The organization of the quinone pool

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Biological aspects of quinones

The widespread occurrence of \( p \)-benzoquinones in biological electron transfer chains had made them the subject of diverse studies. Although their participation in the electron-transfer processes is not disputed, two major views of the way in which they function have emerged. These might be loosely termed the 'liquid-state' and the 'solid-state' models. In the liquid-state model the quinone complement is viewed as mobile and freely diffusing in the lipid phase and electronically connecting the large multiprotein complexes. In the solid-state model the active quinone is envisaged as being permanently protein-bound, there being no interchain electronic mobility, with electrons from a given donor having access to only a single acceptor protein. A third interesting notion, originating from Ragan's group (Ragan & Heron, 1978), lies between these points of view, where it is suggested that interchain electronic mobility occurs, but is provided by collisions between the multiprotein complexes themselves, but with the electron transfers occurring through bound quinone species. Fig. 1 summarizes these models.

A model for the biological quinone

The evidence that there is electronic mobility in many electron-transfer chains seems overwhelming. Evidence includes relative ratios of components (for example, a ratio of complex \( 1/bc \), in mitochondria of \( 1:10 \) is common, and yet NADH will rapidly reduce all of the cytochrome \( c \)), sigmoidal inhibition with tightly binding inhibitors (Kröger & Klingenberg, 1973; Sigel et al., 1972) and double-inhibitor experiments with loosely bound inhibitors (Moreira et al., 1980). Other evidence has been cited previously (Rich, 1981). Molecular mobility of some sort must be invoked if we rule out the possibility of quinone acting as a 'bucket-brigade' for hydrogen atoms, a possibility which is unlikely on the basis of the chemistry of the quinones. Whether mobility is provided by quinones or proteins has been less clear-cut, but several pieces of evidence suggest rather strongly that it is the quinone which is providing the mobility. Firstly, direct kinetic measurements of redox changes of the quinone pool of mitochondria (Kröger & Klingenberg, 1973) and of chloroplasts (Stiel & Witt, 1969) have demonstrated the kinetic competence of the quinone pool. Secondly, quinone extraction–reconstitution studies tend to show that multiple-turnover electron flux rate is proportional to amount of quinone reincorporated, even at levels of quinone in excess of the electron-transfer components with which it interacts (Ernster et al., 1978).

The most reasonable model to emerge from these data, then, is one where the quinone itself act as the mobile carrier between the large protein donors and acceptors. Reactions occur only in environments which are in ionic contact with the outer or inner aqueous phase. During reaction, enzymic intermediates characteristic of 'bound quinones' (Yu & Yu, 1981) may be observed, but reducing equivalents enter or leave the pool by association/dissociation reactions of the quinone itself (Rich, 1981). Such

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**Fig. 1. Possible models of organization of electron-transfer chains involving quinone**

Arrows indicate rapid-diffusion pathways. Abbreviations used: Q, quinone; \( bc \), cytochrome \( b/c \) complex.

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a model is clearly an oversimplification for real electron-transfer systems and must be modified for several specific cases. Discussion of this point is raised in the final section of this presentation.

**Electron transfer from quinol through the bc1 complex**

My view is that the interaction of quinol with the bc1 (or chloroplast b/f) complex is, in the steady state, a second-order process, the rate being limited by diffusion rate. The transient complex formed involves both an electrostatic stabilization of QH2 by a positive charge and also by some hydrophobic bonding between quinol and some hydrophobic site on the bc1 complex (Rich, 1982). Some more recent experiments now allow further definition of the electron-transfer route from quinol through the bc1 complex to cytochrome c. The experiments were performed with purified ox heart mitochondria bc1 complex with trimethylquinol as donor. This quinol was used, since normal electron donor systems and must be modified for several specific cases. Location of components in non-interacting organelles and the high protein contents of some energy-transducing membranes will prevent this ideal situation. Extreme limitation to diffusibility together with rapid electron-transfer-chain activity will cause loss of Q-pool behaviour even although the essential mechanism stays the same. Non-random organization of components could then cause some degree of selectivity of electron transfer from a particular donor to a specific type of acceptor. Such behaviour should, however, be regarded as the exception rather than the rule of organization.

One further possibility for specific quinone-mediated electron-transfer routes arises when several types of quinone occur in the system, such as in the E. coli respiratory chain, particularly since it is likely that equilibration of different quinols and quinones within the membrane is a rather slow process. However, menaquinone and ubiquinone are rather similar in their physical and chemical properties and no evidence for such structural specificity has yet been demonstrated by any acceptor or donor system.

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Vol. 10
DISCUSSION

Peter Garland: What sort of lateral diffusion coefficient would you want for the ubiquinone to do its job in, say, beef heart or rat liver mitochondria?

P. R.: I am sorry, I would not give you a figure on that.

P. G.: I did a little sum and worked out that if it was what you would think it was, like every peoples' lipid, it would go 140 nm in 10 ms, which I think would do you very nicely.

P. R.: There is perhaps a rather more extreme situation which might be of interest. It is in stacked thylakoids, where it is clear that Photosystems II and I become quite separated and one needs a mobile component there over a really very large distance and I worry about the speed required for that particular electron transfer.

Tony Crofts: In the chromatophores we can get a rough idea of what the mobility of the quinone is from its behaviour. We look at the lag before the bc complex gets its electron from the reaction centre at high redox potential, and that gives the diffusion coefficient as of the same order as Peter Garland has just mentioned, 10⁻⁵ cm² s⁻¹: rather lower than what one would expect for a lateral diffusion constant only, and it might include the diffusion of the head group across the membrane.

P. R.: Would you expect that to be as slow?

T. C.: The apparent diffusion coefficient that we measure has several components; the leaving time for the quinone from the reaction centre, the diffusion from the reaction centre to the bc complex and the flip across the membrane of the electron-carrying group, if that is required. I would imagine that the flip is the slowest of those, since we can measure the leaving time, and that is not rate-limiting, and the diffusion time of small molecules is generally rather higher than the apparent diffusion coefficient that we get.

Brian Chappell: You imply that the body of the phospholipid bilayer is an aprotic medium and this is very often found in the literature, but I call this into question. Phospholipid membranes are known to be very permeable to water and water gas would be able to get across those membranes very quickly and therefore to rule out certain mechanisms may not be correct. There is plenty of water there.

P. R.: Well, I think the important point is the effective pH within the membrane. As one goes to more acidic pH, and also as it becomes more difficult to get ionic species...

B. C.: Yes, but they would be ion-paired. If you have got a semiquinone free radical it could react with a water molecule, and produce that plus a hydroxyl, and the things would in fact be balanced, and in that way I think it would be possible to get electron conduction. The second thing I wanted to ask was, why has Nature gone to all this trouble to put this enormously long hydrophobic tail on these things, and then one implies that the quinone can flip across the membrane. If you wanted to do that process it would be better to have a much shorter tail.

P. R.: I think that the hydrophobic tail is one of the most mysterious parts. It seems clear that there isn't a specific side-chain length, based first of all upon the naturally occurring quinones that can vary from Q₁ to Q₁₀, and secondly the reincorporation studies that can usually use anything from Q₁ to Q₁₀ and the electron-transport chain can apparently work just as well.

B. C.: Yes, but Nature has selected for long side chains; both 7 and 10 are long chains in respect to the width of the membrane.

P. R.: Well, until we can find a function which changes when we change the side-chain lengths we are not going to be able to say very much about the role of the side chains.

T. C.: The question has come up in the past of whether there is a bucket-brigade mechanism in the electron pathway through the quinone pool and in experiments with the Melandri's laboratory we have extracted the quinones until there is on average only one mobile quinone per bc complex, and what we find is that the electron-transfer rate from the reaction centre to the bc complex is the same in these preparations as it is in the unextracted chromatophores when the quinone pool is oxidized and only one quinol is introduced into the pool. So this suggests that the presence of the bulk quinone in the pool doesn't affect the rate at which reducing equivalents get to the complex. That is, that it is a diffusional process rather than a bucket-brigade process. This rather supports your argument.

Douglas Kell: If one does the experiment, this has been done in chromatophores, where you do an electrochemical experiment with the electrode and you do the cyclical voltamogram just like you displayed for the quinol: they are entirely electro-inactive unless you disperse them in Triton, and that, would not you think, is somewhat unexpected if there is a lot of bulk quinone with its head groups off the membrane surface.

P. R.: Well, the problem there is that to get an electrochemical process to occur efficiently, the problem is getting something up to the electrode surface. Now, when the thing is in a membrane it depends on the exact structure of the membrane and its surroundings as to whether any electron transfer will occur at all. I must say, I don't find it surprising in any way that something inside a membrane doesn't interact with the electrode surface even although its electrochemical reaction may be occurring at that (the membrane's) surface.

The diffusional mobility of proteins in the cytoplasmic membrane of Escherichia coli

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There are several possibilities whereby individual respiratory-system components can meet with each other in order to effect the transfer of electrons from donor to acceptor. For instance, the components could be organized into multienzymic complexes, each a complete respiratory ensemble. Alternatively the components could be randomly distributed in the membrane bilayer and come together by diffusional collision. Measurements of the rotational and lateral diffusional coefficients (Dₗ and Dₕ) would discriminate between such models just described, and towards this end we have measured Dₗ for two terminal oxidases, cytochrome o and cytochrome aₙ. Both of these oxidases form