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Contribution of Each Complex of the Mitochondrial Respiratory Chain in the Generation of the Proton-motive Force*

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A new parameter is presented for considering the contribution from each complex of the mitochondrial respiratory chain to the creation of the electrochemical gradient. This parameter is the proton gradient generated in each complex per pair of electrons transported ($\Delta H^+/2e^-$) and may be calculated as the sum of the electrons taken up from the N-side and the electrons released at the P-side. The $\Delta H^+/2e^-$ values take into account all the electrons taken up and released in each complex and differentiate between the contribution from protons that are translocated from the N-side to the P-side and the contribution of scalar protons. Using these values, the P/O ratios for the oxidation of NADH and succinate are predicted to be 2.5 and 1.5, respectively.

Keywords: Bioenergetics, mitochondrial respiratory chain, $H^+/2e^-$ stoichiometry, proton-motive force.

OVERVIEW OF THE RESPIRATORY CHAIN

Bioenergetics and the synthesis of ATP are central topics in biochemistry. Since the discovery of cytochromes by Keilin, the work of Warburg about what it called the "respiratory enzyme," and the chemiosmotic theory of Mitchell, a lot of knowledge has accumulated about this and other related processes (see Ref. 1 for a historical review of this subject). We now know that electrons from NADH and other oxidizable substrates pass through a chain of carriers arranged asymmetrically in the inner-membrane of mitochondria or the cell membrane in bacteria. In mitochondria, these carriers are nucleotides of flavine, Fe-S proteins, heme proteins, or Cu atoms, organized in a series of four complexes and two mobile carriers: ubiquinone and cytochrome *c*. The transfer of electrons through these carriers is accompanied by a proton transfer across the membrane, producing a chemical and an electrical gradient. When these protons flow back into the matrix through the complex V, also called ATP synthase, they provide the necessary energy for the ATP synthesis [2].

The availability of the three-dimensional structures for three of the four respiratory complexes [3–5] that transport the electrons in the mitochondrial respiratory chain now allows us to study, with a molecular detail, the transport of electrons and the generation of the proton gradient that impulses the synthesis of ATP [6]. Despite all this information, there still remain several unanswered questions and controversies. Two of the most debated questions are the stoichiometry of the number of protons translocated per pair of electrons transported ($H^+/2e^-$) [7] and the P/O ratio (the number of moles of ATP synthesized per two electrons that flow through a defined segment of an electron transfer).

The accepted values for the number of protons translocated per pair of electrons are 10 for the oxidation of NADH and 6 for the oxidation of succinate, and 4, 4, 2 is the $H^+/2e^-$ stoichiometry for complexes I, III, and IV, respectively [6]. However, when a proton is translocated from the mitochondrial matrix, called the N-side, to the intermembrane space, called the P-side, a gradient (the difference between the two compartments) of two protons is generated. This distinction is important because it is the gradient of protons, called the proton-motive force, that impulses the synthesis of ATP, and in each complex not all the protons that are taken up from the N-side are released at the P-side.

MECHANISM OF PROTON PUMPING IN COMPLEXES I, III, AND IV

Complex I, or NADH-ubiquinone oxidoreductase (EC 1.6.99.3), is the largest of the mitochondrial respiratory complexes with a size of ~900 kDa [6]. This complex catalyzes NADH oxidation and ubiquinone reduction and translocates four protons from the N-side to the P-side [6]. Several models have been proposed to describe how proton translocation is coupled to electron transfer, but the lack of structural information about complex I makes it difficult to verify any of them [6].

Complex III, or cytochrome bc_1 (E.C 1.10.2.2), catalyzes the ubiquinol oxidation and cytochrome *c* reduction by a mechanism known as the Q cycle [8]. The generation of the proton gradient in complex III is intimately related with the

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Q cycle and is an example of a redox loop mechanism in which there are electron carriers (such as ubiquinol) that transport electrons in conjunction with protons, and carriers (such as cytochromes or Fe-S centers) that transport electrons alone [8]. When ubiquinol transfers its electrons to the Fe-S center called Rieske protein or to cytochrome *b* (two subunits of the complex III) at the Q_P site, the protons that were carried in conjunction with electrons are released to the P-side. When ubiquinone is reduced at the Q_N site, protons from the N-side are consumed. So, for every two electrons passing from ubiquinol to cytochrome *c*, complex III releases four protons at the P-side but takes only two from the N-side [4].

Complex IV, or cytochrome oxidase (E.C 1.9.3.1), catalyzes the transfer of electrons from cytochrome *c* to dioxygen. To reduce a molecule of dioxygen into two molecules of water, four electrons from four molecules of cytochrome *c* and four protons from the N-side are needed [5]. Also, for every four electrons passing through the complex, four protons are pumped from the N-side to the P-side through a series of ion channels and in conjunction with some conformational changes in the complex [9]. Therefore, for every two electrons passing through complex IV, four protons are taken up from the N-side but only two are released at the P-side [5].

TABLE I

Stoichiometry of the number of protons taken up from the N-side and released at the P-side, the generated proton-gradient, and the deduced P/O ratio for the mitochondrial respiratory chain

	No. of protons, per pair of e^-			P/O[b]
	Taken up from the N-side	Released at the P-side	ΔH^+[a]	
Complex I	4	4	8	1
Complex III	2	4	6	0.75
Complex IV	4	2	6	0.75

[a] Gradient of protons generated per pair of e^-. $\Delta H^+/2e^-$ is the number of protons released at the P-side plus the number of protons taken up from the N-side.

[b] The P/O ratio (or ATP/$2e^-$) is calculated as the $\Delta H^+/2e^-$ divided by the ΔH^+/ATP ratio. The ΔH^+/ATP ratio has a value of 8, assuming that the movement of three protons from the intermembrane space to the matrix must flow to produce one molecule of ATP, and one proton is needed to transport Pi, ATP, and ADP across the mitochondrial membrane.

STOICHIOMETRY OF THE NUMBER OF PROTONS PUMPED PER PAIR OF ELECTRONS TRANSPORTED

Table I shows the stoichiometry of the numbers of protons, per pair of electrons, taken up from the N-side and released at the P-side for the three mitochondrial respiratory complexes that contribute to the generation of the proton gradient. The 4, 4, 2 stoichiometry of protons pumped out per pair of electrons for complexes I, III, and IV, which appears in most biochemistry books, is a simplification because these are only the numbers of electrons released at the P-side (see Fig. 1). So to calculate the P/O ratios, one uses the number of charges flowing through each complex ($q^+/2e^-$) [10] or the H/O ratios of electrogenic or effective proton translocation [11]. The $q^+/2e^-$ ratios, or the effective number of protons transported for complexes I, III, and IV, are 4, 2, and 4, respectively [10, 11]. Because in complex III two of the protons (called scalar protons) that were carried in conjunction with electrons are released to the P-side without a charge translocation, it is said that the release of these protons is thermodynamically futile and the $q^+/2e^-$ value for this complex is 2 [10]. In addition to the protons pumped in complex IV, electrons are transported from cytochrome *c* (located at the P-side) to the cytochrome a_3-Cu_B binuclear center (located near the N-side), where the dioxygen is reduced. For this reduction, two protons from the N-side (per pair of electrons transported) are needed, so the $q^+/2e^-$ value for complex IV is 4 [10].

$\Delta H^+/2e^-$, A NEW MEASURE OF THE CONTRIBUTION OF EACH COMPLEX TO THE CREATION OF THE ELECTROCHEMICAL GRADIENT

The $q^+/2e^-$ values, or the effective number of protons translocated in each complex, are good measures of the different levels of energy created in each complex and agree with the differences in the redox span of the complexes [7]. However, the scalar protons that originate in the oxido/reduction reactions in complex III and are released to the P-side also help to create the electrochemical gradient. If the contribution of these scalar protons were considered, the best place for it would be complex III. However, the scalar protons contribute less to the creation of the electrochemical gradient than protons translocated from the N-side to the P-side, because scalar protons are released to the P-side but are not taken up from the N-side. Here we present a new parameter for measuring

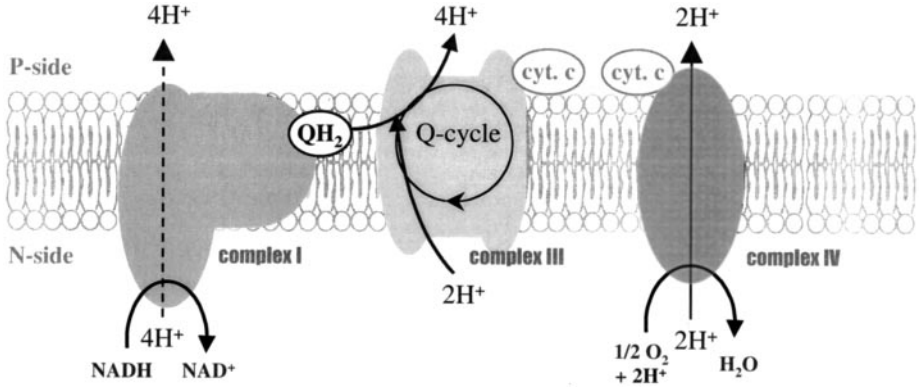

FIG. 1. **Summary of the stoichiometry of the number of protons taken up from the N-side and released at the P-side in mitochondrial complexes I, III, and IV per pair of electrons transported.**

the contribution of each complex to the electrochemical gradient created by the respiratory chain. This new parameter is the proton gradient generated in each complex per pair of electrons transported ($\Delta H^+/2e^-$) and may be calculated as the sum of the electrons taken up from the N-side and the electrons released at the P-side. The proton gradient values take into account all the electrons taken up and released in each complex and differentiate between the contribution from protons that are translocated from the N-side to the P-side and the contribution from scalar protons.

Table I shows the proton gradient generated in each complex. If we assume that the movement of three protons from the P-side to the N-side must flow to produce one molecule of ATP, and one proton is needed to transport Pi, ATP, and ADP across the mitochondrial membrane, the number of protons needed to produce one molecule of ATP is 4 [10]. This number corresponds to a proton gradient of 8, consumed by each molecule of ATP. If we use the proton-gradient stoichiometry that produces or consumes each complex, we can calculate the P/O ratios. For $\Delta H^+/2e^-$ values of 8, 6, and 6 for complex I, III, and IV, respectively, the P/O ratios are 1, 0.75, and 0.75, and the P/O ratios for the oxidation of NADH and succinate are 2.5 and 1.5, respectively. The P/O ratios of 2.5 and 1.5 for the oxidation of NADH and succinate, respectively, are lower than the classical ratios of 3.0 and 2.0, in good agreement with experimental evidences [11]. It has recently been estimated that 10 protons are needed to produce three molecules of ATP in *Escherichia coli* [12]. If this value were generalized to mitochondria, the P/O ratios of complexes I, III, and IV would be 0.92, 0.69, and 0.69, respectively, and the values for the oxidation of NADH and succinate would be 2.3 and 1.38, respectively.

Due to thermodynamic considerations, the P/O ratios of 1, 0.75, and 0.75 for complexes I, III, and IV may be misunderstood because the redox differences in complexes I and III are lower than those in complex IV [7]. However, several factors must be considered. The electron-transfer mechanism, the Q cycle [8], is not usually taken into account when considering the change in redox potential for the reaction catalyzed by complex III. Because complex III catalyzes the oxidation of one ubiquinol molecule and the reduction of two molecules of cytochrome *c*, the difference between the redox potentials of these two carriers is usually taken as the redox difference for complex III. However, when transferring two electrons from ubiquinol to cytochrome *c*, two more electrons are transferred through two cytochromes *b* to a new molecule of ubiquinone [8]. In some way this additional electron transfer may have to be considered when calculating the redox difference for complex III. Also, the different thermodynamic contributions of each complex may reflect the different strategy in the proton transport for each complex. The proton-transport mechanism in complex III is a redox-loop [13], and the mechanism in complex IV is a conformational pump [9]. The release to the P-side of the scalar protons that were transported with electrons requires less energy than the pump of protons (sometimes called vectorial protons) from the N- to the P-side using a conformational pump. Therefore, complex III could create the same proton gradient as complex IV, although complex III has a lower redox potential span.

CONCLUSION

The electron carriers of the respiratory chain form one of the most important systems for transforming energy generated in catabolism into a form that cells can use. This system must be viewed as a whole because all parts are necessary and functionally coordinated. Because an electrochemical gradient of protons is used to impulse the synthesis of ATP and other processes that require energy, when studying the contribution of each complex in the generation of the proton-motive force, we can use the values of the proton-gradient generated in each complex. These values take into account both the protons that are taken up from the N-side and all the protons that are released at the P-side and predict a P/O ratio of 2.5 and 1.5 for the oxidation of NADH and succinate, respectively.

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