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Abstract

As is generally known, the energy transducing reaction in mitochondria is of highly complicated one. Free energy produced by transferring electrons from substrate to oxygen, where many dehydrogenases and respiratory chain of mitochondria are concerned, is transduced to ATP formation or utilized for the ion accmulation reaction, synthesis of various substances, reversal electron transport and the mechanochemical changes of mitochondria. The mechanism of these energy trasducing reactions which is supposed to be closely related with each other, has not yet been clarified. The authors tried to solve these biological energy transducing mechnism by applying physical circuit theory in electronics and elucidate that the energy transduction occurring in mitochondria can be explained theoretically. And some unknown but possible reaction have been postulated from such a physical consideration.

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PHYSICAL ANALYSIS OF THE ENERGY TRANSDUCING REACTION IN MITOCHONDRIA*

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In mitochondria free energy produced by transferring electron from substrate to oxygen will mainly be transduced to ATP formation. ATP may furnish energy for the synthesis and translocation of various substances in the cell as well as in mitochondrion itself. Of course, as is generally understood, the essential energy source is not ATP but the high energy intermediate which will be produced in the way of ATP formation or degradation of ATP[1,2]. But no theoretical explanation has yet been given to the mechanism of transduction of free energy to the energy of ATP formation or other chemical[4-10], physical and mechanochemical reaction[11]. Therefore, we have tried to analyse theoretically the biological energy transducing mechanism in physical term by which the various known biochemical processes may be arranged on the theoretical basement. Besides this, unknown possible biological reactions to be linked to energy metabolism may be postulated through such a consideration.

In this paper the results obtained by physical analysis of the energy transducing reaction in mitochondria are reported.

According to the present concept of respiratory chain in mitochondria every pair of electrons is transferred from a member of Krebs citric acid cycle to molecule of oxygen under aerobic condition, in the course of which three molecules of ATP in average are synthesized from ADP and inorganic phosphate (Pi); meaning that the P/O ratio is 3.0.[3] On the other hand, in the case of the breakdown of substrate, e. g. pyruvate to CO_2 and H_2O, the process can be represented by the following equation:

$$CH_3COCOOH + 5\tfrac{1}{2}\ O_2 + H^+ \longrightarrow 3CO_2 + 2H_2O \qquad \Delta G' = -273.1 \text{ Kcal}$$

That is, 5 atoms of oxygen are required for the completion of the reaction. The free energy is calculated as -273.1 Kcal[12]. As the P/O ratio is 3.0, 15 molecules of ATP are formed on the way to the termination of the reaction. That is:

$$15H^+ + 15\text{pi} + 15\ \text{ADP} \longrightarrow 15\ \text{ATP} + 15\ H_2O \qquad \Delta G' = -104.8 \text{ Kcal.}$$

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In this case, free energy is calculated as -104.8 Kcal. Therefore, the over-all reaction for the oxidation of pyruvate to CO_2 and H_2O is presented by the following equation:

$$CH_3COCOOH + 15\,Pi + 15\,ADP + 5\tfrac{1}{2}\,O_2 + 15H^+ \longrightarrow 3\,CO_2 + 15\,ATP + 17\,H_2O \quad \Delta G' = -168\ \text{Kcal.}$$

Free energy is -168 Kcal. Then the efficiency of ATP formation by using free energy is 38 per cent.

As for the aerobic phosphorylation, it occurs during the electron transfer from primary dehydrogenase to molecular oxygen via the electron carriers arranged in the respiratory chain, such as nicotinamide adenine dinucleotide (NAD), flavoprotein (Fd and Fs) and cytochromes. The $\Delta E_0'$ between $NADH_2$ and molecular oxygen is 1.14 volt (E_0' of the oxygen is $+820$ mv and E_0' of $NADH_2$ is -320 mv). Then the $\Delta G'$ of the transfer of a pair of electrons from $NADH_2$ to molecular oxygen is approximately 52.040 cal/mol, as is calculated from the equation of $\Delta G' = -n\,F\,\Delta E_0'$.[12] As the formation of one molecule of ATP requires an input of 7,000 cal,[12] 7 molecules of ATP should be generated theoretically during the transfer of a pair of electrons from $NADH_2$ to oxygen. As mentioned above, however, the P/O ratio measured by many investigators is nearly 3.0, then the efficiency is calculated as nearly 43 per cent, and some loss in energy may be in the actual reaction. $\Delta E_0'$ equivalent to one "~P" is 0.16 volt. Consequently, the possible sites of phosphorylation in the electron transport pathway on the respiratory chain can be reasonably deduced from the difference in redox potential between two neighboring components (A, B and C or D in Fig. 1). The first site is $NADH_2$—fravoprotein, the second site cyt. b—cyt. c,

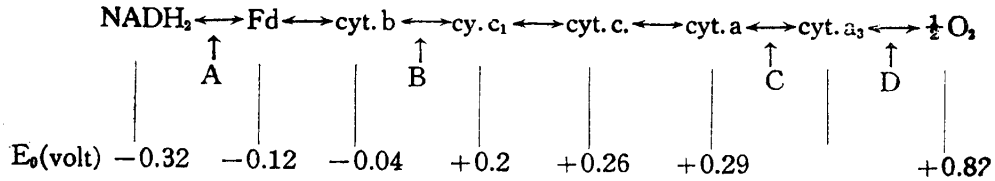


Fig. 1 Possible sites of phosphorylation in the electron transfer pathway on the respiratory chain.

and third site cyt.a—cyt.a_3 or cyt.a_3—$\frac{1}{2}O_2$. These theoretically deduced possible coupling sites of ATP formation to the electron transfer chain coincide partially with those proposed by CHANCE and WILLIAMS[13] from their spectrophotometric investigation (Fig. 2). They postualted that the third coupling site of phosphorylation is cyt.c—cyt.a. But the value of $\Delta E_0'$ indicates the cyt. a-cyt. a_3 as a possible site.

As for the mechanism of ATP formation (Fig. 2) it is postulated by bio-

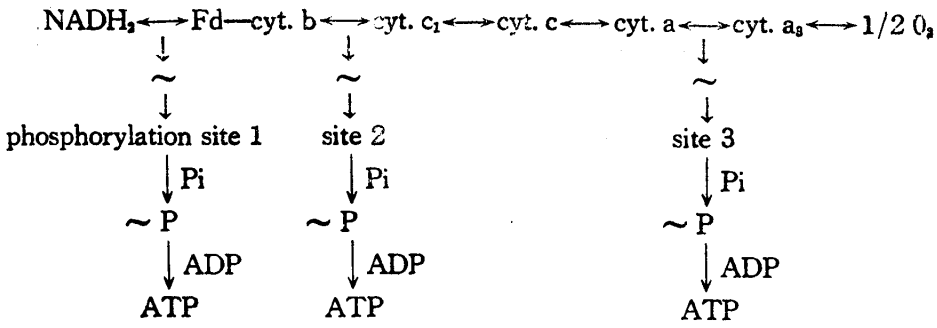


Fig. 2 Diagrammatical representation of phosphorylation sites in mitochondria.

chemical analysis[1,2] that the free energy produced by the electron transfer from $NADH_2$ to molecular oxygen is transduced to the chemical bonding energy as represented by the following equation:

$$Carrier_{red} + X + Oxidant \longrightarrow Carrier_{ox} \sim X + Reductate \quad \cdots\cdots(1)$$

$$Carrier_{ox} \sim X + Pi \longrightarrow Carrier_{ox} + X \sim P \quad \cdots\cdots(2)$$

$$P \sim X + ADP \longrightarrow ATP + X \quad \cdots\cdots(3)$$

Here X is the energy coupling vehicle. Carrier is the electron carrier such as $NADH_2$, cytchromes b and a, ox: oxidated form, red: reduced form. In these reactions, X forms a high energy compound combining with the electron carrier in the course of electron transfer, $Carrier_{ox} \sim X$. Then the high energy ($\sim$) is transferred to phosphate forming $P \sim X$ (a high energy phosphate compound) which in turn donates phosphorus to ADP. $Carrier_{ox} \sim X$ or $P \sim X$ is a common intermediate to the formation or the degradation of ATP and it will be a direct energy source for the mechanochemical reaction and/or the active ion accumulation as well as for the ATP formation.

Now if we suppose the case where succinate is used as energy source, the diagram presented in Fig. 2 may be indicated as that in Fig. 3 in the term of physics by the theory of circuit analog analysis of physical phenomena, [14-20] as electron flow can be understood as an electric current.

As it is well known, in the electron transfer chain of mitochondria the electrons are transferred from $NADH_2$ to oxygen or Fs to oxygen. E_0' of $succinate^{2-}$/$fumarate^{2-}$ is + 0.03 volt. Then the succinoxidase system (E_2) can be postulated as a battery of 0.79 volt. Primary dehydrogenase is consisted of iso-citric dehydrogenase (E_0' of $isocitrate^{3-}$/$oxalosuccinate^{3-}$ = −0.30 volt), α-ketoglutaric dehydrogenase (E_0' of α-$ketoglutarate^{2-}$/$succinyl^{1-}$ CoA = −0.50 volt), malic dehydrogenase (E'_0 of $malate^{2-}$/$oxaloactate^{2-}$ = −0.30 volt), glutamic dehydrogenase (E_0' of $glutamate^{1-}$/α-$ketoglutarate^{2-}$ = −0.12 volt) and β-hydroxybutyric dehydrogenase (β-$hydroxybutyrate^{1-}$/$acetoacetate^{1-}$ = −0.35 volt) can

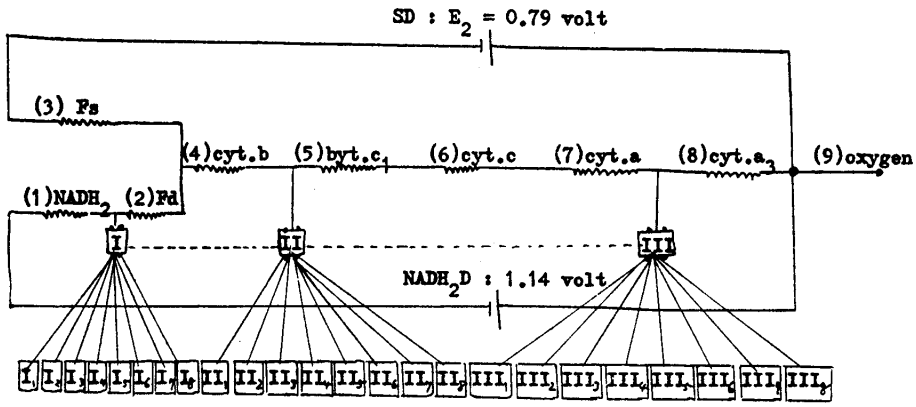


Fig. 3 Diagrammatical drawing of a circuit analog to the energy transducing reaction coupled to the respiratory chain of mitochondria.

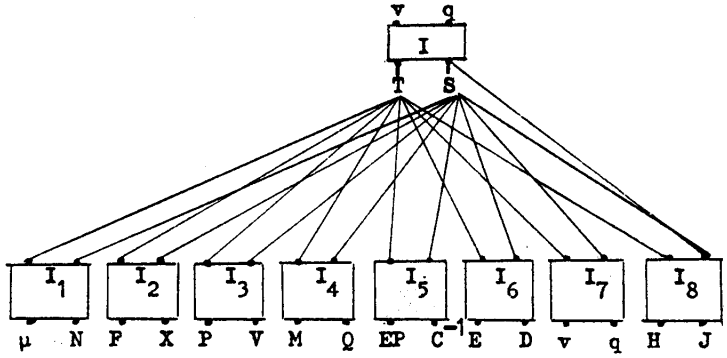
—| |— battery, corresponds to dehydrogenase

—~~~— impedance corresponds to electron carrier.

—:□:— 4 terminal black boxes correspond to coupling factor.

reduce NAD to $NADH_2$.[12] Therefore, primary dehydrogenase complex can be represented by NADH oxidase system.

In the respiratory chain, electric current will run against the direction of electron transfer. Each individual components cyt. a_3, a, b, c and c_1, and fravoprotein can be represented as impedance, because the redox potential of various electric carriers is gradually increased from $NADH_2$ or Fs to oxygen. The energy preserved in the high energy compound which is formed by the transduction of free energy produced by respiration, transforms itself into the chemical,[4-10] physical and mechanical energies. It corresponds to the black box in physics, i. e. the theoretical energy transducing machine in physical terms. Accordingly, by the use of terminology of circuit theory the electron transfer system and the oxidative phosphrylation can be presented as an electron circuit being cascaded by several black boxes. The oxidative phosphorylation can be demonstrated as cascade or tandem connection among the several 4-terminal black boxes in the circuit analog as shown in Fig. 3. The coupling mechanism is indicated by the cascade between two 4-terminal black boxes, whose terminals have specific *degree of freedom* different in opposite site.[16] Thus the cascades among the black boxes as coupling factors (X) are illustrated in Fig. 4 in detail. In this circuit electrical freedoms, v(potential) and q(electric charge) can be transduced by black box I to thermal freedoms, T(absolute temperature) and S(entropy). If the fundamenial matrix(F-matrix)of 4-terminal black boxes is denoted by $\binom{AB}{CD}$, the following equation of matrix is obtained:



Eig. 4 Representation of a possible connection among 4-terminal black boxes as energy transducing reaction.

$$\begin{pmatrix} \Delta V \\ \Delta q \end{pmatrix} = \begin{pmatrix} AB \\ CD \end{pmatrix} \begin{pmatrix} \Delta T \\ \Delta S \end{pmatrix}$$

where, A, B, C and D are matrix elements. According to the circuit theory,[16] the reciprocity theorem is denoted by the following determinant.

$$\begin{vmatrix} AB \\ CD \end{vmatrix} = 1$$

Thus, we obtain $\frac{\Delta V}{\Delta T} = \frac{\Delta S}{\Delta q} = \frac{\Delta Q}{T \Delta q}$ Q: thermal energy. This equation is nothing but the Gibbs-Helmoltz equation. That means the constraint condition between the thermal and electric phenomena. Here the thermal freedoms T and S are transducible to many reactions e. g. chemical, hydrodynamical, electrical and electro-magnetical reactions. Thus the further transduction of the energy given by black box I may be presented by the black boxes I_1, I_2, ……I_8. That is, by black box I_1 the thermal freedoms T and S are transduced to chemical freedoms μ (chemical potential) and N (concentration), by black box I_2 to force and displacement, by black box I_8 to pressure and volume and so on. In the case of black box I_1, the following equation can be obtained as in the case of black box I.

$$\begin{pmatrix} \Delta T \\ \Delta S \end{pmatrix} = \begin{pmatrix} A_1 B_1 \\ C_1 D_1 \end{pmatrix} \begin{pmatrix} \Delta \mu \\ \Delta N \end{pmatrix}, \qquad \text{Assuming that } \begin{vmatrix} A_1 B_1 \\ C_1 D_1 \end{vmatrix} = 1,$$

the following equation is obtained.

$$\frac{\Delta \mu}{\Delta T} = \frac{\Delta S}{\Delta N} \quad \cdots\cdots (I_1)$$

This equation corresponds to the law of Van't Voff or one of Arrehenius, and means that the constraint condition of the changes in chemical equilibrium by the changed temperature and it means ATP formation in mitochondria.

In the black boxes I_{2-4}, the thermal freedoms T and S are transferred to mechanical freedoms F (force) and X (displacement), or P (pressure) and V (volume) and/or M (torque moment of force) and Q (angular). Assuming

that $\begin{vmatrix} A_2 & B_2 \\ C_2 & D_2 \end{vmatrix} = 1 \cdots$, the following equation is obtained, as in the former case.

$$\frac{\Delta F}{\Delta T} = \frac{\Delta S}{\Delta X} \cdots (I_2) \quad \text{or} \quad \frac{\Delta P}{\Delta T} = \frac{\Delta S}{\Delta V} \cdots (I_3) \quad \text{and/or} \quad \frac{\Delta M}{\Delta T} = \frac{\Delta S}{\Delta Q} \cdots (I_4)$$

from black boxes I_2, I_3 and I_4 respectively. The equation I_2 means mechanochemical changes[11] in mitochoadria, I_3 volume change[11] and I_4 unknown but possible changes in mitochondria.[21]

In the black box I_5, the thermal freedoms T and S are transferred to hydrodynamical freedoms EP (Ionization pressure) and C^{-1} (concentration) and we reduces the following equation.

$$\frac{\Delta EP}{\Delta T} = \frac{\Delta S}{\Delta C^{-1}} \quad \cdots\cdots (I_5)$$

This equation corresponds to the equation for osmotic pressure and means the active transport in mitochondria[7].

Furthermore, the thermal freedoms T and S are transferred to electromagnitic freedoms E (electric field) and D (electric displacement) by black box I_6 and v and q by black box I_7, and following epuations are obtained.

$$\frac{\Delta E}{\Delta T} = \frac{\Delta S}{\Delta D} \cdots\cdots (I_6) \qquad \frac{\Delta V}{\Delta T} = \frac{\Delta S}{\Delta q} \cdots\cdots (I_7)$$

These equations correspond to the law of Gibbs-Helmholtz or Onsager's reciprocity, and means the possible fluorescence or vectral property of membrane[22] of mitochondria by I_6 and the reversal electron transfer by (I_7).[10]

By the black box I_8 the thermal freedoms T and S are tranferred to magnetic freedoms H (magnetic field) and J (magnetic intensity) and the following equation is obtained.

$$\frac{\Delta H}{\Delta T} = \frac{\Delta Q}{T \Delta J} \quad \cdots\cdots (I_8)$$

This equation means the DeBye-Jork equation.

The similar theoretical consideration can be applied to the system II to II_1, $II_2 \cdots$ $\cdots II_8$ and III to III_1, $\cdots\cdots III_8$.

SUMMARY

As is generally known, the energy transducing reaction in mitochondria is of highly complicated one. Free energy produced by transferring electrons from substrate to oxygen, where many dehydrogenases and respiratory chain of mitochondria are concerned, is transduced to ATP formation or utilized for the ion accmulation reaction, synthesis of various substances, reversal electron transport and the mechanochemical changes of mitochondria. The mechanism of these energy trasducing reactions which is supposed to be closely related with each other, has not yet been clarified. The authors tried to solve these biological energy transducing mechnism by applying physical circuit theory in electronics

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