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

II. INFLUENCE OF pH ON KINETICS OF ELECTRON TRANSFER

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Summary

pH Dependences of steady-state kinetic parameters of cytochrome chains of submitochondrial particles have been studied. It has been shown that the lifetimes of activated states (τ) of the pairs of cytochromes $b \rightarrow c_1$ and $a \rightarrow a_3$ have different pH dependences; those for the $c_1 \rightarrow c$ and $c \rightarrow a$ cytochrome pairs being similar. The rate constants for the non-activated state of the respiratory chains decreased for the $b \rightarrow c_1$ pair and increased for the $a \rightarrow a_3$ pair when the pH value was increased.

The values of pK calculated from these dependences for the pairs $b \rightarrow c_1$ and $a \rightarrow a_3$ were 7.2 and 8.9, respectively. It has been supposed that the ratio of activated to non-activated electron carriers may be controlled by the local pH value in the mitochondrial membrane, the latter being dependent upon the rate of electron transfer. The kinetic model based on this assumption allows one to explain the experimental dependences on pH of the rate constants for cytochromes $b \rightarrow c_1$ and $a \rightarrow a_3$.

The values of the diffusion rate constants for H^+ and OH^- ions in the mitochondrial membrane estimated from these kinetic data obtained in this study were 10^4—10^5 s^{-1} and 10^2—10^3 s^{-1}, respectively.

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Abbreviations: Cl-CCP, carbonyl cyanide *m*-chlorophenyl hydrazone; FCCP, carbonyl cyanide *p*-trifluoromethoxyphenyl hydrazone.

Introduction

It has been shown in our previous study [1] that the electron transfer through cytochrome chains of submitochondrial particles can be described most precisely by a kinetic model based on the assumption of the carriers' activation during electron transfer; the rate of the carriers' activation being proportional to the overall electron transfer rate and to the proportion of non-activated chains, C/C_0. It has been supposed that activation of chains (or single carriers) may occur due to the electron transfer through adjacent chains (or carriers).

The experimental dependences of Prings' rate constants (k_i') on the steady-state rate of electron transfer have been described by the following equation [1]:

$$k_i' = k_i C_0 + \frac{(k_i^{\star} - k_i)\tau V}{1 + V \cdot \tau / C_0} \tag{1}$$

in which C_0 is a quantity of respiratory ensembles per unit of the volume of the system and $k_i' = V/P_i^{\mathrm{r}} P_{i+1}^{\mathrm{ox}}$, where V is the steady-state specific rate of electron transfer, P_i^{r} and P_{i+1}^{ox} are dimensionless proportions of reduced i-th carriers and oxidized, $(i + 1)$th, carriers, respectively.

In the present study we have analyzed the pH dependences of the following kinetic parameters, obtained from this model: of the rate constants for non-activated and activated states (k_i, $k_i^{\star}$) and of the average lifetimes (τ) of the activated state. The new kinetic model has been developed, assuming that the ratio of activated and nonactivated carriers' forms is controlled by the local pH value in the mitochondrial membrane being determined by the electron transfer rate.

Methods

Experimental methods were as described in our previous paper [1].

Results

Dependences of the rate constants k_i, $k_i^{\star}$ and lifetimes, τ, upon the pH value of the reaction medium

Fig. 1 shows the pH dependences of logarithms of the values of $\alpha_i = k_i C_0$, τ/C_0 and $\lambda = 1/(k_i^{\star} - k_i)C_0$ obtained from ordinate and abscissa intercepts in coordinates k_i' vs. V and $1/(k_i' - \alpha_i)$ vs. $1/V$. On the basis of data given in Fig. 1, the pH dependences of logarithms of the rate constants for activated carriers ($k_i^{\star} C_0$) and the ratios of rate constants for the activated carrier state to those for the non-activated state ($k_i^{\star}/k_i$) have been calculated and are shown in Fig. 2. It can be seen from these figures that there is a significant influence of pH on the rate constants in the case of pairs $b \to c_1$ and $a \to a_3$; in the first case $\log \alpha_i$ monotonically decreases and in the latter case $\log \alpha_i$ increases with an increase in pH.

The dependences of $\log \alpha_i$ on pH for pairs $c_1 \to c$ and $c \to a$, as well as the dependences of the values of $\log k_i^{\star} C_0$ on pH, for all carriers are less apparent.

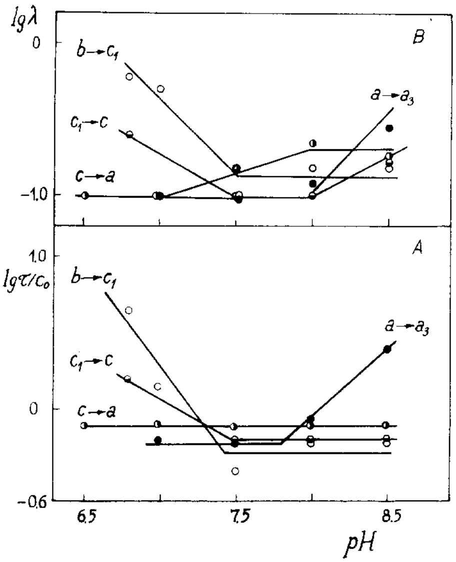


Fig. 1. The pH dependences of logarithms of abscissa intercepts (τ/C_0, Fig. A) and ordinate intercepts (λ, Fig. B) in coordinates $1/(k_i' - \alpha_i)$ vs. $1/V$.

The dependence of the value log τ/C_0 on pH is different for cytochromes $b \rightarrow c_1$ and $a \rightarrow a_3$: for $b \rightarrow c_1$ log τ/C_0 decreases and for $a \rightarrow a_3$ increases with an increase in pH.

The dependences of α_i on hydrogen ion concentration in the medium for cytochromes $b \rightarrow c_1$ and $a \rightarrow a_3$ can be expressed quantitatively in the following way. It is apparent from Figs. 3 and 4 that the experimental curves of these dependences are hyperbolae and may be linearized in coordinates $1/(\alpha - \alpha_{\min})$ vs. $1/[H^+]$ for $b \rightarrow c_1$ and $1/(\alpha - \alpha_{\min})$ vs. $[H^+]$ for $a \rightarrow a_3$. Here $\alpha_{\min}$ is the lower value of α, obtained by extrapolation at $[H^+] \rightarrow 0$ or $[H^+] \rightarrow \infty$, respec-

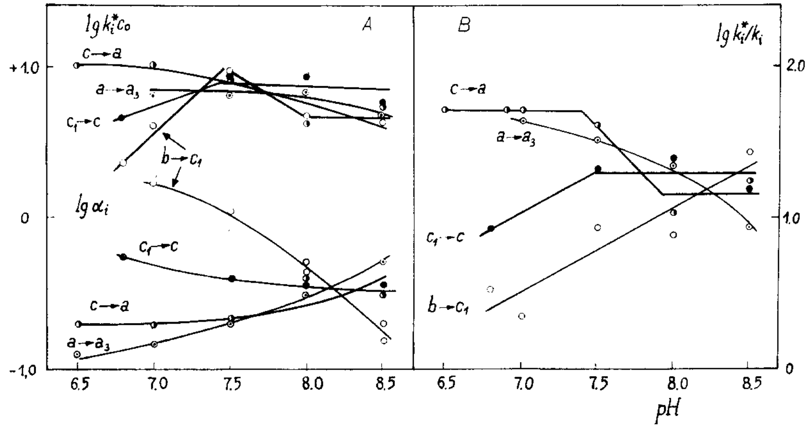


Fig. 2. Dependences of logarithms of $\alpha_i = k_i C_0$ (A, bottom of figure), $k_i^{\star} C_0$ (A, top of figure) and $k_i^{\star}/k_i$ (B). The value of α_i has been found from dependences of k_i' on V, and $k_i^{\star}$Co is calculated from the values of λ_i and α_i according to the equation: $k_i^{\star} C_0 = 1/\lambda + \alpha_i$

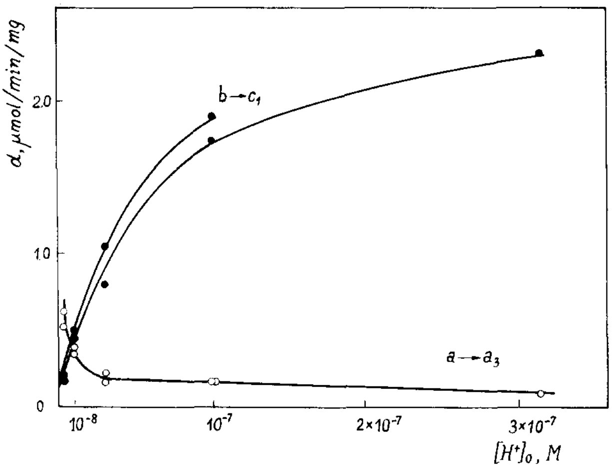


Fig. 3. The dependence of the values of α_i for the cytochrome pairs $b \rightarrow c_1$ and $a \rightarrow a_3$ on H^+ concentration in the medium. The values of α_i are taken from the data given in Fig. 2.

tively. It follows then that α_i for the pair $b \rightarrow c_1$ can be expressed by the following function of $[H^+]$:

$$\alpha = \alpha_{min} + \frac{(\alpha_{max} - \alpha_{min})[H^+]/A}{1 + [H^+]/A} \tag{2}$$

where α_{max} is the maximal value of α at $[H^+] \rightarrow 0$ and A is a constant, having the dimension of M. Accordingly, that dependence for the pair $a \rightarrow a_3$ may be expressed as:

$$\alpha = \alpha_{min} + \frac{\alpha_{max} - \alpha_{min}}{1 + [H^+]/A} \tag{3}$$

where the assignments are the same as in Eqn. 2.

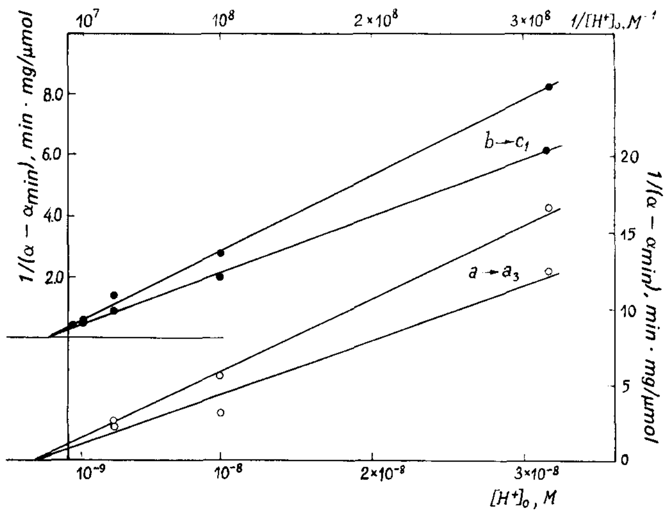


Fig. 4. Linearization of experimental dependences of α_i on $[H^+]$ for cytochromes $b \rightarrow c_1$ in coordinates $1/(\alpha - \alpha_{min})$ vs. $1/[H^+]$ and for cytochromes $a \rightarrow a_3$ in coordinates $1/(\alpha - \alpha_{min})$ vs. $[H^+]$.

Eqns. 2 and 3 may be obtained theoretically, if one supposes that the protonized form of reduced cytochrome *b* (or of oxidized cytochrome c_1) is more active than the deprotonized one and, accordingly, the protonized form of reduced cytochrome *a* (or of oxidized a_3) is less active than the deprotonized one. On the basis of this assumption the following equation (7A), giving the relationship between α_i and hydrogen ion concentration in the medium, may be obtained (see Appendix):

$$\alpha_i = \frac{k_i''' + k_i''[\mathrm{H}^+]/K}{1 + [\mathrm{H}^+]/K} \qquad (7\mathrm{A})$$

where k'' is the rate constant for the protonized form, k''' is the rate constant for the deprotonized one and K is an equilibrium constant between these forms.

For the pair $b \rightarrow c_1$, if the protonized form is more active ($k'' > k'''$) Eqn. 7A may be rewritten as:

$$\alpha_i = k_i'''C_0 + \frac{(k_i'' - k_i''')C_0[\mathrm{H}^+]/K}{1 + [\mathrm{H}^+]/K} \qquad (4)$$

Obviously, Eqn. 4 is identical to the empirically obtained Eqn. 2, when $\alpha_{\mathrm{min}} = k'''C_0$, $\alpha_{\mathrm{max}} = k''C_0$ and $A = K$. Accordingly, for the pair $a \rightarrow a_3$, when the deprotonized form is more active ($k_i''' > k_i''$), Eqn. 7A may be rewritten as

$$\alpha_i = k_i''C_0 + \frac{(k_i''' - k_i'')C_0}{1 + [\mathrm{H}^+]/K} \qquad (5)$$

In this case Eqn. 5 is identical to the empirical Eqn. 3, when $\alpha_{\mathrm{min}} = k''C_0$, $\alpha_{\mathrm{max}} = k'''C_0$ and $A = K$.

Thus, it follows from the data given in Fig. 4 that for the pair $b \rightarrow c_1$ $k'''C_0 = 0.08$ µmol/min per mg, $k''C_0 = 3.0 \pm 0.5$ µmol/min per mg and $K = 6 \pm 1 \cdot 10^{-8}$ M (p$K = 7.2 \pm 0.1$). For the pair $a \rightarrow a_3$ $k''C_0 = 0.08$ µmol/min per mg, $k'''C_0 = 1.2 \pm 0.2$ µmol/min per mg and $K = 1.5 \pm 0.5 \cdot 10^{-9}$ M (pK = 8.7 - 9.0).

From the pH dependences of the rate constants $\alpha_i = k_iC_0$ which characterize the non-activated state of electron carriers it may be concluded that for the pair $b \rightarrow c_1$ the values of α_i are decreased with an increase in pH, and for the pair $a \rightarrow a_3$ they are increased.

In this connection the question arises of what is a degree of activation of the protonized and deprotonized carriers' forms. As the ratio of k_i^*/k_i increases for the pair $b \rightarrow c_1$ and decreases for the pair $a \rightarrow a_3$ with an increase in pH, it may be concluded that, in the former case, the deprotonized form is more activated than the protonized one, the situation being reversed in the latter case.

The results obtained may also be interpreted in another way, assuming that, in the case of electron transfer between *b* and c_1, the activated form of carrier is protonized reduced cytochrome *b* (or oxidized c_1) and, in the case of electron transfer between *a* and a_3, the activated form is deprotonized reduced cytochrome *a* (or oxidized a_3). In such a situation a change in pH of the medium results in changes of ratio of activated and non-activated cytochromes and the degree of activation due to electron transfer (ratio k_i^*/k_i) is dependent on the initial medium pH value. It follows from this consideration that the electron transfer influences in some way the protonization state of carriers. It is logical to suppose that electron transfer is accompanied by changes in pH inside the particles either in local pH in the site of a carrier location in the membrane due to the release or binding of hydrogen ion by some respiratory chain components. Indeed, it is well known that electron transfer is coupled with binding or release of protons by carriers [2—5]. As all our experiments have been carried out in the presence of excess of uncoupler (Cl-CCP) eliminating the pH gradient across the membrane, the activation of carriers may be caused by local changes in the pH value in the membrane. The kinetic model, based on this assumption is given below.

The kinetic model IV

The dependence of local concentration of hydrogen ions in the mitochondrial membrane on the rate of electron transfer. Let us assume that the electron transfer results in consumption or release of hydrogen ions inside the membrane and, consequently, results in an increase or decrease in local concentration of H^+ which will be different from the concentration of these ions in the external medium. In this case the rate of H^+ release or consumption will be proportional to the rate of electron transfer. The rate of diffusion of H^+ or OH^- may be described by the simplest equation of the following form:

$$V_d = \pm k_d(C_{in} - C_{out})$$

Let us assume further that at an i-th step of the electron transfer a reduced i-th carrier or an oxidized $(i + 1)$th carrier may be protonized and that the value of the rate constant for protonized carrier form differs from that for deprotonized carrier. It is necessary for carrier activation that increase (or decrease) in H^+ concentration in the membrane caused by electron transfer will occur at the places of localization of the carriers' protonized form of which is more active (or, respectively, less active) than the deprotonized one. It has been shown (see Appendix) that if one of the carriers is ionized (C_i^r or C_{i+1}^{ox}) non-activated electron transfer may be described as:

$$V = \frac{k_i''' + k_i''[H^+]/K}{1 + [H^+]/K} C_0 P_i^r P_{i+1}^{ox} \tag{6A}$$

where k_i'' and k_i''' are the rate constants for protonized and deprotonized carriers, and K is an equilibrium constant between these forms. Let us assume here that $[H^+]$ is a local H^+ concentration in a part of the membrane containing carriers to be considered. A local H^+ concentration may be assigned as $[H^+]_m$, and an external H^+ concentration as $[H^+]_0$. On the basis of assumptions introduced, the following equations for relationships between $[H^+]_m$ and steady-state rate of electron transfer may be obtained (see Appendix):

In the case of acidification of the membrane at $[H^+]_0^2 >> 6K'_\omega/p^2$.

$$[H^+]_m = p[H^+]_0 + \varphi' V \tag{23AI}$$

and at $[H^+]_0^2 << K'_w/p^2$ and at a small value of V

$$[H^+]_m = K'_w p[H^+]_0/(K'_w - p\varphi'[H^+]_0 V) \tag{22AI}$$

In the case of membrane pH being increased at $[H^+]_0^2 >> 6\,K'_w/p^2$ *and at small V.*

$$[H^+]_m = p[H^+]_0 - \varphi' V \tag{23AII}$$

and at $\quad [H^+]_0^2 << K'_w/p^2$

$$[H^+]_m = K'_w p[H^+]_0/(K'_w + \varphi' p[H^+]_0 V) \tag{22AII}$$

The relationships obtained are illustrated in Fig. 5, which shows the theoretical dependences of $[H^+]_m$ upon V calculated according to the exact equation (18A, see Appendix). It can be seen from Fig. 5 that, in the case of acidification of the membrane, all curves have a linear part at sufficiently high V. On the contrary, in the case of alkalization of the membrane the curves have a linear part only if $[H^+]_o > \sqrt{6K'_w}$ and if V is small enough.

The dependences of k_i' *on V. Acidification of the membrane phase (1)* $[H^+]_0 > 2.5 \cdot 10^{-7}$ *M.* Introducing Eqn. 23AI into Eqn. 7A gives:

$$k'_i = \frac{k'''_i + k''_i p[H^+]_0/K + k''_i \dfrac{\varphi'}{K} V}{1 + p[H^+]_0/K + \dfrac{\varphi'}{K} V} \tag{6}$$

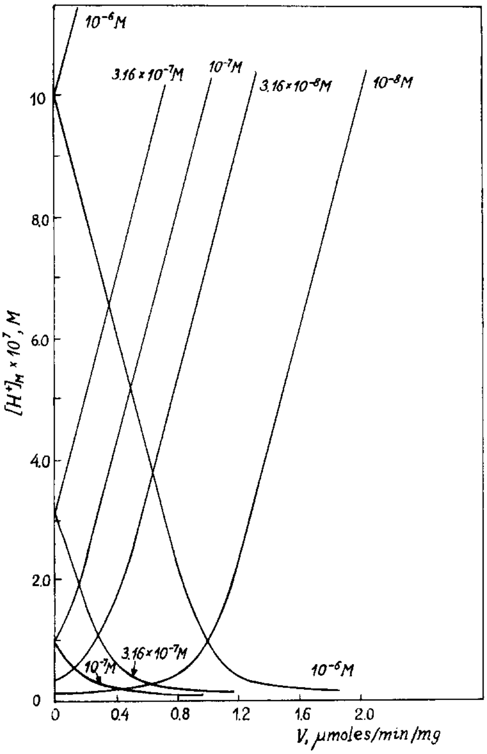


Fig. 5 . Theoretical dependences of local hydrogen ion concentration ($[H^+]_m$) on the steady-state rate of electron transfer and $[H^+]_0$ for the cases of the acidification and alkalization of the membrane phase. Calculations are made according to Eqns. 16A, 17A and 18A assuming that $\varphi' = 1.0$ min · mg per l, $K'_w = 10^{-14}$ M^2 and $\gamma = 1$.

For respiratory chain activation it should be $k_i'' > k_i'''$. Therefore, let us take $k_i'' = k_i^*$ and $k_i''' = k_i$. Moreover, let us assign $\varphi'/K = \varphi$ and $K/p = K'$. Introducing these assignments, we have after transformation:

$$k_i' = k_i C_0 + \frac{(k_i^* - k_i)C_0[\mathrm{H}^+]_0/K'}{1 + [\mathrm{H}^+]_0/K} + \frac{\dfrac{\varphi(k_i^* - k_i)C_0}{(1 + [\mathrm{H}^+]_0/K')^2}V}{1 + \dfrac{\varphi}{1 + [\mathrm{H}^+]_0/K}V} \tag{7}$$

$[\mathrm{H}^+]_0 < 10^{-7}$ M, V is small enough. In this case, introducing Eqn. 22AI into Eqn. 7A we have:

$$k_i' = k_i C_0 + \frac{(k_i^* - k_i)C_0[\mathrm{H}^+]_0/K'}{1 + [\mathrm{H}^+]_0/K'} + \frac{\varphi(k_i^* - k_i)C_0\left(\dfrac{[\mathrm{H}^+]_0/K'}{1 + [\mathrm{H}^+]_0/K'}\right)^2 V}{1 - \varphi\dfrac{[\mathrm{H}^+]_0/K'}{1 + [\mathrm{H}^+]_0/K'}V} \tag{8}$$

Here: $\varphi = \varphi' K/K_w'$

Alkalization of the membrane phase. Let us take the following assignments: $k_i''' = k_i^*$, $k_i'' = k_i$. $[\mathrm{H}^+]_0 < 10^{-7}$ M. Introducing Eqn. 23AII into Eqn. 7A we have, after transformation:

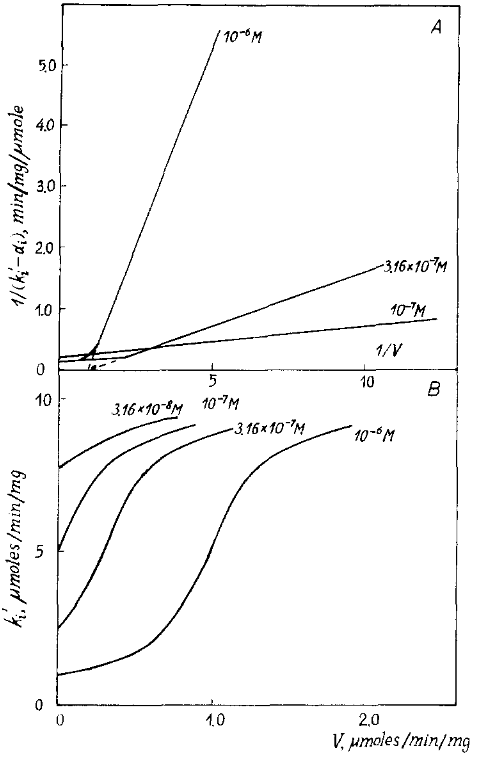
$$k_i' = k_i C_0 + \frac{(k_i^* - k_i)C_0}{1 + [\mathrm{H}^+]_0/K'} + \frac{\varphi(k_i^* - k_i)\left(\dfrac{[\mathrm{H}^+]_0/K'}{1 + [\mathrm{H}^+]_0/K'}\right)^2 V}{1 + \dfrac{\varphi[\mathrm{H}^+]_0/K'}{1 + [\mathrm{H}^+]_0/K'}V} \tag{9}$$

Here: $\varphi = \varphi' K/K_w' V$; $[\mathrm{H}^+]_0 > 2.5 \cdot 10^{-7}$ M, V is small enough. Introducing Eqn. 22AII into Eqn. 7A have, after transformation:

$$k_i' = k_i C_0 + \frac{(k_i^* - k_i)C_0}{1 + [\mathrm{H}^+]_0/K'} + \frac{\dfrac{\varphi(k_i^* - k_i)C_0}{(1 + [\mathrm{H}^+]_0/K')^2}V}{1 - \dfrac{\varphi}{1 + [\mathrm{H}^+]_0/K'}V} \tag{10}$$

Here: $\varphi = \varphi'/K$. With an increase in V Eqn. 10 will not be valid and with V high enough Eqn. 9 will be the valid one.

Theoretical dependences of k_i' on V are shown in Figs. 6A and 7A. To obtain these dependences the calculated relationships between $[\mathrm{H}^+]_m$ and V (see Fig. 5) have been introduced into Eqn. 7A. Obviously, there should be straight lines or curves having two linear parts in coordinates $1/(k_i' - \alpha_i)$ vs. $1/V$. Those dependences are shown in Figs. 6B and 7B. There is one straight line with positive slope value giving the negative abscissa intercept when membrane pH decreases at $[\mathrm{H}^+]_0 > 2.5 \cdot 10^{-7}$ M and the curve has two linear parts, one of which corresponding to the high values of $1/V$ gives a positive abscissa intercept at $[\mathrm{H}^+]_0 < 10^{-7}$ M. However, if the pH of the membrane phase increases the situation is an opposite one. There is one straight line at $[\mathrm{H}^+]_0 < 10^{-7}$ M with a positive slope giving a negative abscissa intercept, and there appears the second linear part of the curve corresponding to high $1/V$ and giving a positive abscissa intercept at $[\mathrm{H}^+]_0 > 2.5 \cdot 10^{-7}$M.



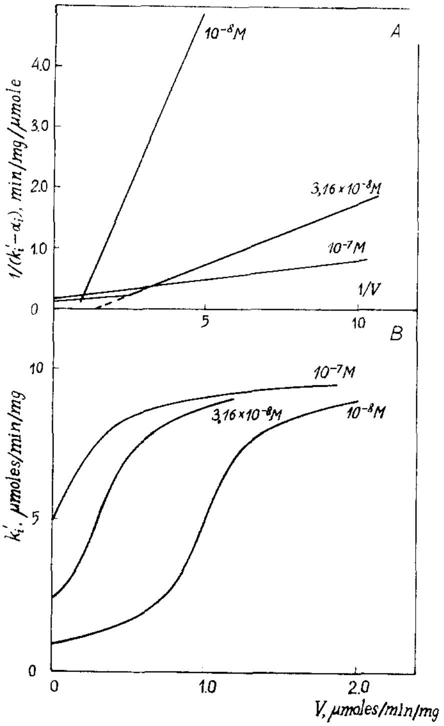


Fig. 6. A. Theoretical dependences of k_i' on V at various $[H^+]_0$ for the case of the acidification of membrane phase. Those are obtained by the introduction of the calculated functions $[H^+]_m$ on V into Eqn. 7A. It is taken that $K = 10^{-7}$ M, $k_iC_0 = 0.1$ μmol/min per mg and $k_i^{\star}C_0 = 10$ μmol/min per mg. B. Linearization of theoretical dependences k_i' on V in coordinates $1/(k_i' - \alpha_i)$ vs. $1/V$.

Fig. 7. A. Theoretical dependences of k_i' on V at various $[H^+]_0$ for the case of the alkalization of the membrane phase obtained by the introduction of the calculated functions $[H^+]_m$ on V into Eqn. 7A. It is assumed that $K = 10^{-7}$ M, $k_iC_0 = 0.1$ μmol/min per mg and $k_i^{\star}C_0 = 10$ μmol/min per mg. B. Linearization of theoretical dependences of k_i' on V in coordinates $1/(k_i' - \alpha_i)$ vs. $1/V$.

The comparison of theoretical and experimental results. It has been shown in our previous study [1] that the experimental dependences of k_i' upon V may be most precisely described by the Eqn. 1 (model III):

$$k_i' = k_iC_0 + \frac{(k_i^{\star} - k_i)\tau V}{1 + V \cdot \tau/C_0}$$

It is obvious that the Eqns. 8 and 10 are not identical to Eqn. 1. At the same time, Eqns. 7 and 9 give Eqn. 1 provided that

$$(k_iC_0)_{III} = \alpha_i = k_iC_0 + \frac{(k_i^{\star} - k_i)C_0[H^+]_0/K'}{1 + [H^+]_0/K'} \qquad (11)$$

for Eqn. 7 and

$$\tau/C_0 = \varphi/(1 + [H^+]_0/K') \qquad (12)$$

Eqns. 9 and 1 are equivalent if

$$(k_iC_0)_{III} = \alpha_i = k_iC_0 + \frac{(k_i^{\star} - k_i)C_0}{1 + [H^+]_0/K'} \qquad (13)$$

$$\tau/C_0 = \frac{\varphi[\mathrm{H}^+]_0/K'}{1+[\mathrm{H}^+]_0/K'} \tag{14}$$

It is obvious that the dependences of α_i on $[\mathrm{H}^+]_0$, predicted by Eqns. 11 and 13 are identical to experimentally determined ones for the pairs $b \rightarrow c_1$ and $a \rightarrow a_3$, respectively. In this case, K' acquires the meaning of apparent equilibrium constant between activated and non-activated carrier forms (pK 7.2 and pK 8.9). In accordance with Eqn. 7 ordinate intercepts of straight lines in $1/(k_i' - \alpha_i)$ vs. $1/V$ coordinates are equal to $(1 + [\mathrm{H}^+]_0/K')/(k_i^\star - k_i)C_0$ (designated as λ) and abscissa intercepts of these lines (τ/C_0) are $\varphi/(1 + [\mathrm{H}^+]_0/K')$.

At the same time, as it follows from Eqn. 9 for the case of membrane alkalization, straight lines give ordinate intercepts equal to $(k_i^\star - k_i)C_0(1 + K'/[\mathrm{H}^+]_0 V)$ and abscissa intercepts equal to $\tau/C_0 = (\varphi[\mathrm{H}^+]_0/K'')/(1 + [\mathrm{H}^+]_0/K')$ in the coordinates indicated. If the theory developed is accurate enough the value of log λ will decrease and log τ/C_0 will increase with an increase in pH for the pair $b \rightarrow c_1$. In the case of the pair $a \rightarrow a_3$ log λ values will grow and log τ/C_0 will drop with an increase in pH. As seen from Fig. 1A, experimentally determined log λ values decrease for the pair $b \rightarrow c_1$ and increase for the pair $a \rightarrow a_3$, with an increase in pH as predicted theoretically.

At the same time, the experimental dependence of log τ/C_0 upon pH is opposite to the predicted one: for the cytochrome pair $b \rightarrow c_1$ log τ/C_0 decreases with an increase in pH instead of its predicted increase; for the pair $a \rightarrow a_3$ there is an increase instead of the predicted decrease. The discrepances between the theoretical and experimental dependences of log τ/C_0 upon pH may be explained by assuming that φ in the expression for τ/C_0 is, in fact, a function of pH. It should be mentioned that in Eqn. 12, $\varphi = \gamma/K \cdot k_\mathrm{H}$, and in Eqn. 14, $\varphi = \gamma K/K_w' k_\mathrm{H}$. Multiplying λ by τ/C_0, $\varphi/(k_i^\star - k_i)C_0$ may be obtained. As follows from Fig. 8A, there is a significant dependence of log $\varphi/(k_i^\star - k_i)C_0$ upon pH. For the pair $b \rightarrow c_1$ this parameter decreases with an increase in pH and increases for the pair $a \rightarrow a_3$. Thus, the model IV allows correct predictions to be made with respect to the dependence of α_i upon pH, and gives a satisfactory description of the dependence of log λ upon pH. At the same time, the dependence of log τ/C_0 on pH differs from the theoretical one. In principal, from the dependences of log λ on pH, the values of $k_i^\star$ may be found. It follows from the data given in Fig. 1A that for the pair $b \rightarrow c_1$ $k_i^\star C_0$ is equal to 8.0 μmol $\cdot$ min^{-1} $\cdot$ mg^{-1} and for the pair $a \rightarrow a_3$ $k_i^\star C_0$ is equal to 10 μmol $\cdot$ min^{-1} $\cdot$ mg^{-1}. pK values may be obtained also from the dependences of log $\lambda(\alpha - \alpha_\mathrm{min})$ on pH. In fact, we have for the pairs $b \rightarrow c_1$ and $a \rightarrow a_3$, respectively:

$$\lambda(\alpha - \alpha_\mathrm{min}) = [\mathrm{H}^+]_0/K' \tag{15}$$

$$\lambda(\alpha - \alpha_\mathrm{min}) = K'/[\mathrm{H}^+]_0 \tag{16}$$

These dependences are shown in Fig. 8B. From these data the following pK values may be determined: 6.8 for $b \rightarrow c_1$ and 9.4 for $a \rightarrow a_3$. These values differ by approx. 0.5 from those determined from the dependences of α_i on $[\mathrm{H}^+]_0$ (7.2 for $b \rightarrow c_1$ and 8.9 for $a \rightarrow a_3$).

It is worth mentioning that the values of $k_i^\star C_0$ determined from the dependences of log λ on pH several times exceed those determined from the depen-

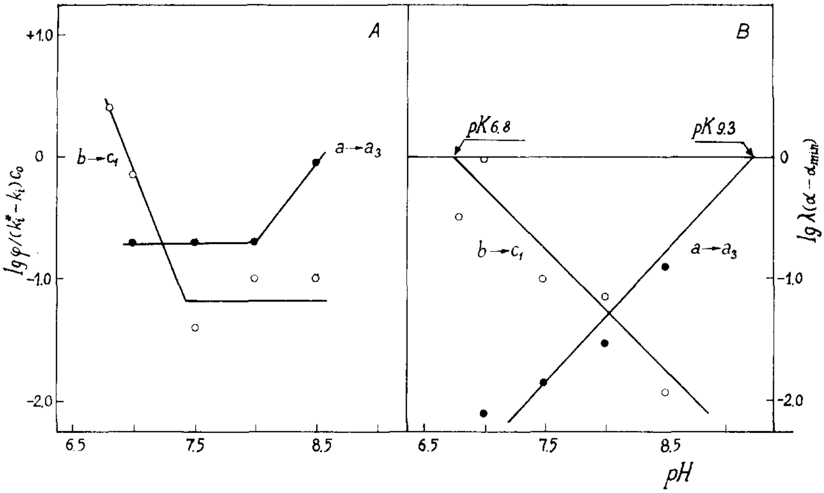


Fig. 8. Changes of values of log $\varphi/(k_i^{\star} - k_i)C_0$ (A) and log $\lambda(\alpha - \alpha_{\min})$ (B) with the changes of medium pH for the pairs of cytochromes $b \to c_1$, and $a \to a_3$. Values of $(\alpha - \alpha_{\min})$ and λ_i are taken from data presented in Figs. 3 and 1, respectively.

dences of α_i on $[H^+]_0$. Thus, for the pair $b \to c_1$ we have 8.0 and 3.5 μmol · $\min^{-1} \cdot mg^{-1}$, respectively, and for $a \to a_3$ 10 and 1.4 $\mu mol \cdot min^{-1} \cdot mg^{-1}$, respectively. One reason for these discrepances may be that changes in pH influence not only the ionization of one ionogenic group responsible for the activation due to electron transfer, but also influence the ionization of other groups of a carrier under consideration; the structure of the membrane may also be changed. In fact, it is known that the changes in pH from 9 to 6 are followed by an increase in light scattering by suspension of submitochondrial particles [6]. Moreover, electron transfer may alter some parameters of the expression for k_i' and is considered to have a constant value in the model. For example, the effective diffusion coefficients for H^+ (k_H) and OH^- (k_{OH}), the proton partition coefficient, (p), and the stoichiometrical coefficient, γ, for proton release or consumption may be functions of the rate of electron transfer and of medium pH. Indeed, it follows from the dependence of log $\varphi/(k_i^{\star} - k_i)C_0$ on pH that some of the parameters mentioned are pH dependent. In connection with this it should be mentioned that the efficiency of the proton pump related to complex III components is dependent on pH in submitochondrial particles [7] and in reconstituted vesicles [8]; the pK value determined from pH dependences being around 7.0 [7,8].

Let us try to evaluate and to compare the rate constants for diffusion of hydrogen (k_H) and hydroxyl (k_{OH}) ions. It follows from Fig. 8A that $\varphi/(k_i^{\star} - k_i)C_0$ varies from approx. 0.1 to 1.0. The value of $(k_i^{\star} - k_i)C_0$ is equal to approx. 10 $\mu mol \cdot min^{-1} \cdot mg^{-1}$ (see Fig. 1). Consequently, φ changes in a range from 1 to 10 min · mg · μmol^{-1}. For the case of pH decrease in the membrane phase $\varphi_{bc_1} = \gamma/k_H K_{bc_1}$, and for the case of its increase, $\varphi_{aa_3} = \gamma K_{aa_3}/K_w' k_{OH}$. Assuming that $\gamma = 1$, $K_{bc_1} = 10^{-7}$ M and $K_{aa_3} = 10^{-9}$ M, and $K_w' = 10^{-14}$ M^2 we may find the effective values of the diffusion coefficients: k_H = 20—200 cm^3/s per mg; K_{OH} = 0.2—2 cm^3/s per mg, or taking the membrane density to be approximately equal to 1 g/cm^3, $k_H \simeq 2 \cdot 10^4$—$2 \cdot 10^5$ s^{-1} and $k_{OH} \simeq 2 \cdot 10^2$—$2 \cdot 10^3$ s^{-1}.

Obviously, effective diffusion coefficients are pseudo first-order rate constants for the appearance or disappearance of the hydrogen ion gradient. Experimentally determined values of the half-time of conversion for uncoupler-induced pH disappearance as well as for changes in the medium pH induced by electron transfer in the presence of FCCP are in a range of tens of milliseconds. That means that the value of the first-order rate constant is equal to approx. 10^2 s^{-1} and, thus is equal to or less than the values of the diffusion rate constant calculated from our data. An important conclusion may be made from these data, namely, that the rate constant for hydrogen ion diffusion is low enough (10^2 s^{-1}) to ensure the appearance of a significant pH gradient between the membrane and the medium. This gradient may be even greater than required to explain the activation of electron carriers in the frames of the model IV.

Discussion

In this study the pH dependences of the constants α_i and log $\lambda(\alpha - \alpha_{min})$ have been used to calculate the values of pK of proton equilibria controlling the rate of electron transfer at stages $b \rightarrow c_1$ and $a \rightarrow a_3$. It has been assumed that the reduced cytochromes b and a, or oxidized forms of c_1 and a_3, are protonized: it may also be that some other components of the respiratory chain in these sites could be protonized. The determined pK values were equal to 6.8 - 7.2 and 8.8—9.3 for pairs $b \rightarrow c_1$ and $a \rightarrow a_3$, respectively. From the results of potentiometric titration of cytochrome b the pK value of 6.8 for the oxidized form and that of more than 8.5 for the reduced form have been obtained [9, 10]. Ionogenic groups of cytochrome c_1 cannot be revealed by a method of potentiometrically. This discrepancy may be caused, first, by the carrier activabasis of kinetic data differs from that for reduced cytochrome b determined potentiometrically. This discrepancy may be caused, first by the carrier activation of the pair $b \rightarrow c_1$, may be determined by a protonized state of another which does not depend on redox state of these carriers, and second, an activation of the pair $b \rightarrow c$, may be determined by a protonized state of another component; for example of (FeS)-protein included in the complex III [12,13].

A similar situation may also be the case for the pair $a \rightarrow a_3$. Potentiometrically-determined pK value for oxidized cytochrome a_3 is approx. 7.0 [14]. In the case of cytochrome a and cupro-protein there is no change in the slope of the dependence of midpoint potentials on pH in the range of pH of 6.5—8.5 [14,15]. However, it may be that the pK for these carriers is higher than 8.5 as it follows from our kinetic data (pK = 8.8—9.3). It should be also emphasized that a potentiometric titration reveals only those ionogenic groups whose pK values are significantly different for oxidized and reduced cytochromes.

The hypothesis of the existence of local high H$^+$ concentrations in the mitochondrial membrane considered in this work, proposed earlier by Williams [16,17] to explain the mechanism of energy transduction. "Local H$^+$-hypothesis" of Williams, has been developed further as the suggestion of the existence of protons in hydrophobic areas of membranes [18]. Indirect evidence for that may be found in the data of the synthesis of ATP from ADP and P_i catalyzed by the ATPase on the interface of water-octan phases and induced by acidification of the hydrophobic phase [19].

The hypothetical mechanism of an activation of the respiratory chain induced by electron transfer may be considered as one of possible ways of regulation of membraneous polyenzyme systems. The central point here is that an enzymic reaction running in the limited membrane phase may be accompanied by changes of membrane properties, such as local pH, electric potential, dielectric constant, microviscosity. Changes in these properties may influence the rate of the enzymic reaction. Such a type of enzyme regulation has been considered for a model system consisting of hydrolytic enzymes immobilized in the collodion membrane [20,21].

It is interesting that a mechanism of carrier activation suggested, which includes local changes in pH, may best be used in the case of cytochromes $b \to c_1$ and $a \to a_3$, involved in H^+ transport across the membrane. It has been assumed also that the membrane will be acidified in sites of cytochromes b and c_1 localization and alkalized in sites of cytochrome c-oxidase localization. These opposite changes in pH may be considered to be realistic if one assumes that they occur at different sides of the membrane.

The kinetic model IV considered in this work has been developed on the basis of most simple assumptions. In particular, local changes in pH were taken to be results of the existence of barriers for H^+ and OH^- diffusion. In principle, the same effect may be achieved by changes in electrical potential of membrane regions in respect to the external medium induced by electron transfer and accompanied by changes in apparent pK of ionogenic groups. In fact, it is known that there is a linear relationship between $\Delta\mu$ for H^+ and the rate of electron transfer in uncoupled mitochondria [22]. Moreover, even in the absence of electron transfer there may be a difference in electrical potentials between the membrane and the external medium as the membrane is a polyelectrolyte (proteins, charged "heads" of phospholipids). In other words, $[H^+]_m = p[H^+]_0$ at $V = 0$, where $p = \exp(-\Delta\psi F/RT)$ and $\Delta\psi$ is the difference in electrical potentials.

Since the experimental dependences allow the values of apparent pK to be estimated while $pK_{app} = pK + \Delta\psi F/RT$, the "real" value of p$K$ will be included only in an expression for φ: $\varphi = \varphi'/K$ and $\varphi = \varphi' K/K'_w$. It follows from this that one of the possible reasons for discrepances between the experimental results and theoretical predictions may be that the partition coefficient of H^+ is a function of medium pH and the rate of electron transfer; this functional relationship not being accounted for in the theory.

Finally, it cannot be excluded that activity of electron carriers may be regulated by the level of reduced ubiquinone [23—28] or another carrier. In fact, it is known that reduced ubiquinone activates succinate dehydrogenase which is not sensitive to oxidized ubiquinone [24]. Further, reduction of ubiquinone is accompanied by changes in the rate of binding of antimycin A with cytochrome b [25]. As suggested by Nelson et al. [25] this may be caused by changes in the degree of protonization of the components of complex III occurring due to reduction of ubiquinone. It is also known that ubiquinone reduction leads to a drop in its mobility in the membrane that may be a result of structural rearrangements in the membrane [26,27].

In respect to the possibility of regulation of electron carriers' activity by a member of respiratory chain being different from ubiquinone, the data of studies in refs. 29—31 are of significant interest. It has been shown in those studies that the rate of cytochrome *b* reduction may be controlled by the redox state of cytochrome c_1 or of another component of complex III.

Various possible mechanisms of activation of electron carriers in the respiratory chain discussed in this study are hypothetical. To solve the problem of the mechanism of activation of electron transfer further experimental investigations are to be carried out. It should be mentioned also that the model IV describes the most simple mechanism and gives satisfactory fitting of theoretical results with the experimental ones.

Appendix

Quantitative description of the pH dependences of constants $\alpha_i = k_i C_0$

Let us suppose that a reduced *i*-th or oxidized (i + 1)th carrier may be in protonized (characterized by rate constant k_i'') and in deprotonized (having a rate constant k_i''') forms and at the same time $k_i'' \neq k_i'''$ and the equilibrium constant between these forms is equal to K. Let us assume for a definity that a reduced carrier may be protonized, we have then:

$$C^r + H^+ \overset{K}{\rightleftharpoons} CH^r \ .$$

It is obvious that

$$[C^r] = \frac{[C^r]_0}{1 + [H^+]/K} \tag{1A}$$

and

$$[CH^r] = \frac{[C^r]_0[H^+]/K}{1 + [H^+]/K} \tag{2A}$$

Here $[C^r]_0$ is the total concentration of a reduced carrier at low values of V when activation may be neglected. Dividing the left and the right in Eqns. 1A and 2A by C_0 we shall have:

$$[C^r]/[C]_0 = P^r_c = \frac{P^r_i}{1 + [H^+]/K} \tag{3A}$$

$$[CH^r]/[C]_0 = P^r_{CH} = \frac{P^r_i[H^+]/K}{1 + [H^+]/K} \tag{4A}$$

The rate of non-activated electron transfer may be written as:

$$V = k'' P^r_{CH} P^{ox}_{i+1} C_0 + k''' P^r_C P^{ox}_{i+1} C_0 \tag{5A}$$

Taking into account Eqns. 3A and 4A, Eqn. 5A may be rewritten as:

$$V = \frac{k_i''' + k_i''[H^+]/K}{1 + [H^+]/K} \, C_0 P^r_i P^{ox}_{i+1} \tag{6A}$$

or, (which is the same):

$$k_i' = \frac{k_i''' + k_i''[H^+]/K}{1 + [H^+]/K} C_0 = k_i C_0 = \alpha_i \tag{7A}$$

Derivation of the dependences of H^+ local concentration in the membrane on the steady-state rate of electron transfer. To simplify the consideration let us introduce some assumptions additional to those described in the text. First, let us take the difference in electrical potential between the membrane and the medium to be other than zero in the absence of electron transfer and therefore $[H^+]_m = p[H^+]_0$ and $[OH^-]_m = [OH^-]_0/p$, where p is the proton distribution coefficient between two phases indicated, $[H^+]_m$, $[OH^-]_m$ are concentrations in the membrane, and $[H^+]_0$, $[OH^-]_0$ are those in the external medium. Second, let us exclude the buffer in the medium from the consideration assuming that buffer anions do not penetrate into the membrane and do not participate in the ionic processes in the membrane.

Under these conditions we can assume that the rate of release or consumption of H^+ (V_H) is directly proportional to the rate of electron transfer $V_H = \gamma V$ where γ is a stoichiometric coefficient. The rate of diffusion of H^+ into the external medium or into the membrane may be taken to be, accounting for an arrangement of respiratory ensembles as monolayer in the membrane plane:

$$V_{DH} = \pm \frac{D_H S}{\delta} \{[H^+]_m - p[H^+]_0\} \tag{8A}$$

where D_H is a diffusion coefficient for H^+, δ is a diffusion layer's thickness, and S is an effective membrane area corresponding to C_0 respiratory chains. To take into account the diffusion of hydroxyl ions, OH^-, we may write in a similar manner:

$$V_{DOH} = \pm \frac{D_{OH} S}{\delta} \{[OH^-]_m - [OH^-]_0/p\} \tag{9A}$$

where D_{OH} is OH^- diffusion coefficient. Further, we shall use effective diffusion coefficients (diffusion rate constants) which are written as $k_H = D_H S/\delta$ and $k_{OH} = D_{OH} S/\delta$.

Let us consider also the following ionic equilibria changing the H^+ concentration in the membrane

$H_2O \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} H^+ + OH^-$ water dissociation

$AH \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} A^- + H^+$ dissociation of membrane ionogenic groups .

An application of steady-state principles for the membrane phase will give:

$$\frac{d[H^+]_m}{dt} = k_1[H_2O] - k_{-1}[H^+]_m[OH^-]_m \pm (\gamma V - V_{DH}) + k_2[AH]$$
$$- k_{-2}[A^-][H^+]_m = 0 \,.$$

$$\frac{d[OH^-]_m}{dt} = k_1[H_2O] - k_{-1}[H^+]_m[OH^-]_m \pm V_{DOH} = 0 \,.$$

$$\frac{d[AH]}{dt} = k_2[AH] - k_{-2}[A^-][H^+]_m = 0\,.$$

Steady-state for the external medium may be not considered due to its volume exceeding that of the membrane many times ($v_{ext}/v_{membr} \approx 10^3$). By solution of this system of equations, we may have

$$\gamma V \pm (V_{DH} - V_{DOH}) = 0 \qquad (10A)$$

or introducing the expressions for V_{DH} and V_{DOH}:

$$\gamma V \pm \{k_H([H^+]_m - p[H^+]_0) - k_{OH}([OH^-]_m - [OH^-]_0/p)\} = 0\,. \qquad (11A)$$

Taking $[OH^-]_m \simeq K_w/[H^+]_m$ and $[OH^-]_0 = K_w/[H^+]_0$ (K_w is the ionic product constant of water) we may write:

$$\gamma V \pm k_H\{([H^+]_m - p[H^+]_0) - \frac{K_w k_{OH}/k_H}{p[H^+]_m[H^+]_0}(p[H^+]_0 - [H^+]_m)\} = 0\,. \qquad (12A)$$

Let us introduce assignments $\gamma/k_H = \varphi'$ and $K_w k_{OH}/k_H = K_w D_{OH}/D_H = K'_w$. Transforming, we may have:

$$[H^+]_m^2 + [H^+]_m\{\pm\varphi' V + (K'_w/p[H^+]_0 - p[H^+]_0)\} - K'_w = 0\,. \qquad (13A)$$

Solution of this equation gives

$$[H^+]_m = \frac{-B \pm \sqrt{B^2 + 4K'_w}}{2} \qquad (14A)$$

where

$$B = \pm\varphi' V + (K'_w/p[H^+]_0 - p[H^+]_0)\,. \qquad (15A)$$

In the case of acidification of the membrane

$$B = -\varphi' V + (K'_w/p[H^+]_0 - p[H^+]_0) \qquad (16A)$$

and in the case of its alkalization:

$$B = +\varphi' V + (K'_w/p[H^+]_0 - p[H^+]_0)\,. \qquad (17A)$$

In eqn. 14A the obvious physical meaning has a solution:

$$[H^+]_m = \frac{-B + \sqrt{B^2 + 4K'_w}}{2} \qquad (18A)$$

At $B^2 >> 4K'_w$ eqn. 18A may be written as:

$$[H^+]_m \simeq -\frac{B}{2} + \frac{|B|}{2}(1 + 2K'_w/B^2)\,. \qquad (19A)$$

If $V = 0$, the limits for application of eqn. 19A may be given as:

$$(K'_w/p[H^+]_0 - p[H^+]_0)^2 >> 4K'_w$$

or after transformation:

$$K'_w/p[H^+]_0^2 + p^2[H^+]_0^2/K'_w >> 6\,.$$

The last relationships may be substituted by:

$[H^+]_0^2 << 6K'_w/p^2$ and $[H^+]_0^2 >> 6K'_w/p^2$.

Eqn. 19A may be transformed into Eqns. 20A or 21A depending on the sign of B. At $B > 0$

$$[H^+]_m \simeq K'_w/B \tag{20A}$$

and at $B < 0$,

$$[H^+]_m \simeq -B\ . \tag{21A}$$

At low V the sign of B is determined by $[H^+]_0$, that is by initial medium pH. When $[H^+]_0^2 << K'_w/p^2 < 6\ K'_w/p^2$ then $B > 0$ and

$$[H^+]_m = K'_w/\{\pm\varphi' V + (K'_w/p[H^+]_0 - p[H^+]_0)\} \tag{20A}$$

but if $[H^+]_0^2 >> 6\ K'_w/p^2 > K'_w/p^2$ then $B < 0$ and Eqn.21A can be used according to which $[H^+]_m$ depends linearly on the rate of electron transfer:

$$[H^+]_m = -B = \pm\varphi' V - (K'_w/p[H^+]_0 - p[H^+]_0)\ . \tag{21A}$$

Eqns. 20A and 21A may be simplified, taking into account the fact that they are valid if $[H^+]_0^2 << K'_w/p^2$ and $[H^+]_0^2 >> 6\ K'_w/p^2$.

In this case we may write:

$$[H^+]_m = K'_w p[H^+]_0/(\pm\varphi' p[H^+]_0 V + K'_w) \tag{22A}$$

at $[H^+]_0^2 << K'_w/p^2$ and

$$[H^+]_m = \pm\varphi' V + p[H^+]_0 \quad \text{at} \quad [H^+]_0^2 >> 6K'_w/p^2\ . \tag{23A}$$

Let us consider now the more common case when V may have any value.

The membrane phase pH decrease. In this case Eqns. 22A and 23A may be rewritten as:

$$[H^+]_m = K'_w p[H^+]_0/(K'_w - \varphi' p[H^+]_0 V) \tag{22AI}$$

$$[H^+]_m = \varphi' V + p[H^+]_0 \tag{23AI}$$

Obviously, at $[H^+]_0^2 >> 6\ K'_w/p^2$ if $B < 0$, an increase in V is accompanied by an increase in $|B|$, therefore B remains less than zero and Eqn. 23AI may be used. At $[H^+]_0^2 << K'_w/p^2$ when $B > 0$, an increase in V is accompanied by a decrease in B that leads to B becoming equal to zero and changing its sign to the opposite one ($B < 0$). Due to this, at low V $[H^+]_m$ is given by Eqn. 22AI, at high V by Eqn. 23AI and at intermediate values of V, when $B^2 \simeq 4\ K'_w$, by the common equation, 18A (see Scheme 1).

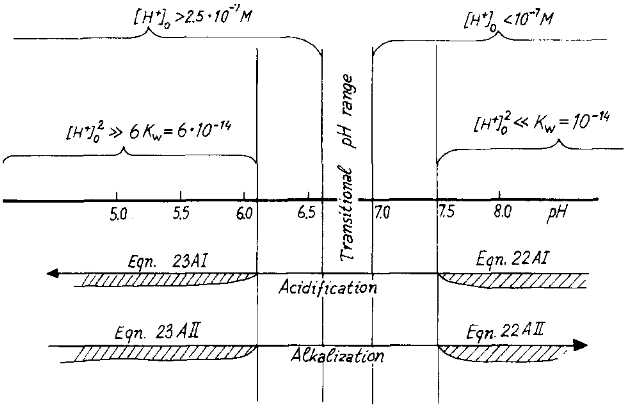
The membrane phase pH increase. In this case Eqns. 22A and 23A may be rewritten as:

$$[H^+]_m = K'_w p[H^+]_0/(K'_w + \varphi' p[H^+]_0 V) \tag{22AII}$$

if $B > 0$ at $[H^+]_0^2 << K'_w/p^2$ and

$$[H^+]_m = -\varphi' V + p[H^+]_0 \tag{23AII}$$

if $B < 0$ at $[H^+]_0^2 >> 6\ K'_w/p^2$. At $[H^+]_0^2 << K'_w/p^2$ ($B > 0$) an increase of V



Scheme 1. Schematic illustration of medium pH ranges for application of the approximate equations (22AI, 22AII, 23AI and 23AII), describing the dependences of membrane H^+ concentration on the rate of electron transfer. The scheme corresponds to the case when $D_{OH}/D_H = 1$ and $p = 1$.

increases B and, therefore, Eqn. 22AII may be used for the whole range of the electron transfer rate values. At $[H^+]_0^2 >> 6\,K'_w/p^2$ ($B < 0$) B is increased with an increase in V and changes its sign to the opposite one ($B > 0$). Thus, at low V Eqn. 23AII can be used and at high V when $B > 0$, Eqn. 22AII can be used. If $B^2 \simeq 4\,K'_w$ no one of these Eqns. may be used to describe the dependence of $[H^+]_m$ on V (see Scheme 1).

Criteria for application of approximate equations. Thus, $[H^+]_m$ as a function of V may be described by one or two approximate equations depending on $[H^+]_0$.

At $[H^+]_0^2 << K'_w/p^2$, for the case of acidification, the dependence of $[H^+]_m$ on V may be approximated by Eqns. 22AI and 23AI, and for the case of alkalization by Eqn. 22AII. At $[H^+]_0^2 >> 6\,K'_w/p^2$ for the acidification the dependence of $[H^+]_m$ on V is linear (Eqn. 23AI), and for alkalization it is described by two equations (23AII) and (22AII) (see Scheme 1).

The criteria for application of these approximate equations have been given by the following inequalities: $[H^+]_0^2 >> 6\,K'_w/p^2$ and $[H^+]_0 << K'_w/p^2$. Remember that $K'_w = K_w D_{OH}/D_H$. It is obvious that if $D_{OH}/D_H = 1$ and $p = 1$, then $K'_w = K_w = 10^{-14}$ and the corresponding inequalities may be rewritten as: $[H^+]_0^2 >> 6 \cdot 10^{-14}$ and $[H^+]_0^2 << 10^{-14}$. These inequalities can be replaced by the following approximate ones which are practically useful: $[H^+]_0^2 < 10\,K_w$ and $[H^+]_0^2 > 60\,K_w$. In the case considered, the transition of Eqn. 22A into Eqn. 23A is in a range of pH approx. 7.0 (see Scheme 1). If $D_{OH}/D_H \neq 1$ the range of transition may be shifted to the more acidic or more alkaline pH. In fact, if for example $D_{OH}/D_H = 10^2$ then the transitional range is given by inequalities $[H^+]_0 > 2.5 \cdot 10^{-5.5}$ M and $[H^+]_0 < 10^{-6.5}$ M, but if $D_{OH}/D_H = 10^{-2}$ then $[H^+]_0 > 2.5 \cdot 10^{-7.5}$ M and $[H^+]_0 < 10^{-8.5}$ M.

Similarly, if the proton distribution coefficient between the membrane and the medium p is not equal to 1, the transitional range will be shifted to more acid pH, at $p > 1$ or alkali pH at $p < 1$ by the value of $\log p$.

Introducing equations for $[H^+]_m$ (22AI, 23AI, 22AII, 23AII) into (7.A) for k_i' gives the dependences of k_i', on V for the cases considered above.

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