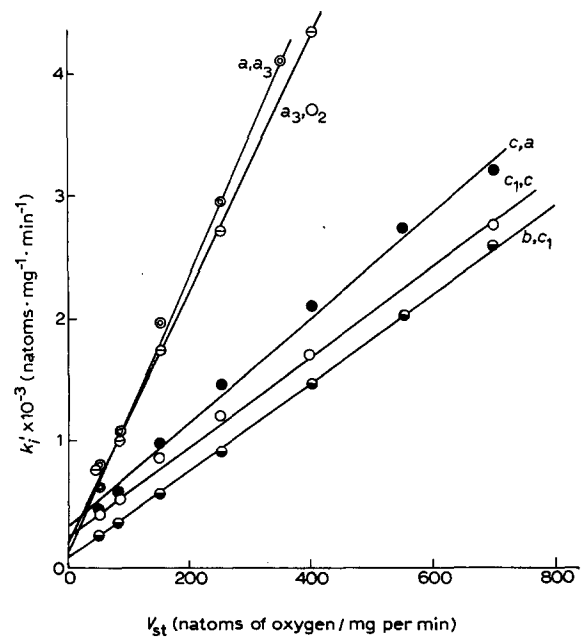

Fig. 2. The change in the rate constant k_i' with change in the rate of NADH oxidation. k_i' was determined from Eqn IV using the data shown in Fig. 1. For the step cytochrome $a_3 \rightarrow O_2$, k' was calculated, assuming $p_{O_2}^{ox} = 1$.

RESULTS

Dependence of the steady-state levels of reduced cytochromes upon the electron transfer rate

Fig. 1 shows the changes in the steady-state levels of reduced cytochromes *b*, c_1, *c*, *a*, and a_3, depending on the rate of electron transfer. It can be seen in the Fig. 1 that all the cytochromes achieve some level of reduction, which changes very little if the electron transfer rate is further increased. On the basis of these data, one can calculate the rate constants for the interaction between the cytochromes at every given rate of electron transfer. Fig. 2 shows the calculated values of the rate constants k_i' (see Eqn IV) at various rates of electron transfer. One can see that k_i' for all the cytochromes increases in a strictly linear fashion as does the steady-state electron transfer rate (V_{st}). This relationship was observed regardless of the mode of altering the electron transfer rate: by controlling the rate of NADH generation or by adding various quantities of amital. A similar dependence occurred when succinate and malonate in various concentrations were used. All these facts point to the universal character of the linear dependence of k_i' on the electron transfer rate.

From the data shown in Fig. 2 the following equation was derived:

$$k_i' = \alpha_i + \beta_i \cdot V_{st} \qquad \text{(VI)}$$

where α_i and β_i are constants.

Introducing Eqn VI in Eqn IV one can find an empirical expression for the dependence between the electron transfer rate in the steady-state and the steady-state levels of any pair of reduced cytochromes:

$$V_{st} = (\alpha_i + \beta_i \cdot V_{st}) \cdot p_i^{r} \cdot p_{i+1}^{ox} \qquad \text{(VII)}$$

or

$$V_{st} = \frac{\alpha_i \cdot p_i^{r} \cdot p_{i+1}^{ox}}{1 - \beta_i \cdot p_i^{r} \cdot p_{i+1}^{ox}} \qquad \text{(VIII)}$$

It follows from Eqn VIII that a plot of $1/V_{st}$ *vs* $1/p_i^{r} \cdot p_{i+1}^{ox}$ will give a straight line from which constants α_i and β_i can be determined. (Fig. 3). These constants, for various pairs of cytochromes, are presented in Table I.

Fig. 4 shows the temperature dependence of constants α and β for the cytochromes pair b–c_1. The unusual character of the log β *vs* $1/T$ plot gives reasons for believing that constant β is a more complex value than the elementary rate constant. This suggestion will be dealt with in detail in the following section. However, to explain the complicated character of the temperature dependence of constant β (the cytochrome pair b–c_1) it should be borne in mind that in the respiratory chain several forms of cytochrome *b* exist[18]. The temperature dependencies of constants α_i and β_i are interesting in themselves and deserve a special study.

The dependence of rate constant k_i' upon the steady-state rate of electron transfer (V_{st}) (Eqn VI) may be interpreted as the activation of the respiratory chain. As the experiments described in this paper were performed with oxygen concentrations far exceeding those necessary for complete saturation of the enzyme[9,19], and as the oxygen content in the medium was the same in all the experiments, the concepts of Pring and Chance[8,9] cannot account for this activation. The dependence of the rate

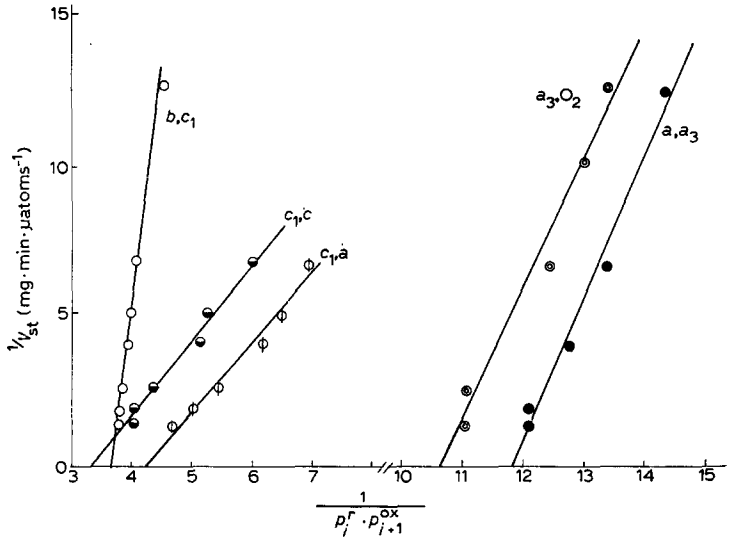

Fig. 3. Plot of $1/V_{st}$ *versus* $1/p_i{}^r \cdot p_{i+1}{}^{ox}$.

TABLE I

VALUES OF THE CONSTANTS α_i AND β_i AND RATE CONSTANTS k_i AND $k_i{}^*$ FOR DIFFERENT PAIRS OF CYTOCHROMES AT 30 °C

The experimental conditions are described in the legend to Fig. 1. α_i and β_i are calculated from the data shown in Fig. 3, $k_i{}^*$ and k_i were determined as described in the text. The constants were calculated for the particle preparation which contained 0.4 nmole haeme aa_3 per mg protein [$\Delta\varepsilon$ at 605–630 nm was assumed to be 24 $mM^{-1} \cdot cm^{-1}$ (ref. 17)].

Pair of cytochromes	α_i (*natoms O/mg per s*)	β_i	*Rate constants* (s^{-1})			
			k_i	$k_i{}^*$	$k_i{}^b$ (*slow*)	$k_i{}^b$ (*fast*)
b, c_1	1.28	3.7	3.2	$\geqslant$ 77	3.336	35.61
	10[a]	3.5[a]				
c_1, c	6.65	3.4	16.6	$\geqslant$ 93	12.37	308.3
c, a	7.5	4.2	18.7	$\geqslant$ 102	3.145	95.93
	7.2[a]	3.1[a]				
a, a_3	3.5	11.8	8.7	$\geqslant$ 238	2.267	197.7
a_3, O_2	3.9	10.7	9.8	$\geqslant$ 226	131.2	

[a] The constants were obtained for the alkaline particles, when the electron transfer rate was varied by adding different quantities of amital. Concentration of NADH, 1.8 mM (see Materials and Methods). The same values of α_i and β_i were observed when 1 mM carbonyl cyanide *m*-chlorophenyl hydrazone was added to submitochondrial particles ETP_H (Mg^{2+}, Mn^{2+}) with slightly coupled oxidation and phosphorylation.

[b] See ref. 8.

constant upon the electron transfer rate allows one to suggest, other things being equal, that the relationship between the activated and non-activated forms of the respiratory chain is controlled by the rate of electron transfer. Described below is a kinetic model based on this assumption.

The kinetic model

Let us assume that every act of electron transfer from a substrate to oxygen results in the appearance of an activated form of a respiratory chain and that the activated form is characterized by the rate constants k_i^* ($k_i^* > k_i$). Let us also assume that the activated form of the respiratory chain is converted to the non-activated form with rate constant k_s. The rate of appearance of activated chains will then equal the rate of electron transfer V and the rate of their inactivation will equal $-k_s \cdot C^*$ (the latter assumption has also been made previously by Pring[8]). The change in the number of activated chains can then be determined from the following equation:

$$\frac{dC^*}{dt} = V - k_s \cdot C^* \tag{IX}$$

It is obvious that in the steady-state $dC^*/dt = 0$, since, if this were not the case, the rate of electron transfer would not have been constant. Therefore, the proportion of activated chains, C^*/C_0, may be determined from the equation:

$$\frac{C^*}{C_0} = \frac{V_{st}}{C_0} \cdot \frac{1}{k_s} = \omega\tau \tag{X}$$

where ω is the frequency of the electron transfer in the steady state across the respiratory chain, and τ can be viewed as the average lifetime of an activated respiratory chain. The proportion of chains in the initial non-activated state then equals $1-\omega\tau$. If we suppose that the activation of respiratory chains does not influence the random fashion of the distribution in the system of reduced or oxidized forms of adjacent carriers, $p_i^r \cdot p_{i+1}^{ox}$ may be considered to be the same for activated and non-activated respiratory chains. In this case the steady-state rate of electron transfer by the non-activated chains can be represented by:

$$V_{st}^{na} = k_i \cdot p_i^r \cdot p_{i+1}^{ox} \cdot C_0(1 - \omega\tau) \tag{XI}$$

The rate of electron transfer by the activated chains by:

$$V_{st}^{a} = k_i^* \cdot p_i^r \cdot p_{i+1}^{ox} \cdot C_0 \cdot \omega\tau \tag{XII}$$

and the total rate of electron transfer at stage $i \rightarrow i+1$ in the steady state by:

$$V_{st} = V_{st}^{na} + V_{st}^{a} = [k_i \cdot C_0 + (k_i^* - k_i) \cdot \tau \cdot V_{st}] \cdot p_i^r \cdot p_{i+1}^{ox} \tag{XIII}$$

or

$$V_{st} = \frac{k_i \cdot C_0 \cdot p_i^r \cdot p_{i+1}^{ox}}{1 - (k_i^* - k_i) \cdot \tau \cdot p_i^r \cdot p_{i+1}^{ox}} \tag{XIV}$$

It is noteworthy that Eqn XIV coincides with Eqn VIII, if

$$\alpha_i = k_i \cdot C_0 \tag{XV}$$

$$\beta_i = (k_i^* - k_i) \cdot \tau \tag{XVI}$$

So, in order to describe the electron transfer across the chain of cytochromes the values of constants k_i, k_i^* and τ should be known.

The constant k_i may be directly calculated from the experimental data (Eqn XV) if one assumes that C_0 is equal to the content of cytochromes aa_3. Constant k_i^* and τ can only be evaluated approximately, using Eqn XVI. It is obvious that $\omega\tau$ is always $\leqslant 1$. For example, $\omega_{max} \cdot \tau \leqslant 1$, where ω_{max} is the maximal frequency of the electron transfer across the respiratory chain (*i.e.* the maximal turnover number of the cytochromes under the experimental conditions). Introducing $\tau \leqslant 1/\omega_{max}$ in Eqn XVI we obtain:

$$k_i^* \geqq \beta_i \cdot \omega_{max} + k_i \qquad \text{(XVII)}$$

As the maximal frequency of electron transfer (ω_{max}) in our experiments was approximately 20 s^{-1}, $\tau \leqslant 50$ ms. The constants k_i^* and k_i, calculated according to Eqns XV and XVII, are shown in Table I where it can be seen that $k_i^*/k_i \geqslant 5$–25. The table also shows, for comparison's sake, the values of the constants obtained by Pring[8] for pigeon heart mitochondria from non-stationary kinetics. It can be seen in Table I that the values of the rate constants obtained by different methods agree satisfactorily.

In terms of our concept, the changes in the steady-state levels of reduced cytochromes due to the alteration in the electron transfer rate can be explained in the following way. It is clear from Eqn XIII that the proportion of respiratory chains in which electron transfer can occur at stage $i \rightarrow i+1$ (see ref. 8), can be determined as $P_{i,i+1}$:

$$P_{i,i+1} = p_i^{r} \cdot p_{i+1}{}^{ox} = \frac{V_{st}}{k_i \cdot C_0 + (k_i^* - k_i) \cdot \tau \cdot V_{st}} \qquad \text{(XVIII)}$$

At small values of V_{st}, when $(k_i^* - k_i) \cdot \tau \cdot V_{st} \ll k_i \cdot C_0$,

$$P_{i,i+1} = \frac{V_{st}}{k_i \cdot C_0} \qquad \text{(XIX)}$$

In this case, the majority of the respiratory chains involved in electron transfer are non-activated and the changes in the rate of oxidation of the substrate in the steady-state* leads to changes in the number of the cytochrome chains containing the $i^r - (i+1)^{ox}$ pairs. At sufficiently high values of V_{st}, when $(k_i^* - k_i) \cdot \tau \cdot V_{st} \gg k_i \cdot C_0$,

$$P_{i,i+1} = \frac{1}{(k_i^* - k_i) \cdot \tau} \qquad \text{(XX)}$$

In these conditions the number of the chains containing $i^r - (i+1)^{ox}$ pairs does not change when the steady-state rate of substrate oxidation increases and it is only the proportion of the chains in the activated state that increases.

We have only studied the behaviour of cytochromes in the steady state, although other components of the cytochrome chain, such as non-haeme iron protein and cuproprotein, possibly take part in electron transfer[20]. Therefore, it should be noted that for the pairs of cytochromes between which these components are located, the

* It should be noted here that the rate-limiting step in electron transfer was always before the cytochrome chain.

calculation of constants k_i and k_i^* by Eqns XVII and XV gives effective values characterizing the overall reaction between the ith and (ith $+1$) components.

Moreover, Eqns I and IV do not take into account the possible reversibility of electron transfer reactions, and recently such a suggestion has been put forward[5,21]. Since we cannot estimate the rates of the reversed stages for the case of an electron transfer which is non-inhibited and far from the thermodynamic equilibrium, we should consider Eqns I and IV as a first approximation.

As follows from Eqn XVI, constant β_i is a composite value. This accounts for the anomalous $\log \beta_i$ *vs* $1/T$ dependence described above (see Fig. 4). In fact, it follows from Eqn XVI that:

$$\beta = (k_0^* \cdot e^{-\Delta E^*/RT} - k_0 \cdot e^{-\Delta E/RT}) \cdot \frac{1}{k_0^s} \cdot e^{\Delta E^s/RT} \qquad \text{(XXI)}$$

or

$$\beta = \frac{k_0^*}{k_0^s}\left[1 - \frac{k_0}{k_0^*} \cdot e^{-(\Delta E - \Delta E^*)/RT}\right] \cdot e^{(\Delta E^s - \Delta E^*)/RT} \qquad \text{(XXII)}$$

From which

$$\log \beta = \log \frac{k_0^*}{k_0^s} + \log\left[1 - \frac{k_0}{k_0^*} \cdot e^{-(\Delta E - \Delta E^*)/RT}\right] + \frac{\Delta E^s - \Delta E^*}{2.3RT} \qquad \text{(XXIII)}$$

An analysis of Eqn XXIII shows that if $\Delta E > \Delta E^*$ and $\Delta E^s > \Delta E^*$ then $d \log \beta / d(1/T) > 0$, *i.e.* constant β increases as the temperature decreases, which corresponds to the experimentally found dependence (see Fig. 4).

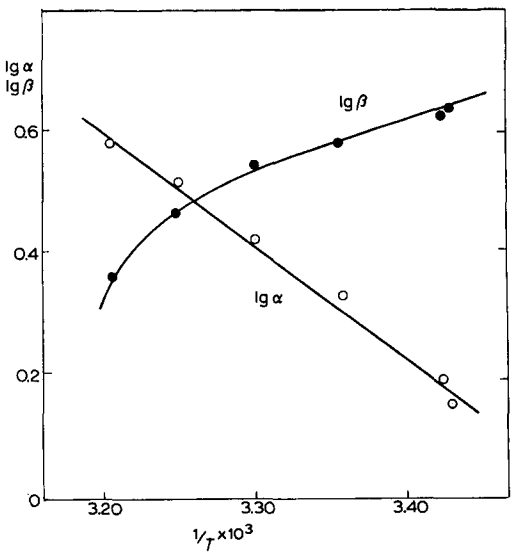

Fig. 4. The temperature dependence of α and β for cytochrome pair b and c_1. For the experimental conditions see Fig. 1.

DISCUSSION

In the present paper a kinetic model has been suggested, which rationalises the dependence of the steady-state levels of reduced cytochromes on the electron transfer rate. This model is based on the assumption that electron transfer is accompanied by activation of the respiratory chain. At present we do not yet know the mechanism of activation in detail. It is not impossible that activation is associated with certain specific conformational changes occurring in the respiratory chain on transfer of electrons. In fact, King *et al.*[22] have previously suggested that reduction of any carrier in the respiratory chain results in a change in its structure and, at the same time, a change in its orientation in relation to its adjacent carrier. As a result the structure of the whole respiratory chain undergoes reversible conformational changes on electron transfer. Morrison[23] put forward a similar point of view concerning the reactions in cytochrome oxidase. In recent years it has been proved experimentally that the structure of some components of the respiratory chain depends on their being in the reduced or oxidized state[24–27]. Moreover, conformational changes in the inner mitochondrial membrane induced by electron transfer have been observed[28–31].

Data have been obtained in this laboratory showing that the electron transfer in the respiratory chain is accompanied by the appearance of a specific form of the latter which is resistant to the action of lytic enzymes: trypsin (or chymotrypsin) and phospholipase A[32–34]. In contrast to such a "functioning" form, both the oxidized and reduced forms of the respiratory chain are sensitive to lytic enzymes. This may be interpreted to mean that on electron transfer the multi-enzyme system acquires a compact conformation in which the labile sites are screened from the environment. There is also evidence that such a conformational transition may improve the contact between the carriers in the membrane and facilitate their interaction[35]. The suggestion concerning the activation of the respiratory chain on electron transfer was also put forward by Cowan *et al.*[36]. It has been shown recently that the resistance of the respiratory chain to proteinases and phospholipase A increases the higher the electron transfer rate[37]. This agrees with one of the major assumptions of this work; that the proportion of respiratory chains in the activated (and more compact) conformation is larger the higher the electron transfer rate.

In spite of the fact that the above experimental facts allow suggestions to be made regarding the nature of the active state of the respiratory chain, no true mechanism of activation can be put forward, since present day concepts about the structural organisation of the respiratory chain and electron transfer mechanisms have not been developed satisfactorily. It is also difficult for the above model to be applied to the non-stationary kinetics of electron transfer, for it must first be known what stages of the electron transfer process are responsible for the activation of the respiratory chain. The latter question requires further elucidation.

As was pointed out above, Pring and Chance[8,9] were the first to suggest the existence of activated and non-activated respiratory chains. According to these authors, the activation is due to the binding of oxygen with cytochrome a_3. The latter assumption does not seem to be well-grounded. For example, Pring[8] failed to obtain from the experimental data the values of the rate constants corresponding to complete activation of the respiratory chains at saturating concentrations of oxygen. It is possible to account for this phenomenon in terms of our kinetic model, according

to which the proportion of activated chains does not depend upon the oxygen concentration, but rather on the rate of electron transfer. This means that a proportion of the activated chains is controlled by the oxygen concentration only when the latter limits the rate of electron transfer. Such a situation may occur only at concentrations of oxygen below those of saturation, *i.e.* below 0.05 μM[9,19].

So, in the present investigation we suggest a simple kinetic model describing electron transfer across the respiratory chain under steady-state conditions. This model allowed an equation to be obtained which is in good agreement with the experimental data. Moreover, the rate constants of electron transfer between cytochromes calculated with the use of this equation are similar to the respective constants obtained by means of a different method[8]. On these grounds, we infer that the basic prerequisites and assumptions of our model are true. Nevertheless, it is beyond doubt that the model requires to be further developed.

ACKNOWLEDGEMENTS

We wish to thank Professor I.V. Berezin for his constant attention and helpful discussions. We are also grateful to Miss T. Kheifets for translating this paper.

REFERENCES

1 B. Chance, *Nature*, 169 (1952) 215.
2 B. Chance, in W. D. McElroy and B. Glass, *The Mechanism of Enzyme Action*, John Hopkins Press, Baltimore, Md., 1954, p. 389.
3 B. Chance and G. R. Williams, *Adv. Enzymol.*, 17 (1956) 65.
4 B. Chance, D. De Vault, V. Legallais, L. Mela and T. Yonetani, in S. Claesson, *Fast Reactions and Primary Processes in Chemical Kinetics*, Nobel Symp. V, Interscience, New York, 1967, p. 437.
5 M. Wagner and M. Erecinska, *Arch. Biochem. Biophys.*, 147 (1971) 666.
6 C. P. Lee, R. W. Estabrook and B. Chance, *Biochim. Biophys. Acta*, 99 (1965) 32.
7 C. P. Lee, L. Ernster and B. Chance, *Eur. J. Biochem.*, 8 (1969) 153.
8 M. Pring, in F. Heinmets and L. D. Cady, *Concepts and Models in Biomathematics*, Dekker, New York, 1969, p. 75.
9 B. Chance and M. Pring, *19 Colloq. Ges. Biol. Chem., Mosbach-Baden*, Springer-Verlag, Berlin, 1968, p. 102.
10 R. E. Beyer, in R. Estabrook and M. Pullman, *Methods in Enzymology*, Vol. 10, Academic Press, New York, 1967, p. 186.
11 F. L. Grane, J. L. Clenn and D. E. Green, *Biochim. Biophys. Acta*, 22 (1956) 475.
12 W. H. Vanneste, *Biochim. Biophys. Acta*, 113 (1966) 175.
13 T. Yonetani, *J. Biol. Chem.*, 235 (1960) 845.
14 A. Kemp, G. S. P. Groot and H. J. Reitsma, *Biochim. Biophys. Acta*, 180 (1969) 28.
15 S. Horie and M. Morrison, *J. Biol. Chem.*, 238 (1963) 2859.
16 T. Yonetani, *J. Biol. Chem.*, 235 (1960) 3138.
17 B. F. Van Gelder, *Biochim. Biophys. Acta*, 118 (1966) 36.
18 J. R. Brandon, J. R. Brocklehurst and C. P. Lee, *Biochemistry*, 11 (1972) 1150.
19 H. Degn and H. Wohlrab, *Biochim. Biophys. Acta*, 245 (1971) 347.
20 S. P. J. Albracht, H. Van Heerikhuizen and E. C. Slater, *Biochim. Biophys. Acta*, 256 (1972) 1.
21 P. L. Dutton, D. F. Wilson and C. P. Lee, *Biochemistry*, 9 (1970) 5077.
22 T. E. King, M. Kuboyama and S. Takemori, in T. E. King, H. S. Mason and M. Morrison, *Oxidases and Related Redox Systems*, Vol. 2, John Wiley and Sons, New York, 1965, p. 707.
23 M. Morrison, in T. E. King, H. S. Mason and M. Morrison, *Oxidases and Related Redox Systems*, Vol. 2, John Wiley and Sons, New York, 1965, p. 639.
24 E. Margoliash and A. Schejter, *Adv. Protein Chem.*, 21 (1966) 113.
25 J. P. Myer, *J. Biol. Chem.*, 246 (1971) 1241.
26 H. Baum, G. S. Rieske, H. J. Silman and S. H. Lipton, *Proc. Natl. Acad. Sci. U.S.*, 57 (1967) 798.
27 T. E. King, P. M. Bayley and F. C. Yong, *Eur. J. Biochem.*, 20 (1971) 103.

28 J. M. Wrigglesworth and L. Packer, *Arch. Biochem. Biophys.*, 128 (1968) 790.
29 A. Azzi, B. Chance, G. K. Radda and C. P. Lee, *Proc. Natl. Acad. Sci. U.S.*, 62 (1969) 612.
30 G. K. Radda, in D. R. Sanadi, *Curr. Top. Bioenerg.*, 4 (1971) 81.
31 J. M. Graham and D. F. H. Wallach, *Biochim. Biophys. Acta*, 193 (1969) 225.
32 V. N. Luzikov, M. M. Rakhimov, V. A. Saks and I. V. Berezin, *Biokhimiya*, 32 (1967) 1234.
33 V. N. Luzikov, V. A. Saks and I. V. Berezin, *Biokhimiya*, 34 (1969) 874.
34 V. N. Luzikov, M. M. Rakhimov and I. V. Berezin, *Biochim. Biophys. Acta*, 180 (1969) 429.
35 V. N. Luzikov, V. A. Saks and I. V. Berezin, *Biochim. Biophys. Acta*, 223 (1970) 16.
36 D. O. Cowan, G. Pasternak and F. Kaufman, *Proc. Natl. Acad. Sci. U.S.*, 66 (1970) 837.
37 V. N. Luzikov and L. V. Romashina, *Biochim. Biophys. Acta*, 267 (1972) 37.