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STEADY-STATE KINETICS OF ELECTRON TRANSFER THROUGH CYTOCHROME CHAIN OF UNCOUPLED SUBMITOCHONDRIAL PARTICLES

I. GENERAL KINETIC ANALYSIS

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SUMMARY

Steady-state kinetics of electron transfer through the cytochrome chain of uncoupled ultrasonic submitochondrial particles at different pH values has been studied. Rate constants calculated from the Pring equation ($k_i' = V/P_i^{r}P_{i+1}^{ox}$) increased with the increase of the rate of the process. As in the previous work (Saks, V. A., Kupriyanov, V. V. and Luzikov, V. N. (1972) Biochim. Biophys. Acta 283, 42–53) this dependence was linear, but only at comparatively low rates of electron transfer. To explain the experimental data several kinetic models, based on the assumption that respiratory chains are activated when functioning, have been proposed and analysed. The best agreement with the experimental data was obtained for the model suggesting that the rate of activation of the carriers is directly proportional to the overall rate of electron transfer and to the proportion of non-activated respiratory chains in the system. Hence it appeared that electron transfer through already activated chains entailed activation of adjacent non-activated chains. This model allowed rate constants for non-activated (k_i) and activated (k_i^*) states of the carriers, as well as the life-time of the activated carriers (τ) to be determined.

INTRODUCTION

To give a quantitative description of electron transfer through the mitochondrial respiratory chain, Chance [1, 2] has proposed a kinetic scheme suggesting that this process is a sequence of bimolecular reactions proceeding in a homogenous medium [1, 2]. Later, Pring [3, 4] developed a kinetic theory which takes into account real properties of the mitochondrial membrane. According to Pring, for a certain *i*-stage of electron transfer

$$V = k_i P_i^{r} P_{i+1}^{ox} \cdot C_0 \qquad (I)$$

where V is the electron transfer rate; k_i is the rate constant (s^{-1}); P_i^{r} and P_{i+1}^{ox} are

Abbreviation: Cl-CCP, carbonyl cyanide *m*-chlorophenyl hydrazone

dimensionless proportions of reduced i-th carriers and oxidized $(i+1)$ carriers, respectively, $(0 \leqslant P_i^r, P_{i+1}^{ox} \leqslant 1)$ and C_0 is the number of respiratory ensembles in the system. It has been demonstrated in our previous work [5] that values of the rate constants of electron transfer between cytochromes in alkaline and ultrasonic submitochondrial particles, calculated on the basis of the Pring equation [3, 4], get higher with increase in the steady-state rate of the process.

This fact was interpreted as activation of respiratory chains induced by electron transfer. A simple kinetic model was suggested assuming that every act of electron transfer leads to the appearance of an activated form of a chain with the rate constant $k_i^*(k_i^* > k_i)$ and the average life-time, τ.

In the present work the phenomenon of the respiratory chain auto-activation has been studied further for the case of completely uncoupled submitochondrial particles. Different kinetic models with detailed mechanisms of activation have been suggested and analyzed.

MATERIALS AND METHODS

Ultrasonic submitochondrial particles were obtained from beef-heart mitochondria by the method of Beyer [6]. Levels of reduced cytochromes were determined in the same way as in the previous work [5]: *b*, 562–577 nm, c_1, 554–540 nm, *c*, 550–535 nm [7], *a* and a_3, at 605–630 nm and 444–455 nm, respectively [8, 9]. Overlapping of absorption bands of cytochromes-*c* and c_1 was not taken into account, since calculation with the help of Vanneste equations [7] showed that in our experiments levels of these reduced cytochromes calculated with a correction for overlapping do not significantly differ from those without this correction. According to Van Gelder [9], for cytochromes-*a* and a_3, the analysis of absorption-band overlapping was also performed with the help of the following equations:

$$P_{a_3}^r = \frac{8P_\gamma^r - 5P_\alpha^r}{3} \quad \text{(A)}; \qquad P_a^r = \frac{5P_\alpha^r - 2P_\gamma^r}{3} \quad \text{(B)}$$

where P_α^r and P_γ^r are proportions of reduced cytochromes *a* and a_3 in α- and γ-peaks, respectively. Proportions of reduced cytochrome *a* (P_a^r) somewhat exceeded the proportions measured at 605–630 nm (P_α^r). At the same time the levels found for a_3 were close to zero and were determined with a great error. For this reason, on calculation of kinetic constants it was considered that $P_a^r = P_\alpha^r$ and $P_{a_3}^{ox} = 1$.

All the measurements were performed on a "Hitachi-356" double beam-two wavelength spectrophotometer in 80 mM phosphate buffer at 30 °C. The rate of electron transfer was varied by changing the concentration of alcohol dehydrogenase from 20 to 800 μg/ml in a NADH-generating system containing from 30 to 60 mM ethanol, 0.1–0.2 mM NAD^+ and 10 mM semicarbazide [5]. The measurement medium also contained 5 μM Cl-CCP.

The Pring constant was calculated according to the equation:

$$k_i' = k_i C_0 = V/P_i^r P_{i+1}^{ox} \quad (1)$$

where k_i is the rate constant (s^{-1}); C_0 is the number of respiratory ensembles per 1 mg of particle preparation, which is regarded as being equal to the content of cytochrome

aa_3 (0.2 nmol/mg of particle protein); V is the specific rate of electron transfer (μmol/min per mg); P_i^r and P_{i+1}^{ox} are proportions of i-th carrier in the reduced state and of $(i+1)$-th in the oxidized state, respectively.

RESULTS AND DISCUSSION

1. Dependence of Pring constants on the steady-state rate of electron transfer

Fig. 1 demonstrates the dependence of rate constants

$$k_i' = k_i \cdot C_0 = V/P_i^r P_{i+1}^{ox}$$

on the steady-state rate of electron transfer (V) at different pH values for the following cytochrome pairs: $b \rightarrow c_1$, $c_1 \rightarrow c$, $c \rightarrow a$ and $a \rightarrow a_3$. It can be seen from the figure that in the case of cytochromes $c_1 \rightarrow c$ and $a \rightarrow a_3$ k_i' are linear functions of V at comparatively low values of the latter: a further increase in the transfer rate leads to a deviation from linearity. It means that the empirical equation

$$k_i' = \alpha_i + \beta_i V \qquad (2)$$

where α_i and β_i are constants (see the previous work, [5]) holds true only for a limited range of electron-transfer rates. We shall designate the kinetic model suggested in the above mentioned work as model I. To find a more general equation valid within the

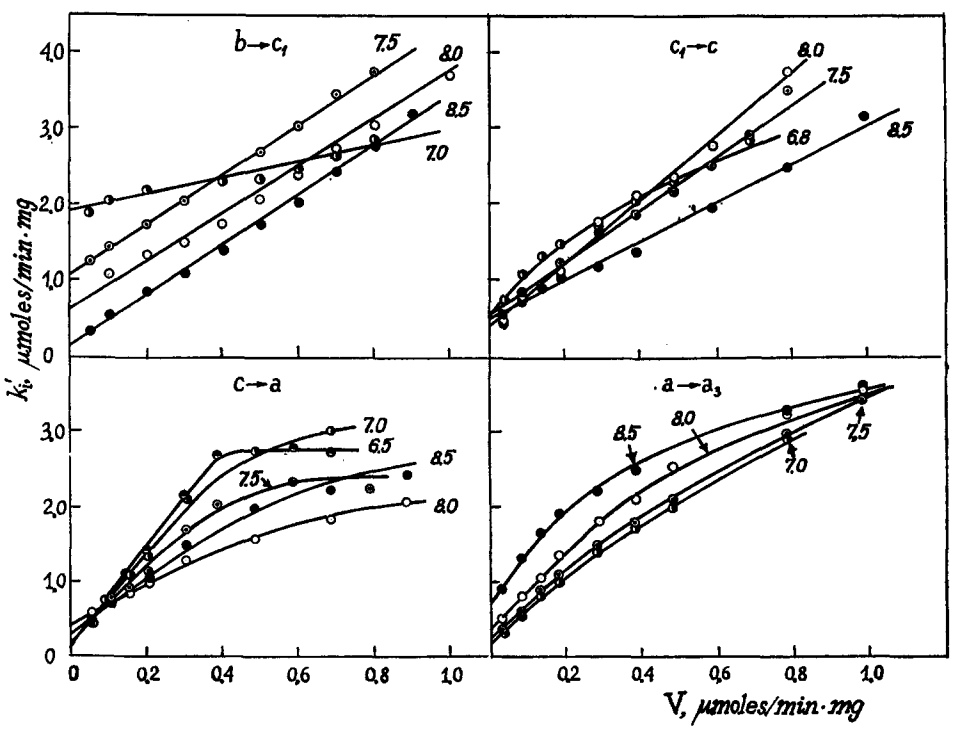

Fig. 1. Dependences of k'_i on V at different pH values of the medium for carrier pairs $b \rightarrow c_1$, $c_1 \rightarrow c$, $c \rightarrow a$ and $a \rightarrow a_3$. Measurements have been carried out as described in Materials and Methods.

entire range of the rates measured, various mechanisms of activation of the respiratory chain are considered below.

2. Kinetic models

Model II. Let us assume that some presumptions of model I are true, namely, (1) that there exist two forms of respiratory chain: an activated one with rate constants k_i^* and life-time τ and a non-activated one with constants $k_i (k_i^* > k_i)$ and (2) that the Pring equation (see above) is valid for both forms of respiratory chain. Let us also assume that activated chains are only formed as a result of electron transfer through non-activated chains (rather than through either type of chain as was supposed earlier [5]) and that the activation rate is equal to the steady-state rate of electron transfer through these chains. In the steady-state the rate of appearance of activated chains equals that of their spontaneous deactivation:

$$V_{na} = k_i C P_i^r P_{i+1}^{ox} = C^*/\tau \tag{3}$$

where C and C^* are concentrations of non-activated and activated forms of respiratory chain, respectively. It is obvious that

$$C + C^* = C_0 \tag{4}$$

Besides,

$$V = V_a + V_{na} = (k_i^* C^* + k_i C) P_i^r P_{i+1}^{ox} \tag{5}$$

which gives

$$k_i' = k_i^* C^* + k_i C \tag{5a}$$

Determining C and C^* from Eqns. 3 and 4 and introducing them into Eqn. 5a, we obtain:

$$k_i' = k_i C_0 \frac{1 + k_i^* \tau P_i^r P_{i+1}^{ox}}{1 + k_i \tau P_i^r P_{i+1}^{ox}} \tag{6}$$

Eqn. 6 may be rearranged as:

$$k_i' = k_i C_0 + \frac{k_i C_0 (k_i^* - k_i) \tau P_i^r P_{i+1}^{ox}}{1 + k_i \tau P_i^r P_{i+1}^{ox}} \tag{7}$$

It is easy to show that Eqn. 7 is linearized in coordinates

$1/(k_i' - k_i C_0)$ vs. $1/P_i^r P_{i+1}^{ox}$.

In fact,

$$1/(k_i' - k_i C_0) = \frac{1}{k_i C_0 (k_i^* - k_i) \tau} \cdot 1/P_i^r P_{i+1}^{ox} + \frac{1}{(k_i^* - k_i) C_0} \tag{8}$$

If the model suggested is correct, the experimental points in coordinates $1/(k_i' - \alpha_i)$ vs. $1/P_i^r P_{i+1}^{ox}$, should give straight lines with negative abscissa intercepts and positive ordinate intercepts (here $\alpha_i = k_i C_0$ is determined by extrapolation of initial linear

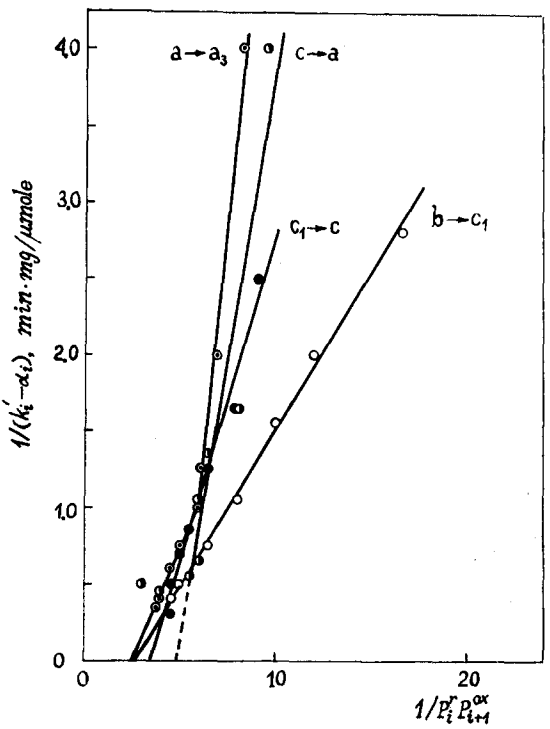

Fig. 2. Presentation of dependences of k'_i on V at pH 7.5 in coordinates $1/(k'_i-\alpha_i)$ vs. $1/P_i{}^rP_{i+1}{}^{ox}$. The values α_i were obtained by extrapolation of linear regions of the curves in Fig. 1 to $V = 0$ (see also text).

regions of k'_i plotted against V to $V = 0$). However, it is clear from Fig. 2 that in the coordinates mentioned above, the linear parts of the experimental curves give only positive abscissa intercepts. Hence it appears that the conclusions from model II are not consistent with the experimental data. In other words, the assumption that activation of electron-transfer chains may only occur as a result of electron transfer through non-activated chains is not true.

Model III. Let us assume now that two forms of the respiratory chain exist, i.e. activated (with rate constants k_i^* and life-time τ') and non-activated (with constants k_i) and that the activation rate is proportional to the overall electron-transfer rate and to a proportion of non-activated chains. Then, for the steady-state case the following equation is valid

$$r \cdot V \cdot C/C_0 = C^*/\tau' \tag{9}$$

where r is a coefficient of proportionality. Solving this equation together with that of material balance (Eqn. 4) and designating $r\tau' = \tau$ we shall obtain expressions for C and C^*.

$$C = \frac{C_0}{1 + V\tau/C_0} \tag{10}$$

$$C^* = \frac{C_0 V\tau/C_0}{1+V\tau/C_0} \tag{11}$$

Introducing values C and C^* into Eqn. 5a for k'_i, we shall see that

$$k'_i = k_i C_0 + \frac{V\tau(k_i^* - k_i)}{1+V\tau/C_0} \tag{12}$$

This expression is transformed into the equation of the straight line in the coordinates $1/(k'_i - k_i C_0)$ vs. $1/V$:

$$1/(k'_i - k_i C_0) = \frac{1}{(k_i^* - k_i)\tau} \cdot 1/V + \frac{1}{(k_i^* - k_i)C_0} \tag{12a}$$

As follows from Fig. 3, experimental data yield straight lines in the above mentioned coordinates for all cytochrome pairs within the pH range from 6.5 to 8.5. Values $k_i C_0$ were determined for these purposes by graphical extrapolation of the curves in the coordinates k'_i vs. V to $V = 0$ ($k_i C_0 = \alpha_i$; $k'_i = V/P_i^r P_{i+1}^{ox}$). It is evident that the theoretical dependence of k'_i on V described in the previous work [5] may be obtained from Eqn. 12 at low V when $V \cdot \tau/C_0 \ll 1$. In fact, if $V \cdot \tau/C_0 \ll 1$, $k'_i = k_i C_0 +$

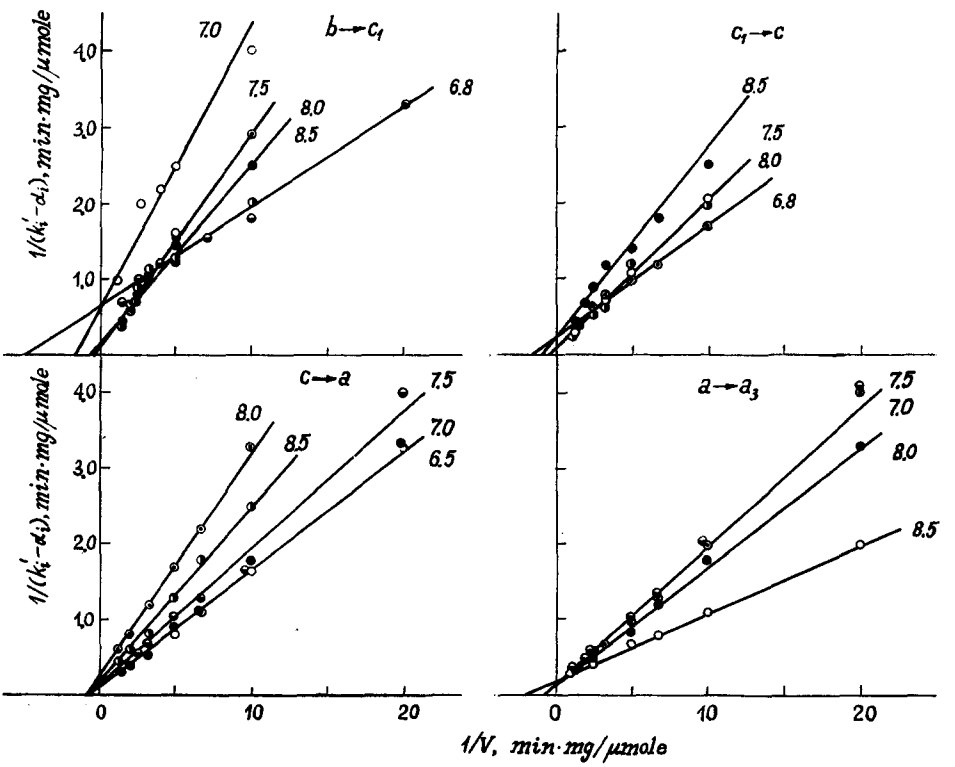

Fig. 3. Dependences of $1/(k'_i - \alpha_i)$ on $1/V$ for cytochrome pairs $b \rightarrow c_1$, $c_1 \rightarrow c$, $c \rightarrow a$, and $a \rightarrow a_3$ at different pH values.

TABLE I

VALUES OF THE CONSTANTS k_i, k_i^* AND τ FOR DIFFERENT PAIRS OF CYTOCHROMES 30 °C

Values k_i, k_i^* and τ were calculated according to model III by Eqn. 12 (see text) for the pairs of cytochromes $b \rightarrow c_1$, $c_1 \rightarrow c$, $c \rightarrow a$ and $a \rightarrow a_3$. Concentration of respiratory ensembles (C_0) was regarded as being equal to that of cytochrome aa_3 in particle preparations, the latter concentration being 0.2 nmol/mg of particle protein. The experiments were carried out at 30 °C, pH 7.5, in the presence of 5 μM Cl-CCP.

Pairs of cytochromes	k_i (s^{-1})	k_i^* (s^{-1})	τ (ms)	k_i^*/k_i
$b \rightarrow c_1$	90	1000	6	11
$c_1 \rightarrow c$	33	500	12	15
$c \rightarrow a$	25	500	12	20
$a \rightarrow a_3$	25	500	12	20

$(k_i^*-k_i)\tau \cdot V$, as follows from Eqn. 12. According to model III representation of the experimental data in coordinates $1/(k'_i-\alpha_i)$ vs. $1/V$ allows the k_i^* and τ values to be determined. Abscissa intercepts in these coordinates are equal to τ/C_0 and ordinate intercepts give the value of $1/(k_i^*-k_i)C_0$. Since $k_iC_0 = \alpha_i$ is derived from dependence of k_i' on V, the latter ratio allows constant k_i^* to be found. The results of these calculations for all cytochrome pairs ($b \rightarrow c_1$, $c_1 \rightarrow c$, $c \rightarrow a$ and $a \rightarrow a_3$) at pH 7.5 are summarized in Table I. The table shows that k_i^* and τ for the majority of pairs are almost the same (probably with the exception of pair $b \rightarrow c_1$). This allows certain conclusions to be made as to the degree of cooperativity of the respiratory-chain activation. In fact, coincidence of the lifetimes of the activated state of various cytochrome pairs may signify that there exists an activated state of the entire respiratory chain (or rather, cytochrome chain). However, it cannot be excluded there exist activated forms of individual carriers. To elucidate this question further experimental work has to be carried out.

Kinetic model I, discussed in our previous work [5], allowed that the activation rate of the cytochrome chain was proportional to the total electron transfer rate only. Hence it appeared, in particular, that electron transfer through activated chains should induce activation both of the non-activated chains and already activated chains. Within the more perfect model III considered in the present work, it is postulated that activation rate depends not only on the total electron-transfer rate, but also on the proportion of non-activated chains (C/C_0) in the system. This means that electron transfer through a certain chain would affect adjacent chains with probability governed by the portion of the chains capable of activation in its vicinity.

Model III differs from model I by an assumption that there is no further activation of already activated chains. It is possible that individual components of the chain are activated, rather than the whole chain. Then, it may be suggested that we observe here activation of some carriers due to electron transfer through others within one chain. In connection with the above statement, the question arises what is the nature of the physico-chemical mechanism of interaction of chains (or their individual components). The next article will deal with a possible mechanism which is based on the assumption that the factor controlling the carrier activity is the local concentration of hydrogen ions in mitochondrial membrane.

Reversibility of electron transfer in experimental conditions. Analysing various kinetic models, we proceeded from two basic assumptions. First, that there exist at least two forms of respiratory chain, i.e. non-activated and activated; the interrelationship between them being regulated by electron-transfer rate. Second, that the Pring equation is valid for any form of carrier. Then the question arises: can the observed increase in the rate constant result from one of the assumptions used for derivation of the Pring equation being wrong? In particular, it is worthwhile to verify the assumption of the irreversibility of electron transfer, since for some cytochromes midpoint redox-potentials do not significantly differ from each other [10].

If electron transfer is reversible then the Pring equation has to be written for direct and reverse reactions. In this case the electron transfer rate at i-stage will be described as follows:

$$V = k_{+i}P_i^{r}P_{i+1}^{ox} - k_{-i}P_{i+1}^{r}P_i^{ox} \tag{20}$$

or, which is the same,

$$k_i' = V/P_i^{r}P_{i+1}^{ox} = k_{+i} - k_{-i}P_{i+1}^{r}P_i^{ox}/P_i^{r}P_{i+1}^{ox} \tag{21}$$

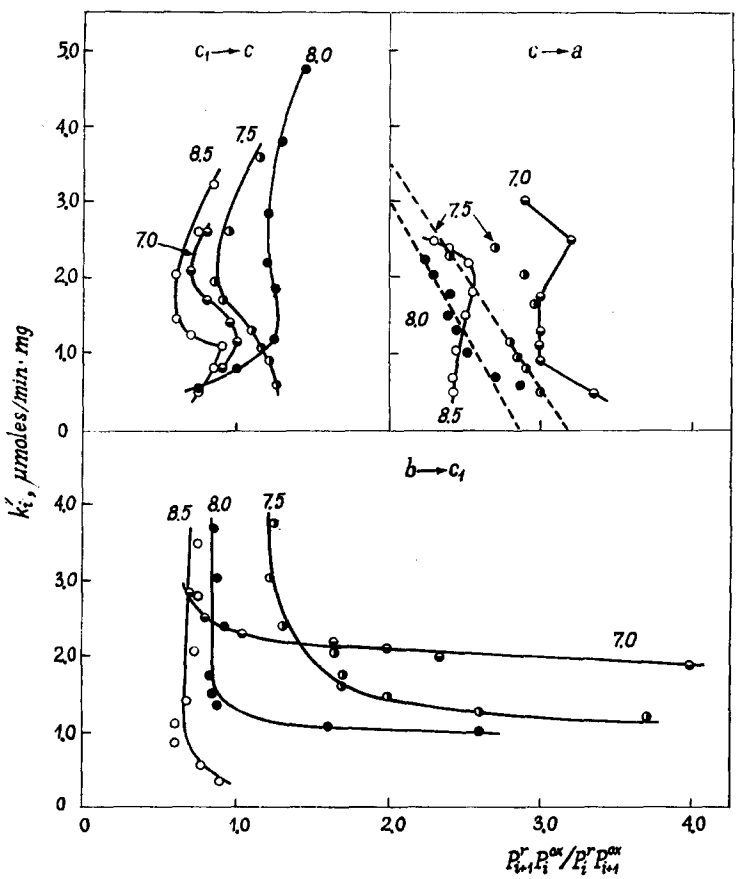

Fig. 4. k'_i as a function of $P_{i+1}{}^{r}P_i{}^{ox}/P_i{}^{r}P_{i+1}{}^{ox}$ for $b \rightarrow c_1$, $c_1 \rightarrow c$ and $c \rightarrow a$ at various pH values.

In coordinates k'_i vs. $P_{i+1}{}^rP_i{}^{ox}/P_i{}^rP_{i+1}{}^{ox}$ the straight line parallel to the abscissa corresponds to the case when electron transfer is almost completely irreversible ($k_{-i} \simeq 0$), whereas that parallel to the ordinate corresponds to the case of electron transfer being reversible with very high (in the limit, infinite) rate constants of direct and reverse reactions. Intersection of the straight line with the abscissa at $P_{i+1}{}^rP_i{}^{ox}/P_i{}^rP_{i+1}{}^{ox} = 1$ means that electron transfer at this stage is equipotential.

Hence it appears that if the k'_i value to be determined experimentally depends linearly upon $P_{i+1}{}^rP_i{}^{ox}/P_i{}^rP_{i+1}{}^{ox}$ and the straight lines have a negative slope, the assumption of reversibility of electron transfer is justified. Fig. 4 demonstrates this dependence for the cytochrome pairs $b \rightarrow c_1$, $c_1 \rightarrow c$ and $c \rightarrow a$ at different pH. As follows from the figure, in most cases experimental points give curves instead of straight lines. Only for the pair $c \rightarrow a$ at pH 7.5 and 8.0 straight lines may be drawn approximately through the experimental points. Thus, on the basis of the above data we may conclude that at least for the pairs $b \rightarrow c_1$ and $c_1 \rightarrow c$ the observed increase in the Pring constants is not accounted for only by reversibility of electron transfer at these stages. Unfortunately, the similar analysis cannot be performed for the pair $a \rightarrow a_3$, since the levels of reduced cytochrome a_3 are determined with great error (see Materials and Methods). Nevertheless the marked difference between midpoint redox-potentials of cytochromes a and a_3 ($\Delta E = +175$ mV [10, 11]) gives good reason for neglecting the reversibility of electron transfer at this stage.

3. Carriers ignored in the kinetic analysis and interpretation of the kinetic data

Studying the electron transfer between cytochromes b and c_1, we regarded the cytochrome b as a carrier, the redox-state of which is determined by light absorption at 562–577 nm. However it is known that there exist at least two types of cytochrome b, one of which (b_K) absorbs preferentially at 562 nm, and the other (b_T) at 566 nm [12, 13]. In this connection, we studied the dependence of the levels of both reduced cytochromes b on the rates of electron transfer. As follows from Fig. 5, these dependences are practically similar at given wavelengths. Moreover, the ratios of absorbance values at the two wavelengths in the steady state ($\Delta A_{562}/\Delta A_{566}$) do not depend on the rate of electron transfer and are equal to the corresponding ratios under anaerobic conditions $(\Delta A_{562}/\Delta A_{566})_{an}$ (Fig. 5). This fact may mean that the cytochrome b_T (b-566) is kinetically identical to the cytochrome b_K (b-562) in our experiments, i.e. in the presence of Cl-CCP. On the other hand, the given dependences may result from the fact that the cytochrome-b_T does not change its redox state significantly in uncoupled particles, remaining oxidized. Interpretation of the data obtained for the cytochrome b_K depends on the assumed sequence of participation of b_K and b_T in electron transfer. According to some authors, cytochromes b are localized in the respiratory-chain scheme in sequence [12, 13], with b_K preceding b_T [12] or vice versa [13]. According to another viewpoint, they are localized in a parallel manner [14]. Considering that the cytochrome b_K is localized after the cytochrome b_T, the kinetic parameters (k_i, k_i^*,τ) determined, in fact describe the interaction between b_K and c_1. If the cytochrome b_K preceeds b_T, the obtained values k_i, k_i^* and τ are effective. It should be borne in mind that between the cytochromes-b and c_1 (FeS)-proteins are localized, and between a and a_3, cuproprotein [15, 16] which takes part in electron transfer. This fact also makes it difficult to interpret the results obtained for the mentioned carriers, since the values to be determined may be effective.

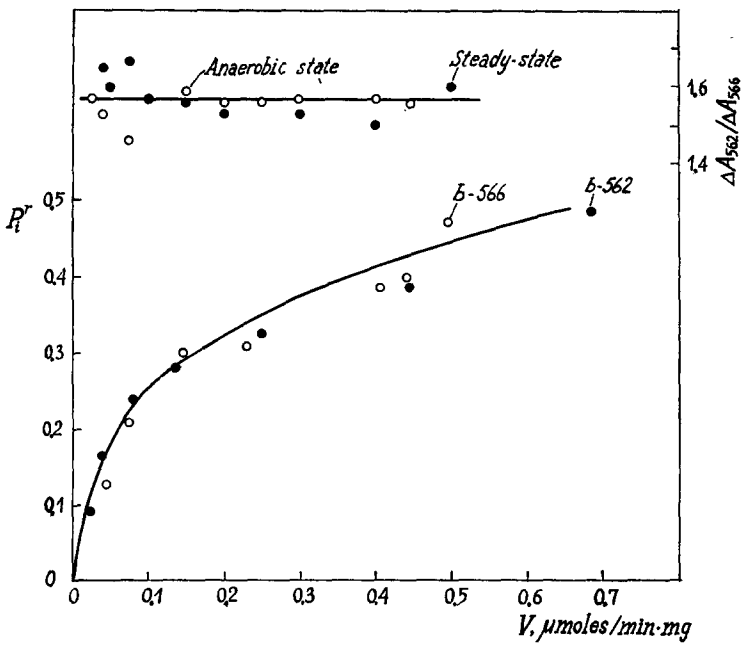

Fig. 5. Increase in the proportions of reduced cytochromes *b* measured at 562–577 nm and 566–577 nm with increase of the rate of electron transfer. Dependence of the ratios of absorbance at 562 nm to that at 566 nm in the steady-state $(\Delta A_{562}/\Delta A_{566})_{st}$ and in the anaerobic state $(\Delta A_{562}/\Delta A_{566})_{an}$, upon electron transfer rate. pH 7.0, 30 °C.

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REFERENCES

1 Chance, B. (1952) Nature, 169, 215–221
2 Chance, B. and Williams, G. R. (1956) Adv. Enzymol. 17, 65–134
3 Pring, M. (1969) in Concepts and Models of Biomathematics, (Heinmetz, F. and Cady, L. D., eds.), pp. 75–104, Dekker, New York
4 Chance, B. and Pring, M. (1968) 19th Colloq. Ges. Biol. Chem. pp. 102–126, Springer-Verlag, Berlin
5 Saks, V. A., Kupriyanov, V. V. and Luzikov, V. N. (1972) Biochim. Biophys. Acta 283, 42–53
6 Beyer, R. E. (1967) in Methods in Enzymology, Vol. 10, (Estabrook, R. W. and Pullman, M., eds.), pp. 186–194, Academic Press, New York
7 Vanneste, W. H. (1966) Biochim. Biophys. Acta 113, 175–178
8 Yonetani, T. (1960) J. Biol. Chem. 235, 845–852
9 Van Gelder, B. F. (1966) Biochim. Biophys. Acta 118, 36–46
10 Wilson, D. F. and Erecińska, M. (1972) in Mitochondria: Biogenesis and Bioenergetics. Biomembranes: Molecular Arrangements and Transport Mechanisms, Vol. 28, pp. 118–132, North Holland/Elsevier
11 Wilson, D. F., Lindsay, J. G. and Brocklehurst, E. (1972) Biochim. Biophys. Acta 256, 277–286
12 Chance, B. (1972) FEBS Lett. 23, 3–20
13 Wikström, M. K. F. (1973) Biochim. Biophys. Acta 301, 155–193

14 Norling, B., Nelson, B. D., Nordenbrand, K. and Ernster, L. (1972) Biochim. Biophys. Acta 275, 18–32
15 Albracht, S. P. J., Van Heerikhuizen, H. and Slater, E. C. (1972) Biochim. Biophys. Acta 256, 1–13
16 Van Gelder, B. F. and Beinert, H. (1969) Biochim. Biophys. Acta 189, 1–24