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Two-dimensional diffusion of F_1F_0-ATP synthase and ADP/ATP translocator. Testing a hypothesis for ATP synthesis in the mitochondrial inner membrane

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We report here the first experimentally determined lateral diffusion coefficients of the F_1F_0-ATP synthase and the ADP/ATP translocator in isolated inner membranes of rat liver mitochondria. Rabbit IgG developed against the F_1F_0-ATP synthase isolated from rat liver mitochondria was determined to be immunospecific for the synthase subunits, notably the α-β doublet, γ and δ subunits of F_1 and subunits two, three and four of F_0. This IgG, conjugated with lissamine-rhodamine, was used as a fluorescent probe to monitor the diffusion of the synthase in the membrane. IgG to cytochrome bc_1 complex, prepared and labeled similarly, was used as a fluorescent probe for diffusion of this redox component. Eosin maleimide was determined to specifically label the ADP/ATP translocator in the isolated inner membrane and was used as a specific probe for the diffusion of the translocator. Using fluorescence recovery after photobleaching, the experimental average lateral diffusion coefficient of the F_1F_0-ATP synthase was determined to be $8.4 \cdot 10^{-10}$ cm^2/s or twice that of cytochrome bc_1 complex while the diffusion coefficient of the ADP/ATP translocator was $1.7 \cdot 10^{-9}$ cm^2/s or four times that of cytochrome bc_1 complex suggesting that all three components are independent two-dimensional diffusants. Using these diffusion coefficients and applying a number of basic assumptions, we calculated the theoretical two-dimensional diffusion-controlled collision frequencies and derived collision efficiencies (protons transferred per collision) between each of the three proton-transferring redox complexes and both the F_1F_0-ATP synthase and ADP/ATP translocator by treating the redox components as proton donors and the synthase and translocator as proton acceptors. These collision efficiencies support the physical possibility of a diffusion-based, random collision process of proton transfer and ATP synthesis in the mitochondrial inner membrane.

Introduction

We have previously measured the rates of lateral diffusion of most of the electron transport components in the mitochondrial inner membrane, and have shown that the random collision of such components can account for the experimentally derived maximal rates of electron transport [1–9]. In this communication, we report the first experimentally determined lateral diffusion coefficients of F_1F_0-ATP synthase and ADP/ATP translocator in the plane of the mitochondrial inner membrane. Our calculations based on these diffusion data reveal that it is possible to account for proton transfer from redox complexes to F_1F_0-ATP synthase for ATP synthesis and from redox complexes to ADP/ATP translocator for nucleotide transfer and/or phosphate/H^+ transporter based on the frequencies of diffusion-based, random collisions between proton-

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donor redox complexes and proton-acceptor synthase and translocator components. Thus, the experimentally determined rates of lateral diffusion, electron transport, proton transfer, and ATP synthesis as well as calculated collision efficiencies can support a diffusion-based, random collision process of proton transfer and ATP synthesis in the mitochondrial inner membrane as hypothesized earlier [10,11]. In such a model, all functionally interacting components in the membrane diffuse independently of one another and collide randomly (Fig. 1).

Materials and Methods

Inner membrane preparations

Rat (male, Sprague-Dawley) liver mitochondria were isolated and intact inner membranes (mitoplasts) were purified using digitonin [12] and suspended in a 40 mosM buffer containing 0.3 mM Hepes (pH 7.4) [13]. For fluorescent, covalent labeling of ADP/ATP translocator, inner membranes were resuspended in the 40 mosM buffer containing 30% (w/v) glycerol, frozen in liquid nitrogen for 5 min, stored overnight at −20°C then thawed for labeling. We determined that the protein (intramembrane particle) distribution, as well as the succinate oxidase activity of glycerol frozen inner membrane preparations remain unaltered.

Immunofluorescent labeling of rat liver F_1F_0-ATP synthase

Rabbits were immunized with purified rat liver F_1F_0-ATP synthase as isolated after McHenry and Pederson [14]. IgG was fractionated using an Affi-gel blue column (Pharmacia) and its titer to isolated rat liver F_1F_0-ATP synthase was determined by the Elisa immunoprecipitation procedure [15]. IgG specificity for F_1F_0-ATP synthase was determined with immunoblots by the procedure of Tijssen [15]. SDS-PAGE was carried out by the procedure of Laemmli [16]. Isolated IgG antibodies were conjugated with 6 μg lissamine-rhodamine (LR) per mg IgG protein [2], and used as a specific label for the F_1F_0-ATP synthase in intact inner membranes.

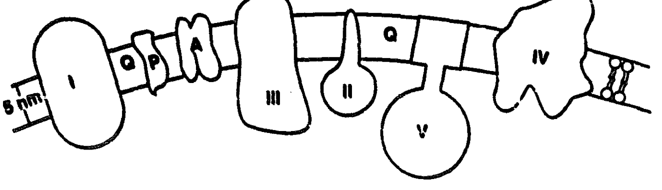

Fig. 1. The inner membrane components that catalyze electron transport, proton transfer and ATP synthesis diffuse two dimensionally and independently of one another and collide randomly. I, NADH dehydrogenase-containing Complex I; II, succinate dehydrogenase-containing Complex II; Q, ubiquinone; III, Complex III or cytochrome bc_1; IV, Complex IV or cytochrome oxidase; *c*, cytochrome *c*; V, Complex V or F_1F_0-ATP synthase; P, phosphate/H^+ transporter; A, ADP/ATP translocator.

Eosin maleimide labeling of ADP/ATP translocator

The glycerol frozen inner membranes were thawed and diluted to 10 mg/ml in H_{40} medium, then prelabeled with 150 μM 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) for 20 min, and finally labeled with 150 μM eosin maleimide (EMA) for 20 min, all at 0°C. The membranes were then centrifuged once at $10\,000 \times g$ for 10 min and incubated with 10 mM dithiothreitol (DTT) for 10 min at 0°C to remove covalently bound DTNB. The EMA-labeled inner membranes were centrifuged and washed three times in H_{40} medium with 0.5% BSA, centrifuged once with 10 mM KP_i (pH 7.4), w/o BSA, and resuspended in 10 mM KP_i (pH 7.4).

Determination of lateral diffusion coefficients (D) of inner membrane proteins

LR-IgG immunospecific for F_1F_0-ATP synthase were added to intact inner membranes. These membranes, or EMA-labeled membranes, were incubated on glass slides at 32°C for 17 min in 10 mM KP_i (pH 7.4) and 10 mM Ca^{2+} for the formation of fused, ultralarge inner membranes [2]. The *D* of the LR-IgG-labeled F_1F_0-ATP synthase as well as of the EMA-labeled ADP/ATP translocator were determined in the inner membranes using fluorescence recovery after photobleaching (FRAP) as described previously [2,8]. Inner membranes immunofluorescently labeled specifically for cytochrome bc_1 complex were used to determine the *D* of cytochrome bc_1 complex in the same membranes as a standard control.

Materials

EMA and LR were purchased from Molecular Probes, DTT and DTNB were purchased from Sigma. All other chemicals were reagent grade.

Results

Specificity of IgG to F_1F_0-ATP synthase in inner membranes

Specific subunits of purified rat liver mitochondrial F_1F_0-ATP synthase were identified with rabbit polyclonal IgG using an immunoblot technique. Immunoblots of rat liver mitochondrial F_1F_0-ATP synthase and rat liver inner membrane using anti-rat liver F_1F_0-ATP synthase IgG revealed that antibodies bound specifically to synthase subunits, notably to the α-β doublet, γ and δ subunits of F_1 and to the two, three and four subunits of F_0, and did not bind to other polypeptides of the inner membrane (Fig. 2). For comparison see also Fig. 1 of Ref. 17. These data, coupled

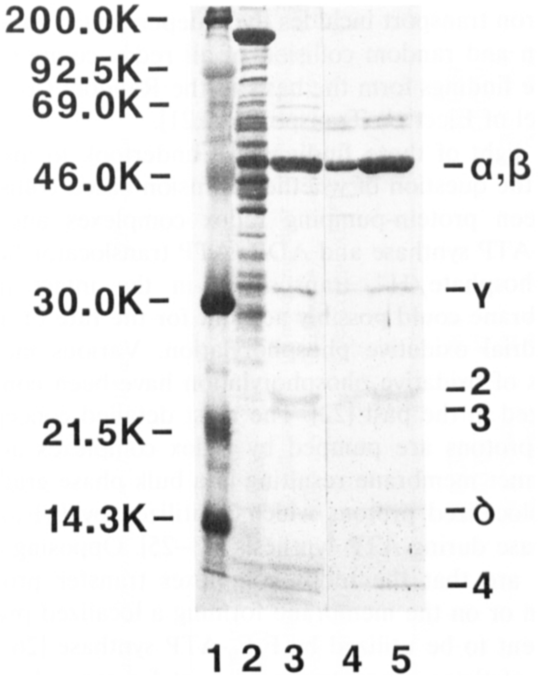

Fig. 2. SDS-PAGE and immunoblots of purified rat liver F_1F_0-ATP synthase and inner membranes. Lane 1: molecular weight standards (top to bottom): myosin, phosphorylase *b*, bovine serum albumin, ovalbumin, carbonic anhydrase, soybean trypsin inhibitor, lysozyme. Lane 2: SDS-PAGE of rat liver inner membranes. Lane 3: SDS-PAGE of purified rat liver F_1F_0-ATP synthase. Lane 4: immunoblot of rat liver inner membranes with anti-rat liver F_1F_0-ATP synthase antibody. Lane 5: immunoblot of purified rat liver F_1F_0-ATP synthase with anti-rat liver antibody.

with fluorescence microscopic observation, showed that the F_0 portion of the ATP synthase could be labeled with our IgG probe from the cytochrome *c* side of the intact rat liver mitochondrial inner membrane for the determination of the *D* of F_1F_0-ATP synthase by FRAP.

Specificity of EMA-labeling of ADP/ATP translocator in inner membranes

Labeling of ADP/ATP translocator with EMA in beef heart mitochondria [18,19] and in rat liver submitochondrial particles [20] has been reported. Houstek and Pedersen [20] concluded that mitochondria and inner membranes are impermeable to EMA, that the EMA-reactive sulfhydryl group of the translocator is located on the matrix side of the inner membrane and, therefore, that the translocator can only be labeled from the matrix side of the inner membrane. Unfortunately, submitochondrial particles labeled by Houstek and Pedersens procedure [20] are too small for diffusional studies by FRAP. Thus we prelabeled glycerol frozen and thawed inner membranes with DTNB, a reversible, sulfhydryl specific reagent. We found that EMA added to the inner membranes after treatment with DTNB specifically labeled the sulfhydryl residue(s) on the ADP/ATP translocator (Fig. 3). The EMA labeling of the translocator was specific at 0°C, but not at room temperature. Post-treatment of the EMA-labeled inner membranes with DTT resulted in reversal of the DTNB prelabel without loss of EMA labeling of the ADP/ATP translocator. Specific labeling of the ADP/ATP translocator was inhibited in the presence of its inhibitor, caboxyactractiloside (CAT) in the presence and absence of ADP. These data showed that the ADP/ATP translocator in the intact, permeabilized inner membrane could be specifically labeled with EMA for the determination of the *D* of the translocator by FRAP.

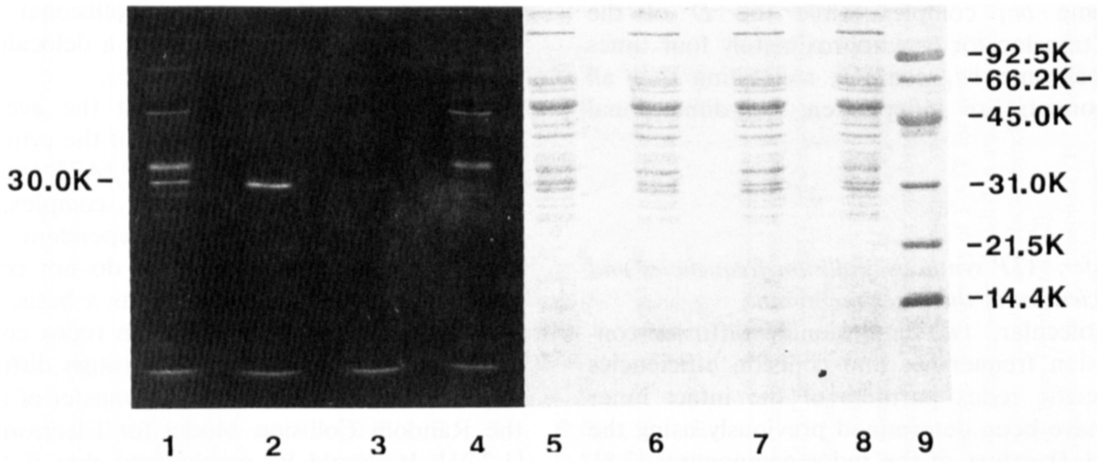

Fig. 3. Fluorographs and SDS-PAGE of EMA-labeled rat liver inner membranes. Lane 1: fluorograph of inner membranes labeled with EMA alone at 0° C. Lane 2: fluorograph of inner membranes labeled with DTNB-EMA at 0° C. Lane 3: fluorograph of inner membranes labeled with DTNB-EMA in the presence of CAT and ADP at 0°C. Lane 4: inner membranes labeled with EMA alone in the presence of CAT and ADP. Lanes 5–8: SDS-PAGE of rat liver inner membranes labeled as in Lanes 1–4. Lane 9: molecular weight standards (bottom to top): lysozyme, soybean trypsin inhibitor, carbonic anhydrase, ovalbumin, bovine serum albumin, phosphorylase *b*.

TABLE I

Lateral diffusion coefficients of F_1F_0-ATP synthase, ADP/ATP translocator, and cytochrome bc_1

Complex	Probe	Diffusion coefficient (cm^2/s) [a]	Number of measurements
F_1F_0-ATP synthase	LR-IgG	$8.4\,(\pm 6.0)\cdot 10^{-10}$	48
ADP/ATP translocator	EMA	$1.7\,(\pm 1.2)\cdot 10^{-9}$	14
Cytochrome bc_1	LR-IgG	$4.4\,(\pm 3.0)\cdot 10^{-10}$	38

[a] Expressed as diffusion coefficient ±S.D.

Lateral diffusion coefficients of F_1F_0-ATP synthase and ADP/ATP translocator

The *D* values of LR-IgG-labeled F_1F_0-ATP synthase, LR-IgG-labeled cytochrome bc_1 complex, and EMA-labeled ADP/ATP translocator were determined by FRAP (Table I) in intact, fused inner membranes [2]. All *D* values were measured on inner membranes prepared on the same day, under similar experimental conditions. The *D* of cytochrome bc_1 complex, was the same as reported previously [2]. The *D* values of cytochrome bc_1 complex and F_1F_0-ATP synthase measured in frozen-thawed membranes were the same as in unfrozen membranes suggesting that the freezing and thawing process, required for labeling the ADP/ATP translocase, had no effect on the *D* of the translocator. (We note that the *D* values for the ADP/ATP translocator and the phosphate/H$^+$ transporter would be expected to be similar due to the similar molecular weights (approx. 30000 and 35000 for monomers, respectively) of these integral proteins and their dimeric nature [19]) Significantly, the average *D* of F_1F_0-ATP synthase was approximately twice that of cytochrome bc_1 complex while the *D* of the ADP/ATP translocator was approximately four times that of cytochrome bc_1 complex suggesting that all three components are independent two-dimensional diffusants.

Discussion

Proton transfer, ATP synthesis, collision frequencies and collision efficiencies in the inner membrane

The bimolecular, two-dimensional, diffusion-controlled collision frequencies and collision efficiencies between specific redox partners of the intact inner membrane have been determined previously using the experimental *D* values of the redox components [2,8]. The maximal rates of electron transfer between all redox partners were found to occur with a high degree of collision efficiency (electron turnovers per collision) suggesting that the overall process of mitochondrial electron transport includes the independent lateral diffusion and random collision of all redox components. These findings form the basis of the Random Collision Model of Electron Transport [1,2,21].

In light of these findings, we undertook to investigate the question of whether diffusion-based collisions between protein-pumping redox complexes and the F_1F_0-ATP synthase and ADP/ATP translocator (and/or phosphate/H$^+$ transporter) in the intact inner membrane could possibly account for the rate of mitochondrial oxidative phosphorylation. Various mechanisms of oxidative phosphorylation have been conceptualized in the past [22]. The most detailed concept is that protons are pumped by redox complexes across the inner membrane resulting in a bulk phase gradient of delocalized protons which is utilized by F_1F_0-ATP synthase during ATP synthesis [23–25]. Opposing concepts are that the redox complexes transfer protons within or on the membrane forming a localized proton gradient to be utilized by F_1F_0-ATP synthase [26–30]. Each of these concepts can account for some, but not for all of the available experimental results [11,30–32]. It is reasonable to consider that if the F_1F_0-ATP synthase, ADP/ATP translocator and phosphate/H$^+$ transporter diffuse laterally in the inner membrane, as do the redox complexes, a transfer of protons from the redox complexes to F_1F_0-ATP synthase for ATP synthesis, and from the redox complexes to ADP/ATP translocator (and or phosphate/H$^+$ transporter) for nucleotide transport, may occur through direct collisional events between the proton donor redox complexes and proton acceptor synthase and translocator components. Although the occurrence of independent two-dimensional diffusion and random collisions between redox complexes and synthase and translocator components may appear to support the concept of a localized proton mechanism for oxidative phosphorylation, such a diffusion-based collisional interaction would not obviate the concept of a delocalized proton mechanism.

Our measurements show that the average *D* of F_1F_0-ATP synthase is twice that of the cytochrome bc_1 complex while the *D* of the ADP/ATP translocator is four times that of cytochrome bc_1 complex, suggesting that these components are independent two-dimensional diffusants such that they do not co-diffuse as aggregates. With these findings as a basis, we considered the transfer of protons from redox complexes to the synthase and translocator through diffusion-based random collisions similar to the transfer of electrons in the Random Collision Model for Electron Transport [1,2,21]. It should be emphasized that if the idea of diffusion-based collisional proton transfer is to be functionally meaningful in terms of the rate control of oxidative phosphorylation, the frequency of collisions between the redox complexes and the synthase and

TABLE II

Diffusion-based collision frequencies and efficiencies of redox complexes (I, III, IV) and F_1F_0-ATP synthase (V) (upper panel), and redox complexes (I, III, IV) and ADP / ATP translocator (A) (lower panel) in proton transfer and ATP synthesis [a]

1 Complex	2 Lateral diffusion coefficient (cm^2/s)	3 Total concentration (molecules/cm^2)	4 Effective concentration (molecules/cm^2)	5 Radius of reactive area [b] (nm)	6 Theoretical diffusion-controlled collision frequency (collisions/ s per cm^2)	7 Theoretical diffusion-controlled collision frequency (collisions/ s per complex)	8 Electron transport-based turnover number (turnovers/ s per complex) [c,d]	9 Collisions/ turnover (% collision efficiency) [d]	10 Electrons transport-based turnover number (turnovers/ s per complex) [c,e]	11 Collisions/ turnover (% collision efficiency) [e]
V	$8.4 \cdot 10^{-10}$	$8.06 \cdot 10^{10}$	$4.03 \cdot 10^{10}$							
I	$7.2 \cdot 10^{-11}$ [f]	$2.69 \cdot 10^{10}$	$1.79 \cdot 10^{10}$	6.5	$1.07 \cdot 10^{12}$	V 56	18.8	3.0 (33)	18.8	3.0 (33)
						I 126	42	3.0 (33)	42	3.0 (33)
III	$4.4 \cdot 10^{-10}$	$8.06 \cdot 10^{10}$	$5.38 \cdot 10^{10}$	5.0	$1.07 \cdot 10^{13}$	V 266	26	10.3 (9.8)	13	20.5 (4.9)
						III 199	19	10.3 (9.8)	9.7	20.5 (4.9)
IV	$3.7 \cdot 10^{-10}$ [g]	$1.88 \cdot 10^{11}$	$1.25 \cdot 10^{11}$	5.0	$2.98 \cdot 10^{13}$	V 740	310	2.4 (42)	465	1.6 (63)
						IV 238	99	2.4 (42)	150	1.6 (63)
A	$1.7 \cdot 10^{-9}$	$5.95 \cdot 10^{11}$	$1.49 \cdot 10^{11}$							
I	$7.2 \cdot 10^{-11}$ [f]	$2.69 \cdot 10^{10}$	$1.79 \cdot 10^{10}$	6.25	$1.64 \cdot 10^{13}$	A 110	5	21.6 (4.6) [h]	5	21.6 (4.6)
						I 912	42	21.6 (4.6)	42	21.6 (4.6)
III	$4.4 \cdot 10^{-10}$	$8.06 \cdot 10^{10}$	$5.38 \cdot 10^{10}$	4.75	$7.2 \cdot 10^{13}$	A 484	7	69 (1.5)	3.5	138 (0.7)
						III 1339	19.4	69 (1.5)	9.7	138 (0.7)
IV	$3.7 \cdot 10^{-10}$ [g]	$1.88 \cdot 10^{11}$	$1.25 \cdot 10^{11}$	4.75	$2.03 \cdot 10^{14}$	A 1366	84	16.3 (6.1)	126	108 (9.2)
						IV 1621	99	16.3 (6.1)	150	108 (9.2)

[a] Theoretical two-dimensional collision frequencies and efficiencies are calculated as described in the text and detailed in Gupte et al. [2].

[b] Sum of the radii of the reactive areas of interacting components. Radii of redox components as in Gupte et al. [2]. The radius of Complex V is 2.5 nm based on F_0 and for dimeric ADP/ATP translocator the radius is 2.25 nm.

[c] Specific electron transport rates were employed for each individual site of oxidative phosphorylation. Experimental rates of coupled electron transport for NADH oxidase at 8.06 e^-/s per heme aa_3 and succinate oxidase at 11.1 e^-/s per heme aa_3 were calculated from the experimental rates in Table III of Lemasters and Billica ([34] based on a determination of 0.62 nmoles heme *a*/mg protein for inverted inner membranes). Rates for cytochrome oxidase at 96 e^-/s per heme aa_3 were derived from Table I of Schwerzmann et al. [35]. These coupled rates were used to calculate the rates of ATP synthesis and ADP/ATP translocation.

[d] Based on the average stoichiometry of 1 ATP for every 2 e^- for each site.

[e] Based on the site stoichiometries detailed in assumption (i) in the Discussion, i.e. 1 ATP/2 e^- at Site I, 0.5 ATP/2 e^- at Site II and 1.5 ATP/2 e^- at Site III.

[f] *D* of Complex I from Chazotte and Hackenbrock [33].

[g] *D* of Complex IV from Gupte et al. [2].

[h] Comparable calculations for the phosphate/H^+ transporter yield collision efficiencies of 6.9%, 4.32%, 7.86% with Complexes I, III and IV, respectively. The effective concentration was calculated from a value of 1.4 nmol transporter per nmol cytochrome *b* [40] and the appropriate assumptions given in the Discussion.

translocator components must be greater than, or close to, the frequency of ATP synthesis.

To test a diffusion-based, random collision hypothesis for proton transfer and ATP synthesis, we followed the rational for our random collision model of electron transport. We previously measured the *D* values of Complex III (cytochrome bc_1) and Complex IV (cytochrome oxidase) to be $4.4 \cdot 10^{-10}$ cm^2/s and $3.7 \cdot 10^{-10}$ cm^2/s, respectively [2], and more recently of Complex I to be $7.2 \cdot 10^{-11}$ cm^2/s [33]. With the lateral diffusion rates of these redox components and the F_1F_0-ATP synthase established, and the rate of ATP synthesis at each of the three proton transferring sites obtained from the literature [34,35], we applied the Hardt equation for two-dimensional diffusion [36], and obtained the theoretical collision frequencies and collision efficiencies between each of the three proton donating redox complexes and the proton-accepting F_1F_0-ATP synthase at *each* of the proton transferring sites (Table II). Similarly, we calculated the collision frequencies and collision efficiencies for proton transfer between each redox complex and the ADP/ATP translocator (detailed calculations for the phosphate/H$^+$ which are similar to the translocator are not presented, see Table II footnote h).

Assumptions for the calculation of the theoretical collision frequencies and efficiencies of proton transfer and ATP synthesis

(a) Each of the three proton-transferring redox complexes exists as monomers in the native inner membrane (as opposed to in two-dimensional protein crystals) in one of three conformations in equal amounts: (i) non-energized, (ii) energized-reduced, and (iii) energized-oxidized. The two energized conformations of each redox complex are capable of storing and transferring protons. Thus, the effective concentration of each of the redox complexes that can donate protons is 66.7% of its total concentration.

(b) F_1F_0-ATP synthase occurs as a monomer in either an energized (with protons) or non-energized (without protons) conformation in equal amounts. Thus, the effective concentration of F_1F_0-ATP synthase that can accept protons is 50% of its total concentration.

(c) ADP/ATP translocator (and phosphate/H$^+$ transporter) occurs as a dimer in either an energized (with protons) or non-energized (without protons) conformation in equal amounts. Thus, the effective concentration of ADP/ATP translocator that can accept protons is 25% of its total concentration.

(d) Cognizant of the long standing controversies on the site stoichiometries, e.g. Lemasters et al. [39], we considered two such possibilities. (1) Each redox complex sequesters 4 charges on average per 2 e$^-$ transferred and stores a variable number of protons at each site. (2) Complex I sequesters 4 net charges per 2 e$^-$ for 1 ATP synthesized; Complex III sequesters 2 net charges per 2 e$^-$ for 0.5 ATP synthesized; Complex IV sequesters 6 net charges per 2 e$^-$ for 1.5 ATP synthesized; all of which considers the effect of substrate (scalar) protons.

(e) A variable number of protons is transferred from each redox complex to an F_1F_0-ATP synthase with each collision.

(f) Of these, three protons are dissipated from the F_1F_0-ATP synthase into the mitochondrial matrix per one ATP synthesized.

(g) A variable number of protons is transferred from each redox complex to an ADP/ATP translocator, (and/or a phosphate/H$^+$ transporter) during each collision of a redox complex with the translocator (and/or the transporter) *.

(h) Of these, one proton is dissipated from the translocator (or transporter) with the transport of a P_i + ADP into the matrix and removal of an ATP from the matrix.

(i) Considering the two aforementioned possibilities (c.f. assumption d) the site stoichiometries can be expressed as: (1) an average of 4 net charges/4 H$^+$/2 e$^-$/ATP at each site, (2) Site I: 4 net charges/4 H$^+$/2 e$^-$/ATP; Site II: 2 net charges/4 H$^+$/2 e$^-$/0.5 ATP;

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* For this calculation we hypothesize the existence of proton utilization in the direct collision of the ADP/ATP translocator or phosphate/H$^+$ transporter with redox complexes. It is believed that the electroneutral 1:1 exchange of internal ATP for external ADP requires the movement of a charge, e.g. a proton into the mitochondrion [37]. Although the ADP/ATP translocator has not been shown to *directly* utilize protons in the (1:1 ADP:ATP) translocation reaction, it is known that when the mitochondrion is energized ADP/ATP translocation is directionally selective, by a factor of approx. 20, in the proper physiological direction [37,38] as opposed to a nonenergized state where ADP or ATP are translocated in either direction in equilibrium fashion. Reconstitution studies have been done with the ADP/ATP translocator alone reincorporated into liposomes with 5% being fully functional in the native configuration, approx. 25% inverted and approx. 70% denatured [38]. The authors found in this case that the ADP/ATP translocation carried out by the 5% of these translocators was linearly related to the applied (valinomycin-potassium) membrane potential and that the pH gradient has a small affect. However, with this 5% functional population the directionality was only affected 3-fold by the applied potassium diffusion potential and not the 20-fold of the native system [38]. Thus it is conceivable that the energized redox proteins might have some direct effect on the ADP/ATP translocator or the phosphate/H$^+$ transporter, e.g. a direct collisional transfer of H$^+$ occurring within the membrane, to control this directionality. Directionality control is also of interest since the size of the adenine nucleotide pool in the mitochondrion is not normally altered during oxidative phosphorylation [37] such that ADP must enter the matrix at a rate comparable to the synthesis of ATP. Therefore we are testing whether collisional interactions are sufficiently rapid to account for an energized proton-donating redox complex directly affecting the ADP/ATP translocator and/or the phosphate/H$^+$ transporter (c.f. Fig. 4) during oxidative phosphorylation.

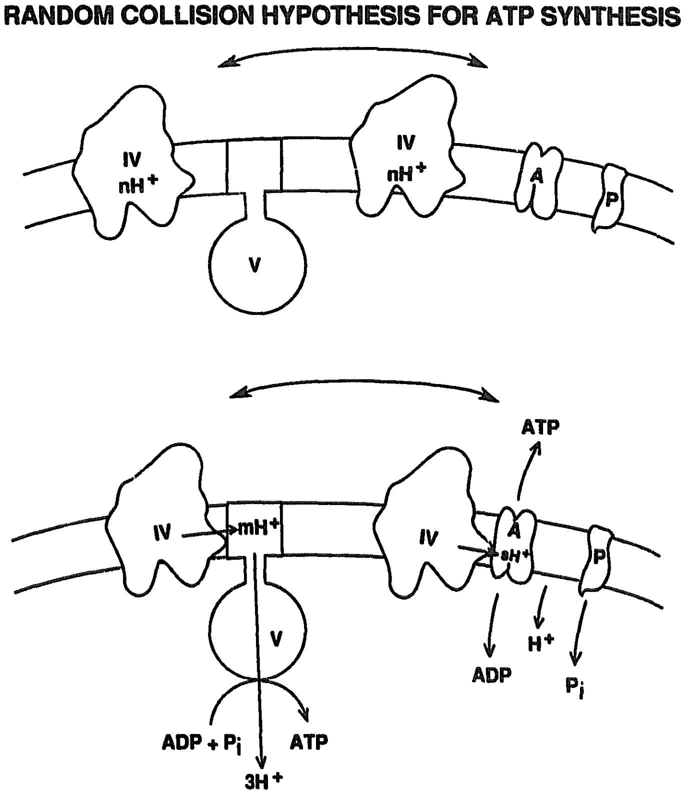

Fig. 4. Random collision hypothesis of proton transfer and ATP synthesis. Top: cytochrome oxidase (IV) is shown in an energized conformation capable of sequestering and storing a variable number of protons (nH^+). Bottom: collision of energized cytochrome oxidase molecules with a non-energized ATP synthase (V) molecule and with an ADP/ATP translocator (A) molecule resulting in a variable number of protons transferred to the synthase (mH^+) and to the translocator (sH^+). Four protons are dissipated into the matrix along with the transport of one molecule each of ADP and P_i during the synthesis of one molecule of ATP.

Site III: 6 net charges/4 H^+/2 e^-/1.5 ATP. In any case the hypothesis is meant to be more mechanistic than stoichiometric and, as can be seen, neither possibility precludes the viability of the proposed mechanism.

These assumptions and the hypothesis for proton transfer and subsequent ATP synthesis are presented diagramatically in Fig. 4. Redox complexes, F_1F_0-ATP synthase (Complex V), ADP/ATP translocator (and presumably phosphate/H^+ transporter) diffuse two dimensionally and independently in the plane of the membrane and collide randomly with each other. In Fig. 4, (upper panel) two cytochrome oxidase (Complex IV) molecules are in an energized conformation capable of storing a variable number of protons (nH^+). When a Complex IV and a Complex V collide (lower panel) a variable number of protons (mH^+) from the energized Complex IV molecule can be transferred to a non-energized Complex V molecule. Three of these protons are dissipated into the mitochondrial matrix during the synthesis of one ATP from ADP and P_i. Also shown is an energized Complex IV molecule as it collides with an ADP/ATP translocator molecule with one proton dissipated into the matrix along with one each of ADP and Pi during the synthesis of one ATP.

Our lateral diffusion coefficients and calculations derived thereof (Table II) are consistent with the possibility that proton transport, and consequently ATP synthesis, is coupled to diffusion-based random collisions between the proton donating redox complexes and proton accepting, F_1F_0-ATP synthase and ADP/ATP translocator (and/or phosphate/H^+ transporter). The efficiency of collisions between the redox complexes and F_1F_0-ATP synthase is calculated from the experimental data to average 28% and between the redox complexes and ADP/ATP translocator dimer, to average 4.1% for the average, unit stoichiometry (Column 9) and slightly higher (34% and 5.8%, respectively) for one of the more complex, non-unit stoichiometries (Column 11) we considered. It should be noted that calculated collision efficiencies greater than 100% would preclude a purely diffusion-based mechanism.

In summary, our calculated collision efficiencies between the redox complexes and the F_1F_0-ATP synthase and ADP/ATP translocator, calculated from experimentally determined lateral diffusion coefficients, can support a diffusion-based, random collision process of proton transfer and ATP synthesis in the mitochondrial inner membrane. This obtains irrespective of the most widely discussed site stoichiometries for ATP synthesis. Also, our diffusion measurements clearly indicate that both proton donors and acceptors are mobile in the plane of the mitochondrial inner membrane.

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