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The Random Collision Model and a Critical Assessment of Diffusion and Collision in Mitochondrial Electron Transport

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Abstract

This review focuses on our studies over the past ten years which reveal that the mitochondrial inner membrane is a fluid-state rather than a solid-state membrane and that all membrane proteins and redox components which catalyze electron transport and ATP synthesis are in constant and independent diffusional motion. The studies reviewed represent the experimental basis for the *random collision model* of electron transport. We present five fundamental postulates upon which the random collision model of mitochondrial electron transport is founded: (1) All redox components are *independent lateral diffusants*; (2) Cytochrome *c* diffuses primarily in *three dimensions*; (3) Electron transport is a *diffusion-coupled* kinetic process; (4) Electron transport is a *multicollisional, obstructed, long-range* diffusional process; (5) The rates of diffusion of the redox components have a direct influence on the overall kinetic process of electron transport and can be *rate limiting*, as in *diffusion control.* The experimental rationales and the results obtained in testing each of the five postulates of the random collision model are presented. In addition, we offer the basic concepts, criteria and experimental strategies that we believe are essential in considering the significance of the relationship between diffusion and electron transport. Finally, we critically explore and assess other contemporary studies on the diffusion of inner membrane components related to electron transport including studies on: rotational diffusion, immobile fractions, complex formation, dynamic aggregates, and rates of diffusion. Review of all available data confirms the random collision model and no data appear to exist that contravene it. It is concluded that mitochondrial electron transport is a diffusion-based random collision process and that diffusion has an integral and controlling affect on electron transport.

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Introduction

Ten years have passed since a comprehensive review of the research from this laboratory on the structure and fluid nature of the mitochondrial inner membrane was published based on a lecture given at the 1976 Nobel Foundation Symposium on the Structure of Biological Membranes (Hackenbrock, 1976). This review put into perspective our observations that cytochrome oxidase and other proteins integral to the inner membrane diffuse laterally and randomly in the membrane plane. These findings prompted us to question the popular notion that a physically associated, macromolecular assembly or ordered (respiratory) chain of electron-transferring components is required for the rapid and sequential transfer of electrons catalyzed in the mitochondrial inner membrane. Based primarily on this early work involving lateral diffusion, the summary of our 1976 review on the mitochondrial inner membrane concluded:

> "The fluid environment provides for lateral translational and rotational mobility of the integral metabolically active membrane proteins which can diffuse laterally, depending on the specific metabolic role, either independent of or in association with other integral proteins. Cytochrome *c* oxidase, a major integral metabolically active protein which occurs in the membrane as a completely transmembraneous polymer, can undergo lateral translational diffusion independent of some, and in association with other, integral proteins. In biogenesis, lateral motional freedom of integral proteins in the energy transducing membrane may permit diffusion of newly incorporated proteins to sites of functional activity. In metabolic functions, lateral motional freedom can account for a diffusional component in the mechanism of electron transport in various segments of the respiratory chain as well as in the mechanism of oxidative phosphorylation."

Thus, ten years ago our model for the relationship of electron transport to membrane structure and lateral diffusion stated that (1) all proteins of the mitochondrial inner membrane *diffuse laterally*, (2) cytochrome oxidase diffuses *independently or* in *association* with other integral proteins, and (3) such lateral diffusion is *intrinsic* to the mechanism of electron transport as well as oxidative phosphorylation.

It is not our intention to review the details of the many studies which led to our views and our model of ten years ago (the reader is referred to Hackenbrock, 1976 for specific studies). Rather, we will focus on our studies since 1976 and the basic postulates upon which our model, now more commonly known as the *random collision model* of mitochondrial electron

transport, is founded. In addition, since diffusion *per se* and the role of diffusion of the membrane components that mediate electron transport and energy conservation are rapidly growing subjects of interest in bioenergetics generally, we shall present some basic concepts, criteria, and experimental strategies that we believe are essential to the discussion of the significance of diffusion in these important metabolic functions. Finally, we will explore, critically, studies from several other laboratories on diffusion of inner membrane components which have appeared since our 1976 review.

Random Collision Model of Electron Transport

The *random collision model* of mitochondrial electron transport (Fig. 1) was formulated gradually from the assessment of our earlier ultrastructural observations (reviewed in Hackenbrock, 1976), combined with our data generated within the past ten years on the reaction and diffusion kinetics of redox components (Höchli and Hackenbrock, 1976, 1977, 1979; Schneider *et al.*, 1980a, b, 1982a, b; Hackenbrock, 1981; Sowers and Hackenbrock, 1981, 1985; Chazotte *et al.*, 1983a, b; Gupte *et al.*, 1984; Höchli *et al.*, 1985; Hackenbrock *et al.*, 1985, 1986). The model, which has been discussed in detail recently (Hackenbrock *et al.*, 1985), rejects the notion that permanent assemblies or transient aggregates of redox components in the inner membrane are necessary to account for the sequence or rate of electron transport.

Five fundamental postulates upon which the random collision model of mitochondrial electron transport is founded can be summarized as follows:

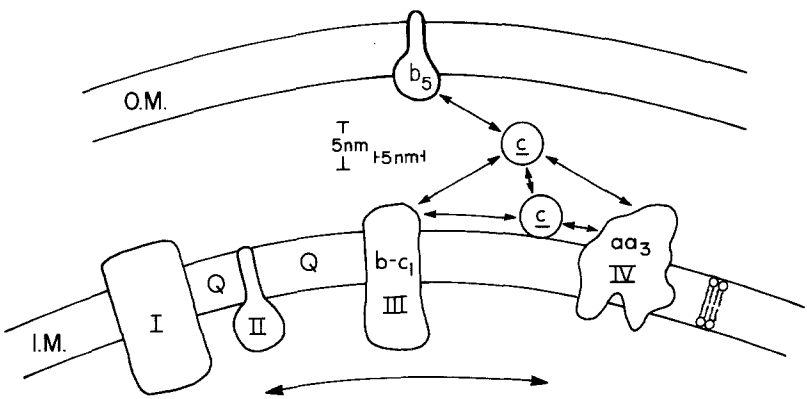

Fig. 1. The random collision model (to scale) of mitochondrial electron transport. O.M., outer membrane; I.M., inner membrane; b_5, cytochrome b_5; I, II, III, IV, complexes I, II, III, IV; *c*, cytochrome *c*; Q, ubiquinone.

(1) All redox components are *independent lateral diffusants.*
(2) Cytochrome *c* diffuses primarily in *three dimensions.*
(3) Electron transport is a *diffusion-coupled* kinetic process.
(4) Electron transport is a *multicollisional, obstructed, long-range* diffusional process.
(5) The rates of diffusion of the redox components have a direct influence on the overall kinetic process of electron transport and can be *rate limiting*, as in *diffusion control.*

The random collision model as currently stated represents an important refinement of our early model of 1976 in two major ways. First, the model reflects our more recent finding that cytochrome *c* diffuses in different modes depending on ionic strength: laterally and pseudo-laterally on the membrane when ionic strength is low, and primarily in three dimensions when ionic strength is high, i.e., physiological (Gupte *et al.*, 1984; Hackenbrock *et al.*, 1985). Second, the model reflects our recent finding that cytochrome oxidase diffuses as a single entity and not in permanent association or transient aggregation with cytochrome b–c_1 complex (Höchli *et al.*, 1985).

There is nothing less valuable in science than an untestable model. In developing the random collision model, we kept in mind the criterion that a fundamentally useful model must lend itself well to the testing of its postulates through experimentation. Thus, by testing our early model through additional studies, we rejected our original notions that cytochrome *c* diffuses in only a lateral mode and that cytochrome oxidase diffuses in an associated or aggregated state.

The following represents the experimental rationales and the results obtained in testing each of the five postulates of the random collision model of mitochondrial electron transport.

Postulate 1. *All redox components are independent lateral diffusants.* We include as all redox components: NADH-ubiquinone oxidoreductase (Complex I), succinate-ubiquinone oxidoreductase (Complex II), ubiquinol-cytochrome *c* oxidoreductase (cytochrome b–c_1, Complex III), cytochrome oxidase (Complex IV), cytochrome *c*, ubiquinone, and a number of other membrane-bound electron transferring flavoproteins, e.g., α-glycerophosphate-, choline-, proline-, and dihydroorotate-dehydrogenases, etc. We also presume nonredox proteins, necessary for the generation of ATP, e.g., ADP-ATP transporter, ATP-synthetase, etc., to be lateral diffusants (c.f. Slater *et al.*, 1985; Rottenberg, 1985).

By 1976 we had combined the technology of differential scanning calorimetry and freeze-fracture electron microscopy to show that the total protein of the inner membrane occupies only one-third to one-half of the membrane surface area (Hackenbrock *et al.*, 1976). This was verified

quantitatively more recently by the finding that the inner membrane proteins migrate into a single patch in the membrane plane under the influence of a strong electric field, leaving 50–60% of the membrane surface area unoccupied by proteins (Sowers and Hackenbrock, 1981). Thus, as a first approximation, 50% or more of the surface area of the native inner membrane is lipid bilayer representing "space" unoccupied by membrane proteins and through which such proteins are free to diffuse laterally.

Our earlier ultrastructural observations that cytochrome oxidase is randomly distributed over the surface of the inner membrane (Hackenbrock and Hammon, 1975) and that all integral proteins in the membrane diffuse laterally as a result of thermotropic lipid phase transitions (Hackenbrock *et al.*, 1976; Höchli and Hackenbrock, 1976, 1977) were sufficient reason to suggest that cytochrome oxidase is a lateral diffusant. The subsequent finding that cytochrome oxidase aggregates into planar clusters in the native membrane as a function of time in the presence of antibody monospecific for the oxidase is significant evidence that cytochrome oxidase is in continuous lateral motion in the membrane and collides randomly with others of its own kind and presumably with all other proteins (Höchli and Hackenbrock, 1979). The use of antibodies as probes of free lateral diffusion and random collision of membrane proteins also resolved recently that cytochrome oxidase and cytochrome b–c_1 complex diffuse independently of one another (Höchli *et al.*, 1985).

The development in our laboratory of a low pH liposome-membrane fusion technique (Schneider *et al.*, 1980a) was a major breakthrough in examining kinetically the independent diffusion of *all* the major redox components in the electron transport sequence. Studies of inner membranes fused with exogenous soybean phospholipid reveal that the rate of electron transfer between the interacting redox partners is clearly dependent on the concentration (two dimensional density) of the interacting partners as would be expected in diffusion-based, bimolecular reactions (Schneider *et al.*, 1980b; Hackenbrock, 1981). Of fundamental significance was the use of freeze-fracture observations which clearly reveal that the average distance between integral proteins increases while electron transfer rates between the redox partners decrease proportional to the degree that the membrane is enriched with exogenous phospholipid. Furthermore, inner membrane enrichment with phospholipid plus ubiquinone restores substantially the decreases in NADH- and succinate-cytochrome c reductase activities observed in membranes enriched with phospholipid alone (Schneider *et al.*, 1982a). Finally, consistent with diffusion-based electron transfer reactions, NADH- and succinate-cytochrome c reductase activity increases when the average distance between the integral proteins of the membrane is decreased (Schneider *et al.*, 1982b).

The preceding summarizes key ultrastructural observations and reaction kinetics which reveal the independent lateral motion of the redox components in the inner membrane. Recently we have taken a more direct approach in assessing the lateral diffusion of redox components utilizing the technique of fluorescence recovery after photobleaching (FRAP).[3] (For details of technique see Jacobson *et al.*, 1976 and Gupte *et al.*, 1984). Using FRAP, we ascertained directly the existence of lateral diffusion and determined the diffusion coefficients of cytochrome *c*, cytochrome oxidase, cytochrome b–c_1 complex, and ubiquinone (Gupte *et al.*, 1984) as well as inner membrane phospholipid (Chazotte *et al.*, 1983a, b; Hackenbrock *et al.*, 1985). The fluorescence recoveries for the transmembrane redox protein complexes and a ubiquinone analogue are greater than 90% in each case, indicating no significant immobile fraction, and are characteristically unicomponent, indicating that all individual components diffuse laterally in a common pool. Cytochrome *c* diffuses in different modes depending on ionic strength and will be considered in detail under Postulate 2. Rat liver mitochondrial inner membranes isolated by the digitonin method (Schnaitman and Greenwalt, 1968) and subsequently fused to give ultralarge, single inner membranes (Chazotte *et al.*, 1985), or inner membranes derived by swelling cuprizone-induced mouse liver megamitochondria, give the same lateral diffusion coefficients (Gupte *et al.*, 1984). Other laboratories have reported on the lateral diffusion of cytochrome *c* (Hochman *et al.*, 1982; 1985; Vanderkooi *et al.*, 1985), cytochrome oxidase (Hochman *et al.*, 1985), ubiquinone analogue (Lenaz *et al.*, 1985, 1986), and phospholipid (Hochman *et al.*, 1985; Vanderkooi *et al.*, 1985). The lateral diffusion coefficients *D* reported for inner membrane components are given in Table I.

Postulate 2. *Cytochrome c diffuses primarily in three dimensions.* Cytochrome *c* is not integral to the inner membrane but interacts electrostatically with the membrane surface. It is known to be dissociated from the membrane at physiological (150 mM) ionic strength (Jacobs and Sanadi, 1960). Depending on ionic strength, cytochrome *c* diffuses laterally, pseudo-laterally, or three dimensionally with respect to the membrane surface (Hackenbrock

[3]Abbreviations: *D*, diffusion coefficient; FRAP, fluorescence recovery after photobleaching; DPH, 1,6-diphenyl-1,3,5-hexatriene; RET, resonance energy transfer; V_{max}, Michaelis maximum velocity; K_m, Michaelis constant; State 3U, uncoupled mitochondrial electron transport; P, poise; cP, centipoise; E_a, apparent activation energy; I, Complex I; II, Complex II; III, Complex III; IV, Complex IV; Q, ubiquinone, coenzyme Q; Q_0C_{10}NBD, NBD hexanoic acid fluorophore conjugated to 2,3-dimethyl-5-methyl-6-(10-hydroxydecyl)quinone; D_r, rotational diffusion coefficient; Q_3, ubiquinone 3; 12-AS, 12-(9-anthroyl)stearic acid; 16-NS, 16-(*N*-oxyl-4′,4′-dimethyloxazolidine)stearic acid; DiI, dihexyl- or dioctyldecylindocarbocyanine fluorescent lipid analogue; NBD, *N*-4-nitrobenz-2-oxa-1,3-diazole fluorophore; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone.

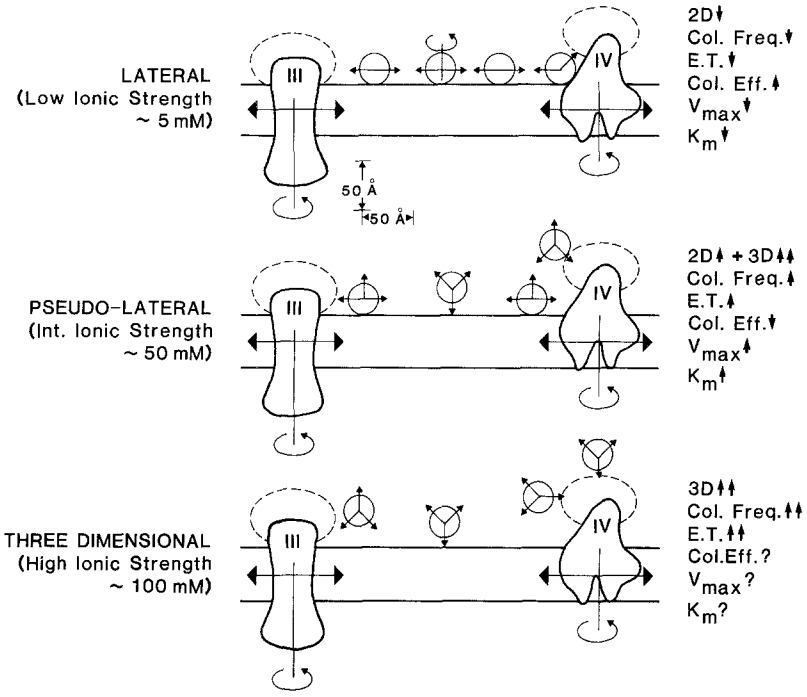

Fig. 2. Model (to scale) of the mode of diffusion of cytochrome *c* (small circles) related to rate of electron transport as a function of ionic strength. III, Complex III; IV, Complex IV; dashed halos represent electric fields of Complexes III and IV; arrows at right indicate lower (↓), higher (↑), and highest (↑↑) rates or quantities; note that Complexes III and IV diffuse in two dimensions while cytochrome *c* diffuses in two and/or three dimensions depending on ionic strength. All three redox components undergo rotational diffusion.

et al., 1985). The random collision model is detailed to show the three modes of diffusion of cytochrome *c* in Fig. 2.

Our FRAP studies reveal that cytochrome *c* exchanges slowly with the surrounding aqueous phase concomitant with its lateral diffusion on the membrane surface (Gupte *et al.*, 1984). More appropriately, this mode of diffusion is termed *pseudo-lateral* (Hackenbrock *et al.*, 1985). At lowest ionic strength (~5 mM) the affinity of cytochrome *c* for the membrane surface is highest, resulting in a predominantly true lateral diffusion with a low apparent *D* (Table II). As ionic strength is increased, the affinity of cytochrome *c* for the membrane decreases, which results in a predominantly pseudo-lateral diffusion and higher apparent *D*'s. At 56 mM ionic strength the apparent *D* for cytochrome *c* at a concentration physiologically stoichiometric with its interacting redox partners is 1.9×10^{-9} cm^2/sec. Above approximately 60 mM ionic strength, the membrane–cytochrome *c*

Table I. *Lateral Diffusion Coefficients of Mitochondrial Inner Membrane Components*

Component	Membrane/source	Probe	Technique	$D \times 10^{10}$ (cm^2/sec)	Recovery (%)	Temperature (°C)	μ^a (mM)	Reference
Complex III	FIM[b] rat liv.	TMRITC-IgG[c]	FRAP	4.4	> 90	23	73	Gupte *et al.* (1984)
	FIM	TMRITC-Fab[c]	FRAP	4.4	> 90	23	73	Gupte *et al.* (1984)
	FIM	TMRITC-IgG	FRAP	4.8	> 90	23	0.3	Gupte *et al.* (1984)
	FIM	TMRITC-IgG	FRAP	6.9	> 90	25	0.3	Hackenbrock *et al.* (1985)
	MEGA[d] m. liv.	TMRITC-IgG	FRAP	3.6	> 90	23	73	Gupte *et al.* (1984)
Complex IV	FIM	TMRITC-IgG	FRAP	3.6	> 90	23	73	Gupte *et al.* (1984)
	FIM	TMRITC-Fab	FRAP	3.7	> 90	23	73	Gupte *et al.* (1984)
	MEGA m. liv.	MRITC-IgG[e]	FRAP-E[f]	1.5	> 85	—	8	Hochman *et al.* (1985)
Integral membrane proteins	IMM[g] rat. liv.	—	FFE[h]	8.3	—	20	—	Sowers and Hackenbrock (1981)
	IMM	—	FFE	1.3–33.5	—	20	—	Sowers and Hackenbrock (1985)
CYTO *c*[i]	FIM	FITC-CYT. *c*[j]	FRAP	0.59	> 85	23	0.3	Gupte *et al.* (1984)
	FIM	FITC-CYT. *c*	FRAP	2.7	> 93	23	23	Gupte *et al.* (1984)
	FIM	FITC-CYT. *c*[k]	FRAP	19	> 83	23	56	Gupte *et al.* (1984); Hackenbrock *et al.* (1985)
	PD-MITO L.I.[l]	PORPH. *c*[m], unwashed[n]	FRAP	0.62	> 95	—	0	Vanderkooi *et al.* (1985)
	PD-MITO L.I.	PORPH. *c*[n]	FRAP	1.6	88	—	25	Vanderkooi *et al.* (1985)
	PD-MITO L.I.	PORPH. *c*[n]	FRAP	3.3	97	—	50	Vanderkooi *et al.* (1985)
	PD-MITO L.I.	PORPH. *c*[n]	FRAP	3.2	96	—	100	Vanderkooi *et al.* (1985)
	PD-MITO L.I.	PORPH. *c*[k]	FRAP	1.6	60	—	0	Vanderkooi *et al.* (1985)
	PD-MITO L.I.	PORPH. *c*	FRAP	1.6	62	—	25	Vanderkooi *et al.* (1985)
	MEGA m. liv.	TMRITC *c*	FRAP-E	1.6	> 90	—	8	Hochman *et al.* (1982)
	MEGA m. liv.	TMRITC *c*	FRAP-E	3.8	89	—	8	Hochman *et al.* (1985)

	MEGA m. liv.	MRITC c^o	FRAP-E	3.5	92	—	8	Hochman *et al.* (1985)
	MEGA m. liv.	MRITC *c*	FRAP-E	7.4	84	—	25	Hochman *et al.* (1985)
Ubiquinone	FIM	$Q_0C_{10}NBD^p$	FRAP	26	> 90	21	0.3	Gupte *et al.* (1984)
	MEGA m. liv.	$Q_0C_{10}NBD$	FRAP	37	> 90	21	0.3	Gupte *et al.* (1984)
	SMP Beef heart	Q3 and 12-AS[q]	Fl. Quenc.[r]	60000	—	25	—	Lenaz *et al.* (1986)
Phospholipid	FIM	DiI[s]	FRAP	39	> 95	21	0.3	Chazotte *et al.* (1983a); Hackenbrock *et al.* (1985)
	FIM	NBD-PE[t]	FRAP	68	> 95	21	0.3	Chazotte *et al.* (1983b); Chazotte and Hackenbrock (1984)
	MEGA m. liv.	DiI	FRAP	33	> 90	21	73	Hackenbrock *et al.* (1985)
	MEGA m. liv.	NBD-PE	FRAP-E	60	—	—	8	Hochman *et al.* (1985)
	PD-MITO L.I.	DiI	FRAP	20	—	—	—	Vanderkooi *et al.* (1985)

[a] μ, ionic strength in mM units.
[b] FIM, fused, ultralarge mitochondrial inner membranes from rat liver.
[c] TMRITC-IgG or -Fab, tetramethylrhodamine isothiocyanate-conjugated rabbit IgG or Fab.
[d] MEGA, megamitochondria isolated from livers of cuprizone-fed mice.
[e] MRITC, morpholinorhodamine isothiocyanate-conjugated IgG.
[f] FRAP-E, fluorescence recovery after photobleaching by the edge technique.
[g] IMM, mitochondrial inner membrane-matrix (mitoplasts) from rat liver.
[h] FFE, freeze fracture–electrophoresis.
[i] Apparent diffusion coefficients (see text).
[j] FITC, fluorescein isothiocyanate-conjugated cytochrome *c* from horse heart.
[k] At stochiometric concentration: cytochrome *c*/cytochrome oxidase (2/1).
[l] PD-MITO L.I., mitochondria from flight muscle of *Lethocerus indicus* with partly damaged outer membrane.
[m] PORPH c, fluorescent cytochrome *c* analogue (i.e., iron free).
[n] Not washed after labeling, as opposed to other cytochrome *c* studies.
[o] MRITC c, morpholinorhodamine isothiocyanate conjugated cytochrome *c*.
[p] $Q_0C_{10}NBD$, fluorescent ubiquinone analogue; NBD hexanoic acid conjugated to 2,3-Dimethyl-5-methyl-6-(10-hydroxyldecyl)quinone.
[q] Ubiquinone-3 and 12-(9-anthroyl)stearic acid quenching pair.
[r] Fl. Quenc, fluorescence quenching technique.
[s] DiI, fluorescent lipid analogue. Dioctyl- or dihexyldecylindocarbocyanine.
[t] NBD-PE, fluorescent lipid phospholipid. *N*-4-nitrobenz-2-oxa-1,3-diazole conjugated to phosphatidylethanolamine.

Table II. Diffusion of Cytochrome *c*

Ionic strength (mM)	FITC-cyto. *c* added (μM)	Cyto. *c* bound to membranes (nmol/mg protein)	Ratio of cyto. *c*/cytochrome oxidase	Diffusion coefficient (cm^2/sec^a)	Fluorescence recovery (%)
0.3	2	0.38	4.0	5.0×10^{-11}	79
	10	—	—	5.1×10^{-11}	90
	20	3.5	36.8	5.3×10^{-11}	96
23	3	—	—	2.6×10^{-10}	96
	10	—	—	3.1×10^{-10}	93
	20	0.68	7.2	2.2×10^{-10}	94
	10	—	—	1.8×10^{-9}	83
56	20	0.2	2.1	1.9×10^{-9}	86

[a]Apparent.

association occurs for too short a time to be detected by FRAP, suggesting that cytochrome *c* diffuses predominantly in *three dimensions* at higher ionic strengths.

It is important to determine precisely the mode of cytochrome *c* diffusion at higher ionic strengths because the cytoplasmic, free K^+ and Cl^- concentrations are 100–150 mM (Hille, 1984). Since the outer mitochondrial membrane is ion permeable, a reasonable assumption is that the free ion concentration in the mitochondrial intermembrane space, in which cytochrome *c* resides, is also 100–150 mM. Thus cytochrome *c* would be expected to be free in solution in the mitochondrial intermembrane space in the intact cell. The volume of the intermembrane space, hence the free cytochrome *c* concentration, varies dramatically as a function of respiratory state. In the condensed configuration, typical of mitochondria freshly isolated or in State 3 (Hackenbrock, 1966, 1968a), this space represents one-half of the total mitochondrial volume (Hackenbrock, 1968b), giving a cytochrome *c* concentration of 100 μM (Hackenbrock *et al.*, 1985). In the orthodox configuration, the volume of the intermembrane space decreases by a factor of approximately 7 (Lemasters, 1978), giving a cytochrome *c* concentration of $\sim$700 μM. Even at this high concentration, cytochrome *c* occupies only 0.6% (v/v) of the intermembrane space. The rate of three-dimensional diffusion of cytochrome *c* would be similar to lysozyme due to their comparable size and shape. For three dimensions, the diffusion coefficient of lysozyme at 700 μM concentration and 150 mM ionic strength has been determined to be 1.4×10^{-6} cm^2/sec (Barisas and Leuther, 1979; Cadman *et al.*, 1981).

To determine the mode of diffusion of cytochrome *c* with respect to the membrane surface at high ionic strengths, we have used resonance energy

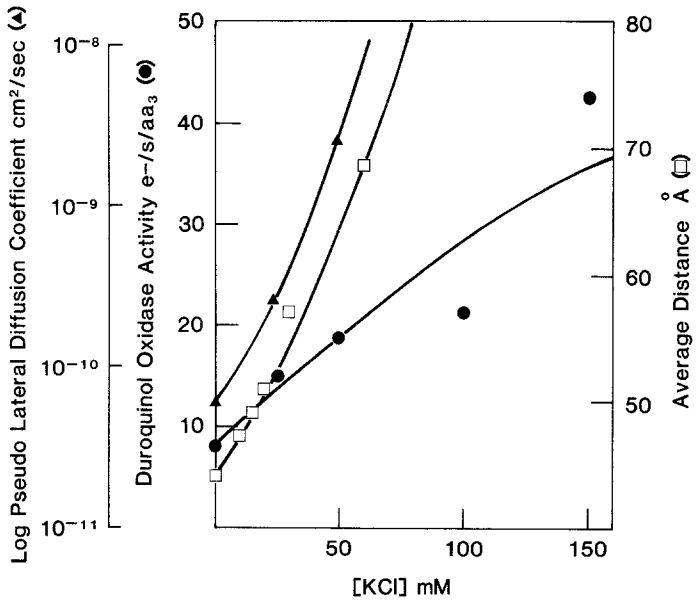

Fig. 3. The pseudo-lateral diffusion of FITC-cytochrome *c* (▲) measured by FRAP; average distance between membrane DPH and cytochrome *c* heme measured by RET (□); and duroquinol oxidase activity (●) of inner membranes as a function of ionic strength. Duroquinol oxidase activity was measured polarographically in the presence of 0.6 mM tetramethyl-*p*-benzoquinone (duroquinol), 1 μM CCCP, 5 μM rotenone, 100 μM horse heart cytochrome *c*, and 40 μg membrane protein at different KCl concentrations, pH 7.4, 300 mosm.

transfer (RET) (Stryer, 1978) to measure the average distance of the *c* heme from the fluorescent probe, 1,6-diphenyl-1,3,5-hexatriene (DPH) incorporated into the bilayer of the inner membrane (Hackenbrock *et al.*, 1985). The average distance between the *c* heme at the membrane surface and DPH in the lipid bilayer increases from 4.3 nm at zero ionic strength to greater than 10 nm at ionic strengths higher than 90 mM. It is especially significant that the V_{max} of cytochrome *c*-mediated duroquinol oxidase and cytochrome oxidase activities, as well as the K_m of cytochrome *c* in these reactions (Table III), increase parallel with the increasing pseudo-lateral diffusion (determined by FRAP) and increasing distance between cytochrome *c* and the membrane surface (determined by RET) which occurs as a function of increasing ionic strength (Fig. 3). Indeed, the activity of duroquinol oxidase in intact mitochondria parallels the increase in activity in inner membranes as ionic strength is increased (Fig. 4).

It follows that maximum electron transfer activity by cytochrome *c* occurs at physiological (150 mM) ionic strength in isolated inner membranes and intact mitochondria when cytochrome *c* is in a state of three-dimensional

Table III. Ionic Strength Dependence of Cytochrome *c*-Mediated Electron Transfer

			Cytochrome oxidase activity			
	Duroquinol oxidase activity		High affinity		Low affinity	
Ionic strength	K_m (cyto. *c*) (M)	V_{max} (e^-/sec/heme *a*)	K_m (cyto. *c*) (M)	V_{max} (e^-/sec/heme *a*)	K_m (cyto. *c*) (M)	V_{max} (e^-/sec/heme *a*)
0.3 mM	1.7×10^{-7}	26.7	2.1×10^{-7}	59.8	6.4×10^{-7}	86.7
23 mM	3.0×10^{-8}	48.1	5.2×10^{-8}	76.7	2.5×10^{-6}	227.3
56 mM	9.2×10^{-8}	52.8	1.1×10^{-7}	63.8	1.0×10^{-5}	375.0

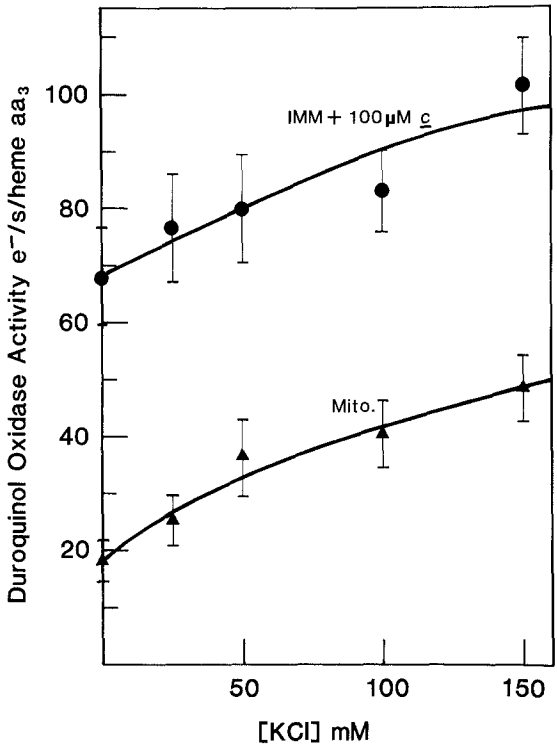

Fig. 4. Duroquinol oxidase activity of intact mitochondria in the presence of endogenous cytochrome *c* (▲) and isolated inner membranes in the presence of 100 μM exogenous horse heart cytochrome *c* (●) as a function of ionic strength. Assay conditions as in Fig. 3.

diffusion. Such mobility during cytochrome *c* function is consistent with the finding that the electron transfer interaction domains of its redox partners, cytochrome oxidase and cytochrome b–c_1 complex in the inner membrane and cytochrome b_5 in the outer membrane, are essentially the same (Stonehuerner *et al.*, 1979; Rieder and Bosshard, 1980). Although cytochrome *c* tends to collide with the membrane surface randomly, it may be guided electrostatically as it approaches the electric field of its membrane redox partners (Koppenol and Margoliash, 1982).

Postulate 3. *Electron transport is a diffusion-coupled kinetic process.* Electron transport is a *diffusion-coupled* kinetic process, i.e., all bimolecular redox reactions in the inner membrane are *preceded* by one or more diffusion-based random collisions between reacting redox partners (Gupte *et al.*, 1984). This was determined from our data on the lateral diffusion coefficients (Table IV, column 1), effective (percent reduced and oxidized) concentrations

Table IV. Collision Frequencies and Efficiencies of Redox Components

	1	2	3	4	5	6
Redox component	Lateral diffusion coefficient (cm^2/sec)	Content[c] (nmol/mg)	Molecules per inner membrane[d]	Concentration[e] (molecules/cm^2)	Effective concentration; % reduction and oxidation of redox partners at steady state[f]	Radius of reactive area[g] (nm)
Complex I	4×10^{-10a}	0.014	1,960	2.77×10^{10}	$I(5_{red}) \rightarrow Q(90_{ox})$	4.0
Complex II	4×10^{-10a}	0.027	3,781	5.35×10^{10}	$II(5_{red}) \rightarrow Q(90_{ox})$	0.7
Ubiquinone	3×10^{-9}	0.86	119,763	1.69×10^{12}	$Q(10_{red}) \rightarrow III(84_{ox})$	2.5
Complex III	4.4×10^{-10}	0.041	5,743	8.12×10^{10}	$III(16_{red}) \rightarrow c(89_{ox})$	4.0
Cytochrome *c*	1.9×10^{-9b}	0.122	17,089	2.42×10^{11}	$c(II_{red}) \rightarrow IV(80_{ox})$	4.0
Complex IV	3.7×10^{-10}	0.095	13,307	1.88×10^{11}	$IV(20_{red})$	—

Table IV. Continued

	7	8	9	10	11
Redox component	Electrons transferred/turnover	Theoretical diffusion-controlled collision frequency[h] (collisions/sec/cm^2)	Theoretical diffusion-controlled collision frequency[i] (collisions/sec/ redox partner)	Experimental turnover number[j] (turnover/sec/ redox partner)	Collisions/ turnover (collision efficiency in %)
Complex I	2	5.04×10^{13}	I 36,274 Q 33	3,360 3	11 (9.1) 11 (9.1)

Complex II	2	2.07×10^{13}	II	7,710	1,680	4.6 (21.6)
			Q	14	3	4.6 (22)
Ubiquinone	2	1.19×10^{14}	Q	707	26.7	26.5 (3.8)
			III	1,752	66.7	26.3 (3.8)
Complex III	1	2.02×10^{13}	III	1,555	700	2.2 (4.5)
			c	94	41.9	2.2 (45)
Cytochrome *c*	1	4.15×10^{14}	*c*	1,561	339.4	4.6 (21.7)
			IV	275	60	4.6 (21.8)
Complex IV	—	—		—	—	—

[a] Based on average D for Complexes III and IV.
[b] Apparent D at 56 mM ionic strength.
[c] Based on 0.19 nmol heme *a*/mg whole mitochondrial protein and stoichiometry after Hatefi and Galante (1978).
[d] Based on 4.3×10^9 mitochondria/mg protein after Gear and Bednarek (1972) and redox components occurring as monomers.
[e] Based on the morphometric analysis (Weibel *et al.*, 1966) of the surface area of the average spherical inner membrane, diameter = 1.5 μm.
[f] After Klingenberg and Kröger (1967), state 3U (uncoupled).
[g] Sum of the radii of the reaction partners. Individiual radii are: Complex I, 4.0 nm; Complex II, 0.7 nm; ubiquinone, negligible; Complex III, 2.5 nm; cytochrome *c*, 1.5 nm; Complex IV, 2.5 nm.
[h] Diffusion-controlled collision frequency = reaction rate = $2\pi N C_A C_B \{D_A/\ln[(\pi C_B)^{-1/2}/a] + D_B/\ln[(\pi N C_A)^{-1/2}/a]\}$, after Hardt (1979).
[i] Collisions/sec/cm^2 (column 8) divided by the concentration of the reduced or oxidized redox partner (column 5).
[j] Based on state 3U (uncoupled) respiratory rate for succinate oxidase = $48\,e^-/\text{sec}/aa^3$ at 56 mM ionic strength and adjusted for the effective concentration of the redox partner.

(Table IV, column 5), and sum of the radii of the reactive areas (Table IV, column 6) of the redox components.

By substituting the experimental quantities of these three parameters, derived from uncoupled (state 3U) inner membranes, into the Hardt equation[4] for a two-dimensional system (Hardt, 1979), we determined the theoretical collision frequency (diffusion-controlled maximum collision frequency) between each pair of redox partners (Table IV, column 9). By comparing the maximum collision frequencies with the experimental maximum turnover numbers (productive collision frequencies) (Table IV, column 10), it is clear that *every potentially reactive redox component undergoes one or more diffusion-based collisions with its potentially reactive redox partner to effect one turnover*. This gives collision efficiencies of 4 to 45% (Table IV, column 11). We have never found turnover numbers to be greater than collision frequencies. These findings reveal that a permanent assembly or transient aggregate of redox components is not required to account for the sequence or maximum rate of electron transport. Since redox components exist in different conformations depending on redox state (Salemme, 1977; Freedman and Chan, 1983), it is likely that a productive redox collision, i.e., electron transfer, requires a conformation-specific association between redox partners. It follows that conformational change occurs in redox partners concomitant with electron transfer, which we believe is the basis for an immediate dissociation of the partners, i.e., an electron-induced, conformation-driven dissociation (Fig. 5).

Having established that electron transport is diffusion coupled, it follows that it could also be *diffusion controlled* if the rates of diffusion, rather than the rates of chemical reaction, of the redox components were rate limiting in the overall kinetic process of electron transport. This will be dealt with in detail under Postulate 5.

Postulate 4. *Electron transport is a multicollisional, obstructed, long-range diffusional process.* Mitochondrial electron transport is on average a long-range (> 10 nm) and not a short-range (< 10 nm) diffusional process (Hackenbrock *et al.*, 1985, 1986). This is consistent with the finding that the integral proteins of the inner membrane occupy 50% or less of the membrane surface area, leaving considerable lipid bilayer through which the randomly distributed redox components must diffuse to effect a consecutive reduction and oxidation. Even the fastest diffusants, ubiquinone and cytochrome *c*, must traverse over considerable distances to be functionally effective (Table V). It is essential to realize that the distances in Table V are absolute,

[4] The Hardt equations for diffusion control assume inert, hard-body collisions, with all collisions being instantaneously productive, and do not explicitly consider rotational diffusion, electrostatic interactions, size and specific location of active sites, and any rate constants other than for diffusion.

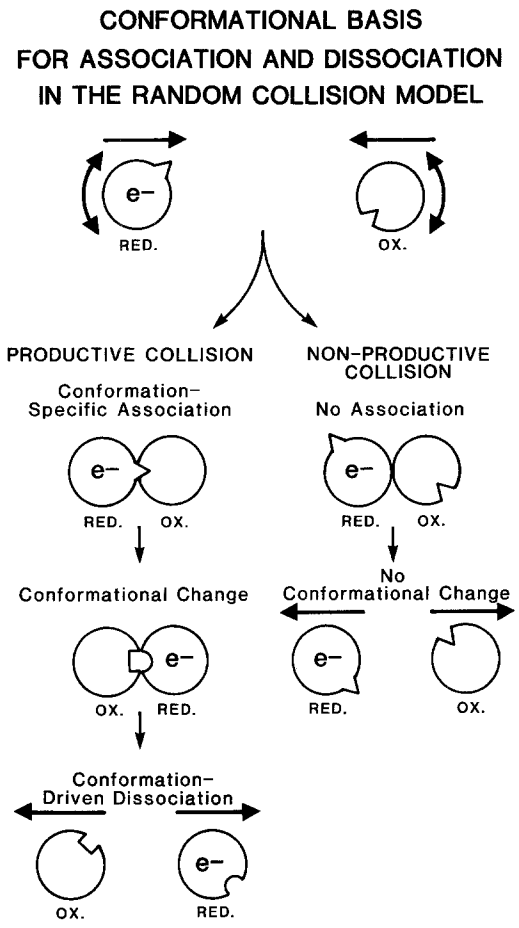

Fig. 5. Membrane surface view of a model for productive and nonproductive diffusion-based collisions between redox partners. Electron transfer-induced conformational change shown for both redox partners as a basis for immediate conformation-driven dissociation. Red, reduced; ox, oxidized.

minimum, straight-line distances between nearest-neighbor redox complexes, and fall far short of the true distances that ubiquinone and cytochrome *c* must traverse in an obstructive, multicollisional, random walk to be functionally effective. Since cytochrome *c* diffuses in three dimensions at physiological ionic strength, its absolute minimum diffusion path length required to effect its consecutive reduction and oxidation is greater than 24.8 nm, the planar distance between reduced Complex III and oxidized Complex IV during steady-state uncoupled electron transport. The minimum path length that ubiquinone is required to diffuse for its consecutive reduction and oxidation

Table V. Minimum Distance between Redox Complexes

Redox complexes	Total concentration of redox complexes (molecules/cm^2 of membrane)	Minimum distance between redox complexes[a] (nm)	% Reduced/oxidized redox complexes[b]	Effective concentration of reduced/oxidized redox complexes (molecules/cm^2)	Minimum distance between redox complexes at effective concentrations[a] (nm)
I III	2.77×10^{10} 8.12×10^{10}	30	I (5 red) III (84 ox)	1.39×10^{9} 6.82×10^{10}	37.9
II III	5.35×10^{10} 8.12×10^{10}	27.2	II (5 red) III (84 ox)	2.68×10^{9} 6.82×10^{10}	37.6
III IV	8.12×10^{10} 1.88×10^{11}	19.3	III (16 red) IV (80 ox)	1.30×10^{10} 1.50×10^{11}	24.8

[a]The distance d is calculated using the equation $d = (c_1 + c_2)^{-1/2}$, where c_1 and c_2 are concentrations of individual redox complexes in a two-dimensional, stationary lattice. From the equation, a nearest neighbor can be either of two redox complexes; therefore, the calculated distance represents the minimum, average distance for two of the redox complexes.
[b]State 3U (uncoupled); from Klingenberg and Kröger (1967).

is 37.6 nm, the planar distance between reduced Complex II and oxidized Complex III. Thus, productive redox collisions are required to occur on average between widely dispersed redox partners. Indeed, we know empirically from the use of FRAP that all redox components diffuse over micron distances and also that a productive electron transfer results from a multicollisional process (Table IV, column 11).

It is evident from these observations that (1) the electron-transferring reactions between all redox components and their respective redox partners at their effective concentrations occur via a long-range diffusional process, (2) the rates of diffusion as well as reactions are affected by nonproductive, obstructive multicollisions among the redox components themselves and among the redox components and other nonredox proteins, and (3) the degree of obstructive multicollisions is proportional to the protein concentration in the inner membrane.

Dilution of inner membrane protein concentration, i.e., the two-dimensional density distribution, by phospholipid enrichment of the membrane results in a decrease in collisional obstruction effecting an increase in the D's, e.g., of Complex III, which approaches the D for phospholipid (Fig. 6). Indeed, we have recently determined that the average D for integral proteins

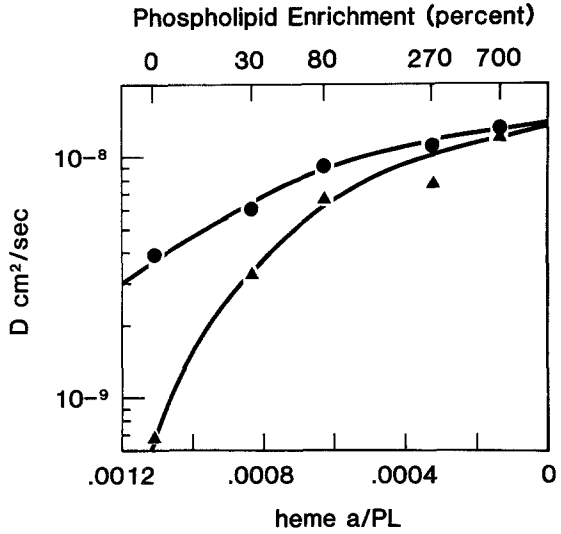

Fig. 6. Effect of inner membrane protein density on lateral diffusion. Integral protein density is expressed in terms of the heme *a*/phospholipid molar ratio. Lateral diffusion coefficients determined by FRAP in fused, phospholipid-enriched and native (nonenriched) inner membranes at 23°C. Lipid diffusion (●) was reported by the fluorescent lipid analogue DiI, incorporated into the inner membrane, and Complex III diffusion (▲) was reported by tetramethylrhodamine isothiocynate-conjugated rabbit anti-complex III IgG bound to Complex III.

collectively in the native inner membrane varies (0.13–3.35 $\times$ 10^{-9} cm^2/sec) inversely with a *naturally occurring variation* in the protein density found in this membrane (Sowers and Hackenbrock, 1985). These results are in agreement with the theory of Saffman and Delbrück (1975) which predicts a weak dependence of D on the radius of the diffusant at dilute concentrations and the theory of Eisinger *et al.* (1986) for obstructed, long-range diffusion in biomembranes. Further, we speculate that the low microviscosity of 0.9 P for the native inner membrane (Feinstein *et al.*, 1975) reflects a requirement for a low resistance to motion in the bilayer since there are a great deal of nonspecific protein–protein collisions. Hence a cholesterol-free, highly unsaturated phospholipid bilayer is found in the inner membrane.

It should be pointed out that although protein dilution by phospholipid enrichment of the inner membrane results in a decrease in collisional obstruction and increase in D's for redox components, such dilution also results in a decrease in the rate of electron transport (Schneider *et al.*, 1980a, b). In addition, increasing the concentration of a single redox component in the membrane, while decreasing its redox partners' concentrations, results in an increase in electron transport activity (Schneider *et al.*, 1982a). Thus the overall kinetic process of electron transport has a redox component concentration dependence as well as a diffusion dependence. When the total protein and redox component concentration of the inner membrane is lowered, one would expect electron transfer activity to increase, owing to a decrease in obstruction which effects an increase in the D's of the redox components, and commitantly to decrease, owing to a decrease in concentration of the redox reactants. In such dilution experiments the increase in the D's of redox components appears not to be great enough to compensate for the decrease in concentration of redox components since the overall kinetic result is a fall in electron transport rate which, by calculation using the Hardt equation, results in an apparent decrease in collision efficiency. Whether this is a real decrease in collision efficiency or an anomalous determination due to the nature of the Hardt equation is not immediately clear. In the more crowded native inner membrane (i.e., nonenriched), more nearest-neighbor collisions per unit time may occur due to a cage effect, by analogy to the Franck–Rabinowitch effect in condensed phases (Franck and Rabinowitch, 1934; Rabinowitch, 1937), than would occur as predicted by the Hardt equation. A second line of reasoning, although less direct, would also suggest that the actual collision efficiency does not change as a function of membrane protein concentration. We demonstrate in Postulate 5 that diffusion is rate limiting in Complex II–ubiquinone–Complex III electron transport and that the decrease in the energies of activation of electron transport activity is proportional to the decrease in the energies of activation of the redox component D's as membrane protein concentration is decreased (Table VI, columns 3

and 4). Under rate-limiting conditions this latter finding suggests little or no change in the real collision efficiencies as a function of protein density.

Postulate 5. *The rates of diffusion of the redox components have a direct influence on the overall kinetic process of electron transport and can be rate limiting, as in diffusion control.* Under Postulate 3, we presented our finding that *every* potentially reactive redox component undergoes *one or more* diffusion-based collisions with its potentially reactive redox partner to effect one turnover. Thus electron transport is a *diffusion-coupled* kinetic process. Electron transport may also be limited by the rate of diffusion-based collisions between potentially reactive redox partners, as in *diffusion control.*

To deal with the question of diffusion control, we set forth the following criteria:

(A) An overall diffusion-coupled kinetic process is composed of a *diffusion step* and a *chemical reaction step.*

(B) When the collision frequency between the reaction partners is *equal* to the frequency of the chemical reaction, the overall kinetic process is, as classically defined, *diffusion controlled.*

(C) When the collision frequency between the reaction partners *exceeds* the frequency of the chemical reaction, the overall kinetic process is *diffusion controlled* when the diffusion step is rate limiting, and *reaction controlled* when the chemical step is rate limiting.

The problem of experimentally determining the rate-limiting step (diffusion vs. chemical reaction) in the electron transport process, which is a series of consecutive reactions, is a difficult one but can be approached in several ways. We have utilized concepts put forth in rate process theory (Johnson *et al.*, 1975) and the theory of absolute reaction rates (e.g., Eyring, 1935) as a rationale for our experimental approach (Hackenbrock *et al.*, 1985, 1986). This approach entails the determination of the temperature dependences, in terms of apparent energies of activation (E_a), for physical and/or chemical reaction rate processes (e.g., diffusion and electron transfer) that comprise the overall kinetic rate process. Following this rate process theory, it can be predicted that the E_a for the rate-limiting step (diffusion or electron transfer) will be the most significant contribution (greatest portion) to the E_a of the overall kinetic process (electron transport).

In practice, we compared the temperature dependence of the overall diffusive steps to the temperature dependence of the overall steps (diffusion plus chemical reaction) in the Complex II–ubiquinone–Complex III (II–Q–III) electron transport process in the uncoupled inner membrane (Hackenbrock *et al.*, 1985, 1986). This was accomplished by experimentally measuring the temperature dependence of the D's of the appropriate redox components by FRAP as well as the temperature dependence of the overall

succinate-linked electron transport rates at various temperatures between 5–40°C and plotting the results of Arrhenius fashion (i.e., log of D vs. $1/T$). The E_a's of D's were determined to be high, e.g., 12.04 kcal/mole for Q and 10.8 kcal/mole for Complex III, which can be expected considering that diffusion is a multicollisional, obstructed process in the native membrane. Applying the Hardt equation for a two-dimensional system (as described under Postulate 3), to the D's of Q, Complex II, and Complex III at various temperatures, the E_a's of the collision frequencies were determined for the II–Q and Q–III redox partners (Table VI, column 2). Following the treatment of Gutman (1980) of assigning separate rate constants to the diffusion steps in electron transport and applying the E_a's of the collision frequencies determined for the II–Q and Q–III partners, we determined an E_a for the overall diffusion steps for II–Q–III (Table VI, column 3). The E_a's of succinate-cytochrome *c* reductase and succinate oxidase activites were also determined (Table VI, column 4).

Table VI. Comparison of Apparent Activation Energies (E_a)[a]

	1	2	3	4	
Inner membrane	Redox sequence	E_a collision frequency	E_a lateral diffusion	E_a uncoupled electron transport activity	
Native	II–Q	11.8			
	Q–III	11.9			
	II–Q–III		12.2	12.87	Succinate cytochrome *c* reductase
				14.3	Succinate oxidase
30% Phospholipid enriched	II–Q				Succinate cytochrome *c* reductase
	Q–III	9.07			
	II–Q–III	9.2	9.55	10.8	
				9.3	Succinate oxidase
80% Phospholipid enriched	II–Q				Succinate cytochrome *c* reductase
	Q–III	8.6			
	II–Q–III	8.9	9.22	10.5	
				9.0	Succinate oxidase

[a] E_a in kcal/mole.

Comparison of the temperature dependence of both overall electron transport processes (E_a = 12.8 and 14.3 kcal/mole) with the temperature dependence of the overall diffusion steps (E_a = 12.2 kcal/mole) reveals that the diffusion steps of Q and its redox partners account for the greatest portion of the E_a of the overall kinetic electron transport process, with the chemical reaction steps (i.e., the actual transfer of electron equivalents during or after collisions) requiring a smaller portion of the total E_a. This finding is compatible with diffusion control as defined in criterion (C) above.

As a further test of diffusion control in electron transport, if lateral diffusion is in fact rate limiting, it follows that any affect on the E_a of diffusion will be approximately the same on the E_a of electron transport. Consistent with diffusion control, when the concentration of the protein of the inner membrane is decreased by enrichment of the membrane with exogenous phospholipid, the E_a's of both lateral diffusion and electron transport decrease approximately proportional to the degree of enrichment (Table VI, columns 3 and 4).

Finally, if lateral diffusion is in fact rate limiting, it follows that the collision efficiencies of redox partners will be virtually temperature *independent* since any effect on the diffusion steps will be approximately the same for the overall electron transport process. To test this prediction for diffusion control, the collision efficiencies of II–Q and Q–III were determined at various temperatures. This is done by plotting, in Arrhenius fashion, the ratio of the turnovers of succinate cytochrome *c* reductase (Fig. 7A), or succinate oxidase (Fig. 7B), at various temperatures per theoretical collision frequencies (= collision efficiencies) for II–Q and Q–III. Consistent with diffusion control, this analysis revealed a very weak or no temperature dependence for collision efficiencies between Q and Complex III (Fig. 7C,D) or between Q and Complex II (not shown). These data on temperature dependences of diffusion and electron transport argue affirmatively for diffusion control [as defined in criterion (C)] in the II–Q–III kinetic process of electron transport.

Approaches to the question of diffusion control in the interaction of cytochrome *c* and its redox partners are considerably more difficult, since the actual *D* for cytochrome *c*, which diffuses in three dimensions, has not been measured in the mitochondrion. More significantly, equations have not been adequately developed to determine collision frequencies between molecules diffusing in three dimensions (cytochrome *c*) with reaction partners diffusing in two dimensions (Complexes III and IV).

Nevertheless, we would point out that the turnover numbers of the reduction of cytochrome *c* by cytochrome b–c_1 and of the oxidation of cytochrome *c* by cytochrome oxidase are three- to four-fold higher than the duroquinol oxidase activity (Veerman *et al.*, 1983; Speck and Margoliash, 1984). This is in agreement with the very rapid oxidation of cytochrome *c* by

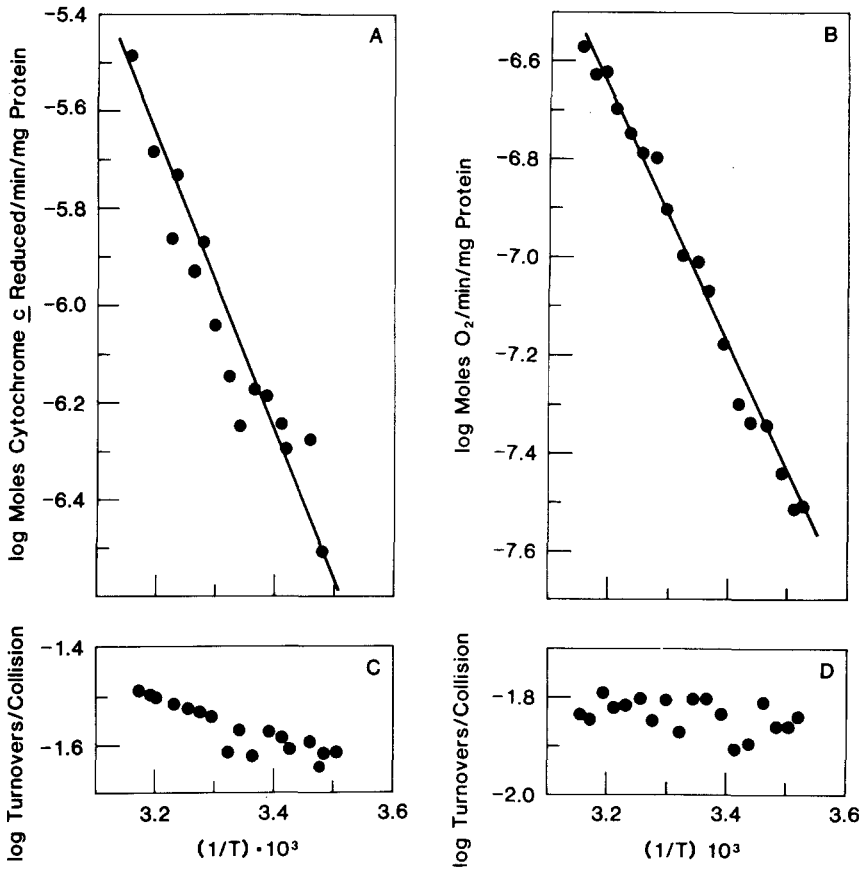

Fig. 7. Temperature dependence of electron transport and collision efficiencies in native inner membranes. (A) State 3U succinate cytochrome *c* reductase activity. Reaction medium: 80 mM potassium phosphate, pH 7.4, 20 mM succinate, 2 μM CCCP, 2 mM KCN, and 50 μM cytochrome *c* to initiate the reaction. Membrane temperature equilibrated in reaction medium prior to assay. (B) State 3U succinate oxidase activity. Reaction medium: 10 mM potassium phosphate, pH 7.4, 5 μM rotenone, 1 μM CCCP, 0.5 mM EDTA, 5.4 μM cytochrome *c*, and 20 mM succinate to initiate the reaction. Membrane temperature equilibrated in reaction medium prior to assay. (C) Collision efficiency as a function of temperature of Q–III redox partners based on turnovers during state 3U succinae cytochrome *c* reductase activity. Turnovers derived from Fig. (A) and theoretical collisions calculated using the Hardt equation (as per text). (D) Same as in Fig. (C), but for state 3U succinate oxidase activity.

cytochrome *a* due to the small driving force for the reaction (< 12 kJ/mol) (Marcus and Sutin, 1985). These data suggest that the slowest or rate-limiting step in the duroquinol oxidase activity is the cytochrome *c*-mediated electron transfer from cytochrome b–c_1 to cytochrome oxidase, i.e., the diffusion step of cytochrome *c*.

As discussed under Postulate 2, at increasingly higher ionic strengths, the rate of electron transport catalyzed by cytochrome *c* increases as the diffusion of cytochrome *c* increases and becomes prediminantly three dimensional. The increase in the rate of electron transfer at higher ionic strengths indicates that the three-dimensional diffusion of cytochrome *c* in the intermembrane space at high, physiological ionic strength results in a greater collision frequency and/or efficiency than would occur if cytochrome *c* was limited to only two-dimensional, i.e., lateral, diffusion. Collision frequency and/or efficiency in electron transfer between cytochrome *c* and its redox partners may be enhanced further through guidance by the strong dipole moments of these components (Koppenol and Margoliash, 1982). These data are compatible with our thinking that the three-dimensional diffusion of cytochrome *c* is rate limiting as in diffusion control in duroquinol oxidase activity at physiological conditions.

Analysis of Other Studies on Diffusion Related to Electron Transport

Since our 1976 review other laboratories have also published reports dealing with diffusion and electron transport in the mitochondrion. In this section we shall examine and critically review the data, the conclusions, and any proposed models from these laboratories.

Rotational Diffusion and Immobile Fraction of Cytochrome Oxidase

Using FRAP we determined that no significant fraction of any redox protein or ubiquinone is immobile, indicating that all redox components diffuse randomly (and, by implication, rotate freely) in the inner membrane as single components in a common pool (Gupte *et al.*, 1984). This is in contrast to the report by Kawato *et al.* (1982) that only 29% (at 20°C) and 35% (at 37°C) of the cytochrome oxidase in the isolated inner membrane is rotationally mobile as determined by measuring the decay of its absorption anisotropy. Hochman *et al.* (1982, 1983, 1985) and Vanderkooi *et al.* (1985) have relied on this result to a significant extent in support of the idea of redox complex formation or aggregation. It should be noted, however, that both Kawato *et al.* (1981), using reconstitution, and Höchli *et al.* (1985), using immunofluorescence, determined that Complex III and IV diffuse independently of one another. Our work (Gupte *et al.*, 1984) and also that of Hochman *et al.* (1985) show that 85–90% of the cytochrome oxidase in the inner membrane is laterally mobile with a D of 1.5–3.7 $\times$ 10^{-10} cm^2/sec. From diffusion theory a high degree of lateral diffusion would predict a comparably high degree of rotational diffusion (Saffman and Delbrück,

1975). In contrast to this theory, Kawato *et al.* (1982) reported that the rotational diffusion coefficient (D_r) for cytochrome oxidase does not increase (although the percent mobile fraction increases) when inner membranes are enriched with exogenous phospholipid by the low pH fusion method of Schneider *et al.*, (1980a). We have recently determined experimentally (Hackenbrock *et al.*, 1986) that D increases dramatically with membrane enrichment and in all cases the mobile fraction is greater than 90% (Fig. 6). Considering the theoretical relationship of D_r and D (Saffman and Delbrück, 1975), and as was experimentally tested (Peters and Cherry, 1982) using reconstituted bacteriorhodopsin, it follows that both D and D_r should increase with protein dilution.

We have considered that the low mobile fraction reported for cytochrome oxidase by Kawato *et al.* (1981, 1982) may be a result of the use of the 60% sucrose solution (55 cP at 22°C) used in their studies to decrease the rate of membrane tumbling and reduce light scattering. Such a high sucrose concentration would have a strong osmotic effect and may have a dehydration effect as well on the inner membrane. This possibility is reinforced by the sucrose-induced aggregation of inner membranes found in their study. In addition, an unusual decrease in cytochrome oxidase activity and intramembrane particle aggregation after membrane enrichment with phospholipid is found in the Kawato *et al.* study (1982) (c.f., Schneider *et al.*, 1980a, b). More directly, using native membranes we have determined recently by FRAP that the mobile fraction at 25°C for Complex III decreases from $\sim 90\%$ to $\sim 25\%$ in 30% sucrose while in 60% sucrose, lateral diffusion is barely perceptible. In agreement with these sucrose-induced decreases in mobile fraction, succinate oxidase activity decreases to $\sim 30\%$ in 30% sucrose and to $\leqslant 17\%$ in 60% sucrose (Chazotte and Hackenbrock, unpublished results). Considering these predictions of diffusion theory and data on lateral diffusion, aggregation of inner membranes and integral proteins, as a result of the high sucrose medium used, can explain the large immobile fraction of cytochrome oxidase reported by Kawato *et al.*

Immobile Fraction and Complex Formation of Cytochrome c

A study on the diffusion of cytochrome *c* by Vanderkooi *et al.* (1985) using FRAP of a nonfunctional porphyrin cytochrome *c* at various ionic strengths is in qualitative agreement with our observations on cytochrome *c* diffusion. Their apparent average D at approximately zero ionic strength and excess cytochrome *c* concentrations is virtually identical to the D reported earlier by Gupte *et al.* (1984) at various concentrations of cytochrome *c* and at 0.3 mM ionic strength (Tables I and II). Vanderkooi *et al.* also verified the finding of Gupte *et al.* that the apparent D for cytochrome *c* increases with

increasing ionic strength (Table I). However, Vanderkooi *et al.* reported an approximately 50% immobile fraction of cytochrome *c* on inner membranes at zero ionic strength after addition of quantities of cytochrome *c* stoichiometric with cytochrome oxidase or after washing the membranes before FRAP measurement. Hochman *et al.* (1985) and Gupte *et al.* found no substantial immobile fraction of cytochrome *c* in their washed membranes. In the latter study no significant immobile fraction could be detected even when quantities of cytochrome *c* were stoichiometric with cytochrome oxidase (Tables I and II). It should be noted that Vanderkooi *et al.* found that approximately 3 nmol of the porphyrin cytochrome *c* remained tightly bound per mg of mitochondrial protein (or 3.5 : 1 cytochrome *c* : cytochrome oxidase) in the presence of 150 mM KCl, contrary to the virtually complete dissociation of native cytochrome *c* from inner membrane at 150 mM KCl. Vanderkooi *et al.* have suggested that their immobile fraction of cytochrome *c* is due to binding to its redox partners, which may be immobile in the membrane. However, earlier studies on the *D*'s of Complex III (Gupte *et al.*, 1984) and Complex IV (Gupte *et al.*, 1984; Hochman *et al.*, 1985) found no significant immobile fraction of these redox proteins (Table I).

It is our thinking that the large immobile fraction of cytochrome *c* reported by Vanderkooi *et al.* (1985) is due to an unusually strong binding of the nonfunctional, perhaps denatured, porphyrin cytochrome *c* to the membrane which is irreversible, even in high salt. This unusually strong binding explains why the porphyrin cytochrome *c* was detected on the membrane at 100 mM ionic strength by FRAP, since native cytochrome *c* does not remain bound to the membrane at this ionic strength for sufficient time to be detected by FRAP.

Ubiquinone Diffusion and Complex Formation

Ragan and Cottingham (1985) have reviewed the kinetics of Q pools in electron transport and proposed a kinetic model designed to refine the pool equations originally presented by Kröger and Klingenberg (1973a, b) in order to deal with conditions (nonphysiological) that induce deviations from the predicted behavior of the original equations. Ragan and Cottingham observe that the evidence for the concept of Q as a mobile pool of laterally diffusing molecules linking the dehydrogenases and the cytochromes (Complex III in mitochondria) is substantial, and the question remaining to be answered is which step (e.g., diffusion, binding, electron transfer) is rate limiting for electron transport in this region. In this regard they point out that earlier experiments from Ragan's laboratory (Ragan, 1978; Ragan and Heron, 1978; Heron *et al.*, 1978) in which an apparent 1 : 1 association of Complex I and Complex III occurred as a result of reconstitution in low phospholipid

(low phospholipid/protein ratio) were performed under extreme nonphysiological or "peculiar" conditions. Further, they state that these conditions may induce an abnormal organization of inner membrane components although Q could still diffuse, albeit in a restricted manner. Ragan and Cottingham conclude, with respect to the formulation of their kinetic model, that the assumption of a diffusion-limited reaction (Rich, 1984) is not a prerequisite for the development of rate equations. They further conclude that *no* association of the dehydrogenases and Complex III is indicated in native inner membranes from experimental evidence or by the requirements of the kinetics. Finally, they suggest that models requiring transient redox complex formation to account for electron transport are unnecessarily complicated. These data and views are compatible with the random collision model of electron transport.

Dynamic Aggregates of Heme Proteins

Hochman *et al.* (1982, 1983) have made calculations interpreted by them to show that the D for cytochrome c is too low for diffusion to sustain the maximum electron transport rate and proposed a dynamic aggregate model for electron transport. This model is a hybrid of the classical aggregate respiratory chain model and the random collision model, i.e., it incorporates transient aggregates as well as free lateral diffusion of redox components to account for the rate of electron transport. The model proposes that multielectron transfers occur during the time course of temporary aggregations of redox complexes. The correct bi- or multimolecular redox complexes are proposed to aggregate through diffusion-based random collisions to give the correct sequence and rate of electron transport. Hochman *et al.* do not specify the percent, duration, or statistical probability for diffusion-based transient aggregation of specific redox components for any metabolic state or experimental condition. In addition, the model offers no clue for the rationale or regulatory basis (e.g., temporary forces involved) for temporary aggregation or subsequent disaggregation of redox partners, or indeed of nonredox partners such as Complex III and IV. Since the model relies on transient aggregates, during which successive, multielectron transfer occur, the model does not conform to the concept of redox-induced conformation-specific association or conformation-driven dissociation as a basis for electron transfer (Fig. 5).

The model of Hochman *et al.* appears to be very similar to that proposed earlier by Ragan (Ragan and Heron, 1978) for Complex I–Q–Complex III electron transfer but who has suggested recently (Ragan and Cottingham, 1985), based on kinetic considerations (see previous section), that such models requiring transient bi- or multimolecular redox complexes are

unnecessarily complicated. In agreement with this thinking, it should also be pointed out that Kawato *et al.* (1981) concluded from reconstitution experiments that their immobile fraction of cytochrome oxidase was not due to its aggregating with Complex III. More recently Höchli *et al.* (1985) determined by immunofluorescence that Complexes III and IV diffuse independently in the inner membrane.

In addition to these points, we find that the data on which the dynamic aggregate model is based leaves the model untenable. In their earlier work Hochman *et al.* (1982) calculated a theoretical minimum D for cytochrome c of 4×10^{-9} cm^2/sec which they proposed was required to satisfy the experimental maximum rate of electron transport by a random diffusion mechanism. Their experimentally measured D at 8 mM ionic strength was 1.6×10^{-10} cm^2/sec which is essentially the same as found by Gupte *et al.* (1984) at 23 mM ionic strength (Table I). However, the theoretical (required) D as calculated by Hochman *et al.* is high compared to their measured D owing to their underestimated concentration of cytochrome oxidase in the inner membrane of cuprizone-induced mouse liver megamitochondria. Hochman *et al.* determined that their megamitochondria contained levels of cytochromes and activites comparable to normal rat liver mitochondria. They determined the heme *a* of megamitochondria to be 0.24 nmol/mg, which is similar to the 0.2 nmol/mg determined for rat liver mitochondria in most laboratories. However, they assumed that cytochrome oxidase occupies only 2% of the total inner membrane protein and calculated 1.7×10^{10} monomers/cm^2 of membrane surface area. In contrast, Gupte *et al.* measured the heme *a* to be 0.19 nmol/mg protein and the surface area of the inner membrane of the average liver mitochondrion to be 7.1 μm^2, and based on 4.3×10^9 mitochondria/mg protein (Gear and Bednarek, 1972), calculated the concentration of cytochrome oxidase to be 1.88×10^{11} monomers/cm^2. Thus cytochrome oxidase more reasonably comprises ~8% of the inner membrane protein or 13,307 monomers per average liver mitochondrion (Table IV, column 3). This determination is in close agreement with that of Estabrook and Holowinsky (1961) who determined cytochrome oxidase at 17,000 monomers per average liver mitochondrion, which by calculation is ~8% of the innermembrane protein. Using morphometric analysis, Schwerzmann *et al.* (1986) recently claimed 15,656 cytochrome oxidase monomers per liver mitochondrion and comprising an even greater amount (15.9%) of the inner membrane protein.

Based on the Hochman *et al.* calculation of 1.7×10^{10} oxidase monomers/cm^2, the average liver mitochondrion would contain only 1100–1200 monomers, which is at least 11- to 14-fold less than determined by others (Estabrook and Holowinski *et al.*, 1961; Gupte *et al.*, 1984; Schwertzman *et al.*, 1986). Therefore, Hochman *et al.* considerably overestimated the

average distance of 63 nm between any cytochrome oxidase or Complex III monomers. From the data of Gupte *et al.* this distance is only 19.3 nm. This is in good agreement with Capaldi's review (1982) which by calculation gives 16.7 nm for this distance based on monomers in bovine heart mitochondrial inner membrane in which cytochrome oxidase by calculation comprises ~ 13% of the total membrane protein. This distance and percentage obtains even though there is much more inner membrane present and approximately 84,000 cytochrome oxidase monomers per heart mitochondrion. At effective (percent reduction and oxidation) concentrations, Hochman *et al.* calculated that a cytochrome *c* would have to diffuse from a reduced Complex III to an oxidized cytochrome oxidase and back to a reduced Complex III, or 284.5 nm, to effect one turnover. Using the appropriate (effective) concentrations of redox components (Table V), this distance is only 50 nm (i.e., 24.8 × 2). Clearly, the underestimated concentrations of cytochrome oxidase and consequently Complex III, calculated by Hochman *et al.* yielded an unrealistically high theoretical *D* requirement for cytochrome *c* to account for the known maximum rate of electron transport. Substituting the correct distances, based on the correct concentrations for cytochrome oxidase and Complex III, into the mean-square displacement equation used by Hochman *et al.* (1982), we calculate a required *D* of 1.3×10^{-10} cm^2/sec which is essentialy identical to their measured *D* of 1.6×10^{-10} cm^2/sec. This agreement would support a classically defined diffusion-controlled mechanism for electron transport.

One additional important point bearing on their treatment is that Hochman *et al.* did not consider the *D*'s of cytochrome oxidase and Complex III in their calculations since they assumed these components were relatively *immobile*. As it turns out, their *D* for cytochrome *c* is essentially *equal* to the *D*'s for cytochrome oxidase and Complex III (Table I). If considered, this would result in an even lower required *D* for cytochrome *c* to sustain maximum electron transport. Collectively or separately, these data support the random collision model of electron transport.

In their second report, Hochman *et al.* (1985) recalculated the cytochrome oxidase concentration to be 4.5% of the total membrane protein and 3.8×10^{10} monomers/cm^2 of membrane. Although doubling the concentration used in their first paper, this still underestimated by approximately fivefold the number of oxidase monomers/cm^2 found by other laboratories (Estabrook and Holowinski, 1961; Capaldi, 1982; Gupte *et al.*, 1984; Schwertzmann *et al.*, 1986). Hochman *et al.* determined that the *D* for cytochrome *c* was 3.5×10^{-10} cm^2/sec at 8 mM ionic strength to 7.4×10^{-10} cm^2/sec at 25 mM ionic strength, which is greater than what they reported earlier but similar to the *D*'s obtained in our laboratory at various ionic strengths (Gupte *et al.*, 1984). They also determined the *D* for

cytochrome oxidase to be 1.5×10^{-10} cm^2/sec, similar to our earlier determination of 3.7×10^{-10} cm^2/sec (Gupte *et al.*, 1984). Using their data, Hochman *et al.* calculated that a D of $1.0–1.6 \times 10^{-9}$ cm^2/sec is required for cytochrome *c* to account for a theoretical diffusion-controlled collision frequency (using a turnover number of $20\,e^-$/sec/aa_3). Their calculated requirement of a D of $1.0–1.6 \times 10^{-9}$ cm^2/sec (or $10–16 \times 10^{-10}$ cm^2/sec) *overlaps* significantly, in agreement with their experimental D of $7.4 \pm 4.1 \times 10^{-10}$ cm^2/sec (or $3.3–11.5 \times 10^{-10}$ cm^2/sec) at the highest ionic strength (25 mM) used. At an ionic strength of 8 mM, their experimental D for cytochrome *c* approached closely the calculated required D. Use of the correct cytochrome concentrations would have yielded an even lower calculated required D of 1.0×10^{-10} cm^2/sec for cytochrome *c*. These findings not only support the random collision model of electron transport, but, in terms of the overlap, are compatible with the classical criterion for diffusion control, i.e., one collision results in one reaction or 100% collision efficiency. Hochman *et al.* interpreted the agreement of their calculated and experimental D's as an indication that the mobility of cytochrome *c* is not sufficient to explain electron transfer by a completely random diffusion mechanism.

In light of our analysis of the Hochman *et al.* data (1982, 1983, 1985) and the conditions in the Kawato *et al.* (1981, 1982) studies regarding the D_r of cytochrome oxidase, as well as Ragan and Cottingham's (1985) evaluation of redox complex formation in electron transport, we find no experimental evidence for the existence of transient redox aggregates for electron transport in the mitochondrial inner membrane nor any rationale or need to invoke their existance.

Rate of Ubiquinone Diffusion

Lenaz and co-workers (Fato *et al.*, 1985; Lenaz *et al.*, 1985, 1986), concentrating their efforts solely in the Q region, have generated results which are in agreement with the results of our laboratory showing that Q is a mobile carrier and with the basic precepts of the random collision model. However, there is disagreement over the rate of Q diffusion and consequently the role of diffusion with regard to rate control in electron transport. Lenaz and co-workers used fluorescence quenching (Lakowicz and Hogen, 1980) to measure the lateral diffusion of ubiquinone homologues of various lengths in lipid vesicles and, in one experiment, Q_3 in submitochondrial particles. In their studies the D's for the various ubiquinones were determined to be in the 10^{-6} cm^2/sec range, and based on these data, the authors concluded that the D for ubiquinone is too high for diffusion to be rate limiting in mitochondrial electron transport. It must be pointed out, however, that the D measured by

Table VII. Lateral Diffusion Coefficients of Lipoidal Molecules in Native Membranes and Phospholipid Bilayers

Component	Membrane/source	Probe	Technique	$D \times 10^{10}$ (cm^2/sec)	Temperature (°C)	Reference
Cholesterol	DMPC-CHOLES.[a,b]	NBD-CHOL.[c]	FRAP[d]	14	26	Alecio *et al.* (1982)
Phospholipid	PD-MITO[e] L.I.	DiI[f]	FRAP	20	—	Vanderkooi *et al.* (1985)
Phospholipid	Red blood cell	DiI	FRAP	20	25	Kapitza and Sackman (1980)
Ubiquinone	FIM[g] Rat. liv.	Q_0C_{10}NBD[h]	FRAP	26	21	Gupte *et al.* (1984)
Phospholipid	MEGA[i] M. liv.	DiI	FRAP	33	23	Hackenbrock *et al.* (1985)
Ubiquinone	MEGA M. liv.	Q_0C_{10}NBD	FRAP	37	23	Gupte *et al.* (1984)
Phospholipid	FIM	DiI	FRAP	39	23	Chazotte and Hackenbrock (1983*a*); Hackenbrock *et al.* (1985)
Phospholipid	Plasma memb. sciatic nerv.	PL[j]	NMR[k]	50	31	Lee *et al.* (1973)
Phospholipid	MEGA M. liv.	NBD-PE[l]	FRAP-E[m]	60	—	Hochman *et al.* (1985)
Phospholipid	FIM	NBD-PE	FRAP	68	23	Chazotte *et al.* (1983*b*); Chazotte and Hackenbrock (1984)
Phospholipid	Plasma memb. fibroblast	DiI	FRAP	80	25	Jacobson *et al.* (1981)
Phosphatidyl choline	PC[n]	PC	NMR	100	20	Horowitz *et al.* (1972)
Phospholipid	Plasma memb. *E. coli*	PL	NMR	180	31	Davis (1972)
Phospholipid	Sarcopl. reticulum	PL	NMR	180	25	Davis and Inesi (1972)
Cholesterol	DMPC	NBD-CHOL.	FRAP	200	26	Alecio *et al.* (1982)
Phospholipid	Asolectin[o]	DiI	FRAP	300	23	Chazotte *et al.* (1983)
Lyso-phospholipid	DMPC	NBD-LysoPE	FRAP	480	24	Derzko and Jacobson (1980)
Fatty acid	Red blood cell	PDA[p]	EXCIMER	540	25	Eisinger *et al.* (1986)

Ubiquinone	DMPC	Q_0C_{10}NBD	FRAP	550	30	Hackenbrock *et al.* (1985)
Phospholipid	DMPC	DiI	FRAP	600	30	Hackenbrock *et al.* (1985)
Phospholipid	DMPC	DiI	FRAP	670	24	Derzko and Jacobson (1980)
Phospholipid	DMPC	NBD-PE	FRAP	670	24	Derzko and Jacobson (1980)
Fatty acid	DMPC	NBD-C12[q]	FRAP	720	24	Derzko and Jacobson (1980)
Stearic acid	Microsomes	16-NS[r]	ESR	980	25	Stier and Sackman (1973)
Stearic acid	Asolectin	12-AS[s] and 16-NS	Fl. Quenc.[t]	8500	25	Fato *et al.* (1985)
Ubiquinone	Asolectin	Q1-10 and 12-AS[u]	Fl. Quenc.	60000	25	Fato *et al.* (1985)
Ubiquinone	SMP beef heart	Q3 and 12-AS[v]	Fl. Quenc.	60000	25	Lenaz *et al.* (1986)

[a] DMPC, dimyristoylphosphatidylcholine.
[b] CHOLES, 10 mole % cholesterol content.
[c] NBD-CHOL, *N*-4-Nitrobenz-2-oxa-1,3-diazole conjugated to cholesterol.
[d] FRAP, fluorescence recovery after photobleaching.
[e] PD-MITO L.I., mitochondria from flight muscle of *Lethocerus indicus* with partly damaged outer membrane.
[f] DiI, fluorescent lipid analogue. Dioctyl- or dihexyldecylcarbocyanine.
[g] FIM, fused ultralarge mitochondrial inner membranes from rat liver.
[h] Q_0C_{10}NBD, fluorescent ubiquinone analogue, NBD, hexanoic acid conjugated to 2,3-dimethyl-5-methyl-6-(10-hydroxyldecyl)quinone.
[i] MEGA, megamitochondria isolated from livers of cuprizone-fed mice.
[j] PL, phospholipid.
[k] NMR, nuclear magnetic resonance.
[l] NBD-PE, NBD fluorophore conjugated to phosphatidylethanolamine.
[m] FRAP-E, fluorescence recovery after photobleaching by the edge technique.
[n] PC, phosphatidylcholine.
[o] Asolectin, soybean phospholipids.
[p] PDA, 1-pyrene decanoic acid.
[q] NBD-C12, NBD fluorophore conjugated to lauric cid.
[r] 16-NS, Nitroxide spin label of stearic acid: 16-(*N*-oxyl-4,4′-dimethyloxalolidine)stearic acid.
[s] 16-NS and 12-AS, nitroxide stearic acid and fluorescent 12-(9-anthroyl)stearic acid quenching pair.
[t] Fl. Quenc, fluorescence quenching technique.
[u] Q1-10 and 12-AS, ubiquinones 1–10 with 12-(9-anthroyl)stearic acid as quenching pairs, average D in 10^{-6} cm^2/sec range.
[v] Q_3 and 12-AS, ubiquinone-3 and 12-(9-anthroyl)stearic acid quenching pair.

fluorescence quenching is a nonobstructed, short-range measurement, i.e., it determines D's over a distance where on average the diffusant (ubiquinone) would not encounter an obstruction (proteins) even though the latter are present. Therefore, using fluorescence quenching, the D measured for ubiquinones will be the same in the protein-rich inner membrane and protein-free lipid bilayers which Lenaz *et al.* found to be the case. Thus, high D's are obtained for small molecules such as ubiquinones using fluorescence quenching which cannot be related realistically to rates of electron transport since obstructive multicollisions with proteins, when they are present, are not included in the measurement, and since electron transport is a long-range diffusional process and indeed requires collisional interaction between redox partners.

In addition to this problem, D's in the 10^{-6} cm^2/sec range as measured by Lenaz's group for various ubiquinones are in themselves inordinately high and at variance with numerous reports on D's for small, lipoidal molecules in native membranes and lipid bilayers (Table VII). At temperatures similar to that used in the studies of Lenaz (25°C), the D's measured by various techniques in other laboratories, including our own, for phospholipids, cholesterol, ubiquinone analogue, and indeed fatty acids, have not exceeded the 10^{-8} cm^2/sec range, even in protein-free lipid bilayers (Table VII). It should be pointed out that a D in the 10^{-6} cm^2/sec range is equivalent to the D of sucrose in water at infinite dilution.

Various possible sources of error can explain the unusually high D's obtained by Lenaz's group for various Q's. Lenaz's group used the stearic acid probe 12-AS as a primary standard in determining the D's for Q's. They used 8.5×10^{-7} cm^2/sec as the D for 12-AS determined from the spin-labeled stearic acid, 16-NS, at 25°C in asolectin. This value is 8.6-fold greater than that reported by Stier and Sackman (1973) for 16-NS at the same temperature (Table VII). This D is also 28-fold greater than that of DiI in asolectin at 23°C and 11- to 18-fold greater than that of other fatty acids in membranes (Table VII). Clearly, the D used by Lenaz's group for their primary standard is not in agreement with established values.

Another source of error can arise from contributions of static quenching to dynamic quenching. Only the latter is related to the diffusion coefficient of the probes. There is clear evidence in the Stern–Volmer plots published by Lenaz's group (Fato *et al.*, 1985) of a static quenching contribution using the Q : 12-AS probe pair which is manifested as an upward curvature of the plots. Chance and Erecinska (1975) found previously an approximately one-third contribution of static quenching in both phospholipid bilayer and mitochondrial inner membranes using the same probe pair. In the presence of static quenching the bimolecular quenching constant results in anomalously high diffusion coefficients. It is our opinion that, although fluorescence

quenching is a sophisticated technique, it can be susceptible to various sources of error in determining diffusion coefficients.

FRAP, a proven and widely used technique for determining D's, is an ideal technique for measuring the lateral diffusion of ubiquinone in the inner membrane since the measurement includes the obstructive, multicollisions with proteins which affect both diffusion and electron transport rates. Accordingly, using FRAP we determined (Hackenbrock *et al.*, 1985, 1986) the D for the fluorescent ubiquinone analogue, $Q_0C_{10}NBD$ (Q) partitioned in the bilayer of the inner membrane to be 3×10^{-9} cm^2/sec, and in protein-free DMPC bilayers to be 5.5×10^{-8} cm^2/sec (Table VII). This finding is consistent with the factor of 10 difference predicted for two such membrane systems (Eisinger *et al.*, 1986). The D for Q determined by Gupte *et al.* (1984) has been questioned by Lenaz's group (Fato *et al.*, 1985) on the contention that an unphysiological ubiquinone analogue incorporated into the mitochondrial inner membrane was used. It should be pointed out that virtually all studies reported by Lenaz's group were carried out with various Q analogues partitioned into asolectin (phospholipid vesicles); one experiment was performed using Q_3 (and not naturally occurring Q_{10}) in submitochondrial particles which is far from physiologial.

We determined further by FRAP (Hackenbrock *et al.*, 1986) that the D for Q is equal to the D for the lipid analogue DiI, which is routinely used as a probe to determine the D of membrane phospholipid (Table VII). It is not surprising that the D's for Q and phospholipid in the inner membrane are essentially identical, since the phospholipids of the bilayer represent the medium for lateral diffusion, therefore setting the upper limit for D in the 10^{-8} cm^2/sec range for all membrane components. It is almost certain that the benzoquinone head group of Q oscillates toward both surfaces of the membrane (Stidham *et al.*, 1984; Aronada and Gomez-Fernandez, 1985). This permits Q to react with the active sites on NADH-, succinate-, choline-, electron-transferring protein-, α-glycerophosphate-, proline- and dihydroorotate-dehydrogenases in the inner membrane and of course to react with Complex III, its only known native oxidant in the membrane. Considering that the terminal portion of the isoprenoid chain of Q may lie in the hydrophobic center of the membrane with the head group of Q oscillating between the phospholipid monolayers, at least the head group must intercalate between the fatty acyl chain of both monolayers. Thus, as a molecule of Q diffuses laterally, it must exchange places with a molecule of phospholipid, the D of which will be limiting for the D of Q. To argue that Q diffuses only in the membrane midplane and yet require it to react near the membrane surface at times in order to function (Lenaz *et al.*, 1985, 1986) is to implicitly agree that Q will experience greater membrane microviscosities than believed to exist at the bilayer midplane.

Summary

The data and analyses presented in this review codify, expand, and confirm the random collision model of mitochondrial electron transport. We have presented five postulates upon which the model is founded and presented in detail the supporting data. We have critically reviewed the current literature devoted to diffusion of redox components. We have shown that the preponderance of evidence supports the random collision model and that there is no data that contravene the model. We conclude that mitochondrial electron transport is a diffusion-based random collision process and that diffusion has an integral and controlling affect on mitochondrial electron transport.

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