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Energy transduction in electron transport

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SUMMARY

Mathematical formulae and computations are presented which may be useful in understanding equilibrium phenomena at an energy transduction site in an electron transport chain, such as variation of apparent midpoint potential with phosphate potential, crossover phenomena, and respiratory control. The model used has been published previously without the present calculations and is intended to be the simplest that can be conceived on purely physicochemical principles. Several alternative explanations of the Wilson–Dutton data are given.

The discovery by Wilson and Dutton that addition of ATP can lower the apparent midpoint potential (E_A) of cytochrome a_3 as much as 100 mV (ref. 1) and raise the E_A of cytochrome b as much as 200 mV (ref. 2) may prove to be a breakthrough in our understanding of the mechanism of phosphorylation and energy conservation in electron transport. One cannot tell at this stage to what further discoveries this may lead, but it may be of some help to call attention to the fact that simple physicochemical considerations lead to a conclusion that such variations of E_A should occur. This idea was outlined briefly in an earlier attempt to apply it to electron transport in photosynthetic bacteria[3]. A more complete explanation is in preparation. Here I wish to take only the space necessary to review the essential principles and to exhibit new mathematical conclusions pertinent to the findings of Wilson and Dutton[1,2].

The overall process at an energy conservation site in an electron transport chain is one of taking electrons from a donor at redox potential E_1, giving them to an acceptor at potential E_2, while taking the free energy available from the electrons and giving it to a hypothetical entity, I, and converting it to a high energy form, I*, able to do useful work:

$$ne^-(E_1) + I \rightarrow ne^-(E_2) + I^* \quad (1)$$

The free energy (ΔG) per mole of I available from the electron flow is $nF(E_2 - E_1)$, where F is the Faraday constant. The free energy carried away by I* is $\Delta G^\circ(I \rightarrow I^*) + RT \ln((I^*)/(I))$, where R is the gas constant and T the absolute temperature. In biological systems the energy of I* is passed to ATP:

$$m\mathrm{I}^* + \mathrm{ADP} + \mathrm{P_i} + \nu\mathrm{H}^+ \rightarrow m\mathrm{I} + \mathrm{ATP} \qquad (2)$$

Eqn. (2) is to be taken in a formal sense only. Its purpose is to relate the unknown I and I* to the measurable phosphate potential. It is intended to include the case that I and I* could actually be ADP + P_i and ATP, respectively. The number of electrons per ATP is $n \cdot m$.

The energy transduction theory outlined previously[3] is based on five postulates:

(1) Potential matching. The energy-transducing entity (transductase?) should receive the electron at E_1 in a state in which its standard redox potential, E_0', approximates (±60 mV) E_1 under conditions of maximal rate of energy transduction, such as the mitochondrial State 3. It should give the electron to the external acceptor in a state in which its E_0' approximates E_2. (The detailed arguments for this and other postulates are left to the expanded paper.) Let E_0' of the receiving form be E_ℓ°, and that of the donating form be E_h°. Note that the term "midpoint potential" is not used here, because it can be defined in terms of a summation of indistinguished species with various ligand bindings as we have defined "E_A". For the sake of studying mechanism, any ligand-binding steps are separated from electron transfer steps. Thus E_0' represents the attractiveness for an electron of a redox couple whose reduced form is equal to the oxidized form *plus n* electrons. The prime indicates that the degree of protonation is not considered (unless I* = H^+), and so E_0' could be a function of pH.

(2) Identity. The low potential couple and the high potential couple should be alternate forms of the same entity, "C". By changing its E_0' from E_ℓ° to E_h°, a single substance bridges the gap at the energy transduction site with an approach to equilibrium on both sides. Thus, the data enable us to identify the energy-transducing entity at Site III as a_3 itself, acting between *a* and oxygen, and that at Site II as a special *b* cytochrome (now called b_T by Chance *et al.*[4]), acting between ordinary *b*, perhaps, and c_1.

(3) Complexing with I or I.* This phenomenon produces the changes between E_ℓ° and E_h°. The six different ways in which this can be done give rise to the six different cases shown in Fig.1. Each involves five steps: (a) electron reception; (b) electron donation;

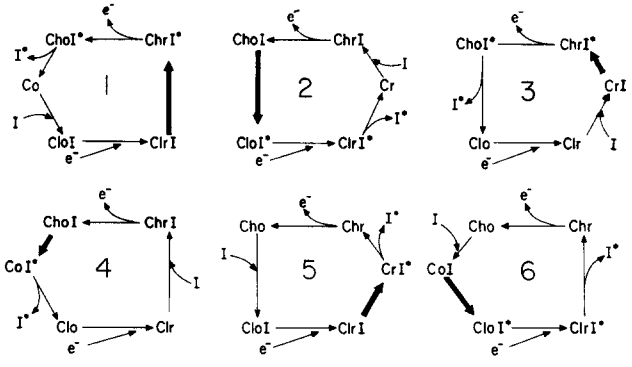


Fig.1. Six ways to arrange an energy transducer with the minimum five steps. The notation is the same as that of ref. 3. "C" stands for "carrier" or "catalyst" or "complex". "h" added to it indicates a high standard potential form; "l", low potential. "o" indicates the oxidized form and "r" the reduced form.

(c) contact with I; (d) release of I*; and (e) the change from I to I*. These occur between the five states or forms of the entity which, if we drop the "I's", may be written: Cho, Chr, Clo, Clr, and either Co or Cr (see figure legend).

(4) Electrical charges. Charges on I or I* or on a part of C moving with I or I* toward or from the vicinity of the exchangeable electrons of C, could alter the electrical potential of its environment enough to produce the required change in E_0'. This postulate is not essential to what follows, but it proposes that it is not necessary to postulate a change of ligand to the iron atom.

(5) Specificity. In the tightly coupled state it is necessary that the low potential form of the transducer can exchange electrons only with the donor, and the high potential form only with the acceptor. If, for example, the low potential form could alternately receive electrons from the donor and give them to the acceptor without doing anything else, the system would be "uncoupled"★.

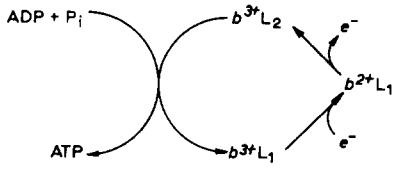
In all coupled mitochondrial states except State 3, the electron flux is low, and we can expect that near-equilibrium conditions will prevail, as they must if the energy transduction is efficient. The calculations in the rest of this paper will assume equilibrium. The equilibrium relations governing the quantities in Eqns. 1 and 2 are well-known and are repeated here only for reference:

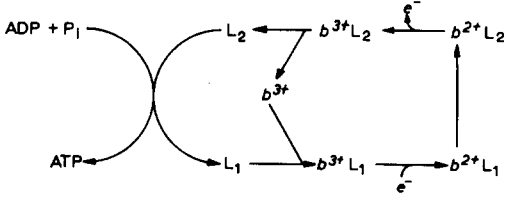
$$E_2 - E_1 = E_p = E_p^\circ + \frac{RT}{nmF} \ln \frac{(ATP)}{(ADP)(P_i)(H^+)^\nu} = \frac{\Delta G^\circ(I \rightarrow I^*)}{nF} + \frac{RT}{nF} \ln \frac{(I^*)}{(I)} \qquad (3)$$

E_p stands for the "phosphate potential" and $E_p^\circ = \Delta G^\circ(ADP \rightarrow ATP)/nmF$. Eqn. 3 makes it clear that increasing the ratio of ATP to ADP always tends to increase E_2 and/or decrease E_1. Increase of E_2 causes the acceptor to become more oxidized, and decrease of E_1 causes the donor to become more reduced. This is the well-known phenomenon of "reversed electron transport"[5] and is independent of the mechanism operating between E_1 and E_2 to accomplish Eqn. 1. It assumes the equilibrium explanation[6,7] for respiratory control as well.

The fraction of transducer in each of its five forms is given by:

★If the scheme suggested by Wilson and Dutton[2] were put into the format of Fig.1, we would have the first diagram below. In this form, it fails to show why $b^{2+}L_1$ has a high E_0' and changes to $b^{3+}L_2$ on giving an electron to the acceptor, but has a low E_0' on reversibly giving an electron to the donor. This also seems to violate Postulate (5) and fails to exhibit the various steps involved in the conversion from $b^{3+}L_2$ to $b^{3+}L_1$ which we wish to analyze in this paper. With slight changes, we could have the second diagram below which, with identification of L_1 with I, and of L_2 with I*, is the same as Case 1 of Fig.1.





$$f(\mathrm{Clr}) = 1/D \tag{4}$$

$$f(\mathrm{Clo}) = B_1/D \tag{5}$$

$$f(\mathrm{Chr}) = X/D \tag{6}$$

$$f(\mathrm{Cho}) = B_2 X/D = B_1 \Pi^2 X/D \tag{7}$$

$f(\mathrm{Cr}) = Yr/D$ (= 0 in Cases 1, 4, and 6) (8)

$f(\mathrm{Co}) = B_1 Yo/D$ (= 0 in Cases 2, 3, and 5) (9)

where: $B_1 = \exp\left[nF(E_1 - E_\ell^\circ)/(RT)\right]$; $B_2 = \exp\left[nF(E_2 - E_h^\circ)/(RT)\right]$; $D = 1 + B_1 + X(1 + B_2) + Yr + B_1 Yo$; $\Pi^2 = B_2/B_1$; and X, Yo and Yr depend upon the case as follows:

In case:	*1*	*2*	*3*	*4*	*5*	*6*
X =	K	$1/(K\Pi^2)$	$\Lambda\sqrt{K}/\Pi$	$\Lambda/(\Pi\sqrt{K})$	$\sqrt{K}/(\Lambda\Pi)$	$1/(\Lambda\Pi\sqrt{K})$
Yr =	0	$1/(\Lambda\Pi\sqrt{K})$	$\Lambda/(\Pi\sqrt{K})$	0	K	0
Yo =	$\Pi\sqrt{K}/\Lambda$	0	0	$\Lambda\Pi\sqrt{K}$	0	$1/K$

K = the equilibrium constant for the step in which I changes to I*, indicated by the heavy arrow in Fig.1: $\Lambda = \sqrt{(\mathrm{I})(\mathrm{I}^*)/(K_1 K^*_1)}$. K_1 = the "dissociation constant" for the reaction in which I combines with C. K^*_1 = the "dissociation constant" for the dissociation of I* from C.

If, as seems to be the case, the high and low potential forms are spectroscopically indistinguishable, any measurement will lump all the oxidized forms together and likewise all the reduced forms. If one measures some ambient potential E and applies the Nernst equation:

$$E = E_A + \frac{RT}{nF} \ln \frac{\Sigma \mathrm{Ox}}{\Sigma \mathrm{Red}} \tag{10}$$

one obtains an "apparent midpoint potential", E_A.

From above:

$$\frac{\Sigma \mathrm{Ox}}{\Sigma \mathrm{Red}} = \frac{B_1 + B_1 \Pi^2 X + B_1 Yo}{1 + X + Yr} = B_1 \beta = B_2 \beta/\Pi^2 \tag{11}$$

where $\beta = (1 + \Pi^2 X + Yo)/(1 + X + Yr)$.

Substituting Eqn. 11 into Eqn. 10 and rearranging:

$$E^1_A = E^\circ_\ell - \frac{RT}{nF} \ln \beta \tag{12}$$

$$E^2_A = E^\circ_h - \frac{RT}{nF} \ln(\beta\Pi^{-2}) \tag{13}$$

where E_A^1 is E_A if the potential measured is E_1, and E_A^2 is E_A if $E = E_2$. Both β and Π and, therefore, E_A^1 and E_A^2 are functions only of the phosphate potential, of Λ and of constants. This means that at a given phosphate potential and Λ, the system titrates apparently like a single redox couple. It will give a straight line for E *vs.* $\ln(\Sigma Ox/\Sigma Red)$.

Fig.2 shows examples of how E_A will vary with phosphate potential for each of the six cases of Fig.1, with K taken as 10 and Λ as 1. It is seen that except for the regions of Cases 1 and 2, which approach horizontal asymptotes, the E_A can be indefinitely high or low. Its measure does not readily give a clue as to the actual standard potentials, E_h° and E_ℓ°, existing in the transducer. In Fig.2A, the E_A's all decrease with increasing phosphate potential, while the opposite is true in Fig.2B. Although Wilson and Dutton attempted to equilibrate both sides with their electrode simultaneously, their results could just as well be interpreted as equilibrating through E_1 (cytochrome *a* or *c*) when they observed a_3, and through E_2 (c_1 or *c*) when they observed b_T. If we knew the actual phosphate potentials for measurements of E_A at several values, we should be able to tell which one of the six cases and what values for K and Λ apply.

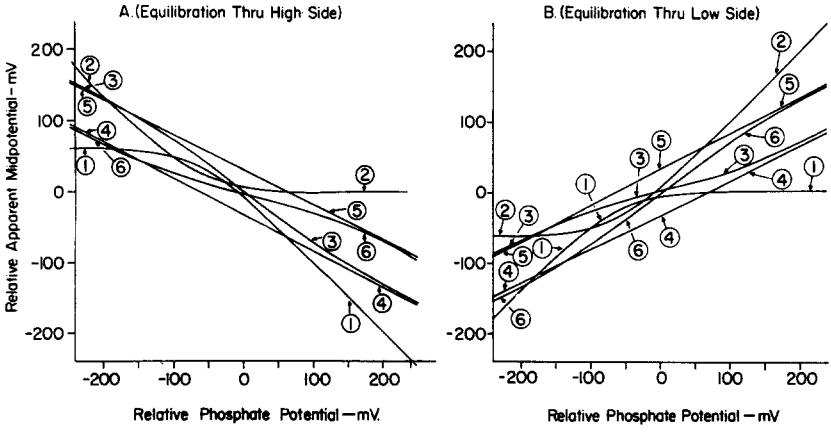


Fig.2. A. Plot of $(E_A^1 - E_\ell^\circ)$ *vs.* $(E_p - E_h^\circ + E_\ell^\circ)$. B. Plot of $(E_A^2 - E_h^\circ)$ *vs.* $(E_p - E_h^\circ + E_\ell^\circ)$. In both cases, $K = 10$ and $\Lambda = 1$. Numbers in circles indicate the case of Fig.1 to which each curve belongs.

If cytochrome *b* is a donor to cytochrome b_T and equilibration with the electrode is only through the high side of b_T, its apparent midpoint potential will also vary with the phosphate potential. This leaves open the question as to which E_A's in the Wilson–Dutton[2] data refer to *b* and which to b_T. Table I shows examples of different ways in which their data could be fitted. In all cases, the parameters E_h° and E_ℓ° were taken arbitrarily as +230 and +30 mV, respectively. The E_0' of cytochrome *b* was taken as +30 mV in the first four rows of Table I where it is assumed to equilibrate with E_1, and it can be +35 mV in the last four rows where it is assumed to equilibrate with E_2. In Table I, E_A^D stands for the apparent midpoint potential of cytochrome *b* acting as a donor to b_T and "(*1*)" refers to the case of low phosphate potential and "(*2*)" to the case with ATP added. The data in rows 3 and 4

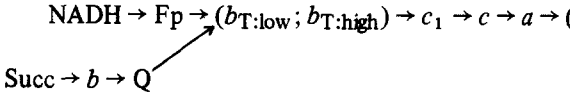
TABLE I

EXAMPLES OF CYTOCHROMES *b* AND b_T DATA[2] FITTED WITH $E_h^\circ = +230$ mV AND $E_Q^\circ = +30$ mV

Data				*Case*	*Parameters to fit*			
$E_A^2(1)$ (*mV*)	$E_A^D(1)$ (*mV*)	$E_A^2(2)$ (*mV*)	$E_A^D(2)$ (*mV*)		*K*	Λ	$E_p(1)$ (*mV*)	$E_p(2)$ (*mV*)
−55	35	35	245	4	$1.41 \cdot 10^5$	7.16	5	215
35	−55	35	245	4	$1.98 \cdot 10^3$	10^4	−85	215
−35	30	87	256	6	$4.20 \cdot 10^{-4}$	12.7	0	226
30	−35	87	256	4	$1.82 \cdot 10^3$	11.8	−65	226
−55	*	245	*	1	$2.91 \cdot 10^{-2}$	0.0228	−85	335
−55	*	245	*	2	$6.63 \cdot 10^4$	434	−205	215
−55	*	245	*	3	1.26	0.0198	−205	215
−55	*	245	*	5	$2.36 \cdot 10^{-2}$	3.85	−205	215

are consistent with the Wilson–Dutton experimental points as published[2], while that in the other rows assume the values which they choose.

It may be noted that *b* could be associated with the low side of b_T *in vivo* and still equilibrate with E_2 in the Wilson–Dutton experiment, if something like Scheme 1 prevailed:



Scheme 1

and if the electrode equilibrated at "*c*" and at "Succ" while the rate of electron transport through Q were too slow to allow *b* to equilibrate with $b_{T:low}$ in the experiments.

One concludes that any of the six schemes of ref. 3 can explain the Wilson–Dutton data without assigning standard potentials to $b_{T:low}$ and to $b_{T:high}$ equal to the extreme E_A's (*e.g.* −55 and +245 mV) observed. Table I used +30 and +230 mV which match donor and acceptor E_0''s more closely. One notes also that because of the assumed coupling of *b* to the electrode through the transducer in the first four rows of Table I, *b* is titrated in a redox titration at a higher potential than b_T in the cases in which $E_A^D > E_A^2$.

The considerations of this paper are strictly physicochemical and intended to apply to any specific chemical schemes which others may offer, such as the very recent glutathione model[8–11]. I hope to give proper credit to authors of other or similar theories of energy conservation in the more complete presentation now in preparation. Dr. Britton Chance has encouraged me in working out this theory since 1964, and the arrangement of Scheme 1 was partly his suggestion. This research was supported by National Science Foundation GB 6556 and Public Health Service GM 12202.

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