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CONCLUDING REMARKS

On the Role of Physical Parameters in the Regulation of Electron Transport: Diffusion, Collision, and Complex Formation

Martin Klingenberg[1]

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The mechanism of electron transport has been one of the most fascinating problems in biology. Only recently a stage has been reached where molecular parameters have become defined as a prerequisite for an understanding of the physics involved and where theoretical considerations and calculations become meaningful. These developments are: (i) The elucidation of the molecular composition of the components involved; (ii) the emergence of the three-dimensional atomic resolution of an electron transport complex; (iii) the analysis of the molecular architecture of the lipid phase of the involved membrane; (iv) high-resolution techniques for understanding primary events in electron transport; and (v) new physical methods to determine the vicinal interaction of electron-transferring nuclei.

On this basis a rebirth of interest in this classical field of biochemistry can be seen. Therefore, it is timely to assemble the viewpoints of leading scientists in this field. The presently discussed reviews deal only with a small part of the whole problem the diffusional and collisional parameters in the regulation of electron transfer.

Electron transport first evolved in photosynthetic systems in a kind of "solid state" because of the short-lived excited states of the initial electron donor. In the photosynthetic bacterial reaction centers the orientation of the electron donor and acceptor systems is now precisely known (Deisenhofer *et al.*, 1984) and it is here that accurate measurements of the early

[1] Institut für Physikalische Biochemie der Universität München, Goethestrasse 33, 8000 München 2, FRG.

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electron-transfer events and concomitant calculations are possible within the framework of the structural and electronic data (Michel-Beyerle, 1985).

Structurally organized electron transport within multicomponent protein complexes is also found at later evolutionary stages, i.e., in the respiratory chain. The homology between the mitochondrial bc_1 complex and the b_6f complex shows that the mitochondrial complexes are obviously derived from the early photosynthetic electron transport complexes. The cytochrome oxidase complex in oxygen-dependent prokaryotes is still structurally simple before it develops into a multicomponent complex in the mitochondria. Here again occur unstable intermediates during the stepwise oxygen reduction which are coped with by the "solid-state" electron transfer between the heme and copper centers.

Diffusional electron and hydrogen transfer is considerably slower and facilitates transfer between stable redox states of donor and acceptor. We can differentiate between two types of diffusional processes: (a) those by relatively small mobile molecules between larger, less mobile complexes [see Ragan (1986), Allred *et al.* (1986), Lenaz (1986), and Crofts (1986)] and (b) direct collisional interaction between these complexes within the membrane [see Hackenbrock *et al.* (1986) and J. Hochman *et al.* (1985)]. Earlier, diffusional electron transport was postulated simply on the basis that there is a large stoichiometric excess of some small transfer components. To these belong primarily the quinones, then probably also plastocyanines, and to a certain extent the *c*-type cytochromes. For some time, even for quinones diffusional processes have been negated and stacking of these components in a "bucket brigade" proposed, for example, of plastoquinone molecules (Rich, 1984). These proposals, however, disregarded basic chemical evidence that quinones do not readily interact with each other. Instead, in the reaction center a primary acceptor, quinone, is shown to exist, which can mix with the quinone pool [cf. Crofts, (1986)]. A most thoughtful review considering in particular these aspects has been presented by Rich (1984).

The best defined case for a small diffusional component are the quinones. Being largely unbound and dissolved in the lipid layer, they can efficiently diffuse in a two-dimensional space. After the discovery of ubiquinone (UQ) in mitochondria, it took considerable time to remove objections against its role in the respiratory chain, because, at first, due to technical difficulties, erroneous conclusions were drawn. Work from our laboratory established the role of UQ as a main electron pathway component on the basis of commensurable redox rates (Kröger and Klingenberg, 1966, 1967). With about 10 molecules UQ per cytochrome aa_3, UQ was considered to form a hydrogen pool. In analogy, menaquinone in bacteria was then shown to have a similar hydrogen transfer role (Kröger and Klingenberg, 1970; Kröger and Unden, 1985).

The proposal of a membrane-diffusible pool of Q was substantiated by several types of evidence. Most important was the homogeneous redox behavior of most of the mitochondrial Q, as elucidated by steady-state and kinetic analyses (Kröger and Klingenberg, 1967, 1973). The projection of the electron component densities on the mitochondrial membrane and the low molar ratio of the membrane-bound dehydrogenases to the cytochromes also argued for the existence of a linking diffusible pool. In one striking experiment a membranous cytochrome chain preparation devoid of succinic dehydrogenase, but still complemented with UQ, was titrated with SDH. Already at a molar ratio of 1 : 500 per cytochrome *a* all cytochromes and also most of the UQ present were reduced, illustrating the large diffusional distances which can be covered by UQ across the membrane (Kröger and Klingenberg, 1970). A postulated microcompartmentation of UQ between SDH and NADH-DH (Lenaz *et al.*, 1968; Gutman *et al.*, 1971) could be refuted and was shown to be due to different K_m of the two dehydrogenases (Kröger and Klingenberg, 1973).

The probing of the UQ diffusion rate by determining the collision-dependent fluorescent quenching of a lipid probe [see G. Lenaz (19??)] is consistent with the pool function where diffusion rates of UQ far exceed those required for electron transport. Therefore, diffusional control by UQ can be excluded.

Diffusion-mediated electron transport by cytochrome *c* between the complexes III and IV is much less stringently suggested by the same arguments as for UQ, based on the stoichiometric excess of cytochrome *c* over *a* and the substoichiometry of the bc_1 complex to the oxidase. Earlier, the variability of *c* between liver and heart, for example, suggested a non-ordered type arrangement of *c*. Moreover, the ratio of only 0.5 bc_1 complex per oxidase (Hatefi and Galante, 1981) would violate the solid-state chain concept unless one assumes that one bc_1 complex is paired with two oxidase complexes. However, in view of symmetry considerations, there are difficulties to visualize how both molecules of the bc_1 dimer would associate with one oxidase dimer. Extensive studies of Hackenbrock and his school (see chapter by C. Hackenbrock) on the diffusibility of the oxidase and then later on the bc_1 complex, and the possibility to segregate these complexes even macroscopically in the membrane, strongly favor their separate existence.

Based on early stoichiometric measurements, the distribution of the respiratory components, in particular the cytochromes and the dehydrogenases, as projected on the mitochondrial membrane, indicated considerable distances to be bridged by diffusion. The earlier calculated occupancy of one oxidase per 6000 $Å^2$ still forms the basis for current calculations of diffusion rates (Klingenberg, 1968). Nevertheless, Ferguson and her associates do not concur with the random collision model for cytochrome oxidase, but assume

dynamic aggregates in which electrons are transferred [see Ferguson (1985) and Hochman *et al.*, (1982)]. In other words, collisional complexes with a long lifetime are formed. Also the suggestion of immobile cytochrome complexes based on flash photolysis (Utsuma and Packer, 1967) and of an undisturbed electron transport in cross-linked proteins (Kawato *et al.*, 1981) may actually be explained by the heteorgeneity of the preparations. The data on cytochrome *c* diffusional rates [see Hackenbrock (1986)], however, question whether direct interaction between oxidase and bc_1 complex, either by random collision or by dynamic aggregates, is at all necessary.

Cytochrome *c*, in contrast to ubiquinone, is easily dissociable from the membrane. Considering that in the cristae the two outer surfaces of the inner membranes are quite closely apposed, cytochrome *c* should be able to transfer electrons to cytochrome oxidase of the opposite membrane. Since the distance between the two membrane surfaces may not exceed 60 Å, whereas the lateral distances between bc_1 and oxidase complex are on the order of 300–400 Å, one may propose that intermembrane electron transfer by cytochrome *c* is more efficient than the lateral transfer.

All considerations on the organization of electron transport have to keep in mind that a major purpose is energy transfer, i.e., the creation of a proton electrochemical potential. First, this function is linked to the transverse asymmetric arrangement of the electron carriers. It may also implicate a limited transmembrane diffusion or oscillation of UQ. The requirements for the transmembrane reaction are built into the structure of electron transfer complexes since any membrane protein complex should have the symmetry axis traversing the membrane. The lateral diffusion is less relevant to the transmembrane H^+ or change shift. UQ is considered also to either diffuse or only oscillate between the two redox centers of the bc_1 complex. To what extent these UQ molecules may be momentarily bound or free, remains unknown.

In conclusion, investigation of the electron transport system in mitochondria reveals a large dynamic variability between an organized solid state and a diffusional interaction. Within the often large solid-state building blocks, electron transfer is organized either to preserve short-lived or unstable intermediates and/or to provide the transmembrane charge shift necessary for the energy transfer via the proton electrochemical potential. The mere transfer of reducing equivalents between the more or less mobile, but segregated and stoichiometrically diverse, complexes is probably mediated by small electron and hydrogen carriers through diffusion.

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