Possible Function of Vitamin K and Related Quinones in Oxidative Phosphorylation

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WE HAVE BEEN interested for some time now in the formation, transformation, utilization, distribution and control of energy at the subcellular level. During the past few years we have focused our attention on the energy reactions associated with rat liver mitochondria and more specifically those coupled with the oxidative processes utilizing the respiratory chain.

We have found that if one irradiates mitochondria with ultraviolet light the energetic efficiency; i.e., the amount of ATP formed per atom oxygen utilized, was lowered accompanying the oxidation of beta-hydroxybutyrate, whereas it was not altered when reduced cytochrome c was the substrate. It was further shown that added vitamin K_1 was able to restore the depressed system to near normal.[1,2] From these investigations it was concluded that (1) vitamin K_1 was either directly or indirectly concerned with the phosphorylative mechanism accompanying oxidation and (2) the phosphorylative site(s) affected was between DPN^+ and cytochrome c.

Recently we have extended these irradiation studies and attempted to correlate the irradiation effect with certain fairly well established energetic reactions that occur in mitochondria. In Figure 1 we see that phosphorylation associated with oxidation is inhibited by irradiation with ultraviolet light to a lesser extent than is the P_i-ATP exchange reaction. This indicated, as has been suggested by others, that the limiting reaction in the sequence of reactions concerned with the phosphorylative processes is the initial set of reactions concerned with the transfer of energy to some intermediate compound; i.e., steps 1 and 2, Figure 2. In preliminary comparative experiments it has also been found that DNP stimulated ATPase is inhibited to a lesser degree while magnesium stimulated ATPase to a greater degree than either oxidative phosphorylation or P_i-ATP exchange. These recent findings appear to have certain implications regarding the over-all question of phosphorylative reactions related to oxidation but will not be elaborated.

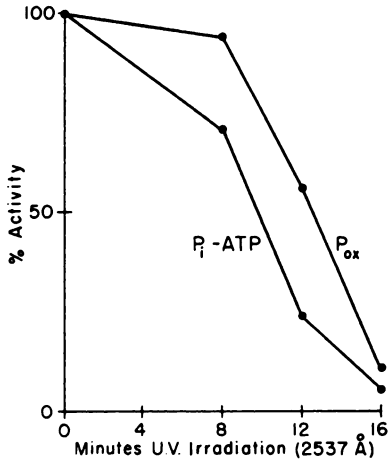

FIG. 1.

1) $AH_2 + X \rightleftarrows XH_2 + A$
2) $XH_2 + B \rightleftarrows \sim X \sim + BH_2$
3) $\sim X \sim + Pi \rightleftarrows X \sim P$
4) $X \sim P + ADP \rightleftarrows ATP + X$

FIG. 2.

Let us now turn our attention to the question of how vitamin K_1 could participate in these

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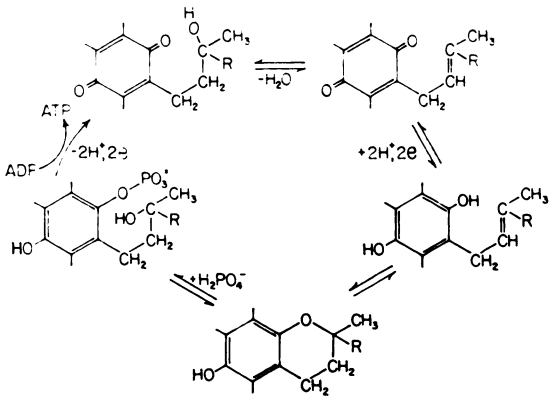

FIG. 3

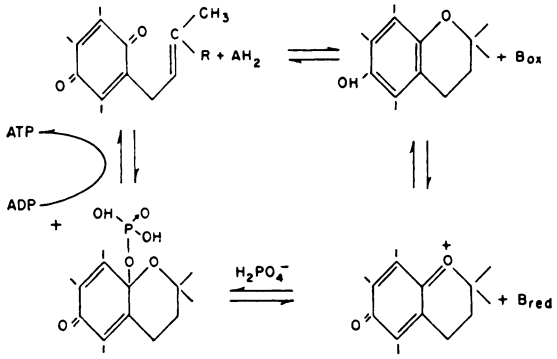

FIG. 4

coupled energy reactions. Several other investigators have speculated that vitamin K and quinones, in general, could act as a cofactor in the production of ATP by oxidation of quinol phosphate in the presence of a suitable acceptor such as ADP. Professor Taylor and I approached this phase of our work by first considering the problem of how inorganic phosphate could combine with quinones to form a phosphoquinol, a step which must necessarily precede the oxidation-energy transfer reaction.[3]

Examination of the structure of several quinones which have been implicated in biological oxidation and to a lesser extent in the accompanying phosphorylations, vitamin K, ubiquinone and vitamin E, reveals several common features; all are para quinones; all are completely substituted, and all possess a side chain with basically the same carbon skeleton. Although two of these, vitamin K_1 and ubiquinone possess unsaturated open side chain and vitamin E is a ring structure, it is well known that the two side chain structures, open and ring, are readily interconvertible chemically. For example, the quinone molecules with an open isoprenoid side chain can be reduced to their respective hydroquinones but if this is carried out in acid solution one obtains the cyclic derivatives of the tocopherol type. It should be emphasized that these three compounds are able to cyclize during reduction to form the tocopherol derivative. Thus, we see that these three quinones have similar structures and are capable of undergoing similar chemical transformations. May these similar chemical reactions in any way afford a pathway for the incorporation and transfer of energized inorganic phosphate to ADP during mitochondrial oxidative phosphorylation?

Considerations of the energetics of any proposed mechanism absolutely require the introduction of inorganic phosphate into the hypothetical intermediate in some manner which does not require a great deal of energy; i.e., there must be an equilibrium between inorganic phosphate and the organic phosphate which is not too far to either side and, of course, ATP cannot be used up. Once this can be done, the possibilities of oxidation of the hydroquinone phosphate to a high energy compound presumably could follow the reaction pathway suggested by others.

One such hypothetical mechanism proposed by us is seen in Figure 3. Here we see that a quinone with the proper prerequisites, such as vitamin K, ubiquinone or trimethyl-phytyl benzoquinone (oxidized form of vitamin E) can undergo reduction to the quinol and then cyclize to the tocopherol. Inorganic phosphate might then be capable of splitting the chroman ring by phosphorolysis resulting in the formation of the hydroquinone monophosphate ester with a hydroxylated side chain. Attention should be focused on a primary requisite of this and other schemes we have proposed; i.e., cyclization of the side chain to form a chroman ring precedes inorganic phosphate combination with the quinone. Preliminary experiments have indicated that cyclization of the quinone is probably an essential in the functioning of vitamin K in the irradiated systems. When the beta-gamma dihydrophtyl derivative of vitamin K_1,

TABLE I
Effect of Benzoquinones on Oxidative Phosphorylation

Compound	Molar Concentration	P_i (μM)		O (μatoms)		Activity* (%)	
		Control	Quinone	Control	Quinone	Phos.	Ox.
U_0	$1x10^{-3}$	3.31	0	2.64	0	0	0
	$2.2x10^{-4}$	2.91	0	2.45	0	0	0
	$5x10^{-5}$	3.32	2.23	2.90	2.42	80	83
	$1x10^{-5}$	3.32	3.03	2.80	2.65	93	95
U_5	$1x10^{-3}$	4.11	1.52	3.17	2.90	37	93
	$1x10^{-4}$	5.00	4.50	3.36	3.20	90	96
	$1x10^{-5}$	4.00	3.80	3.50	3.40	95	96
Trimethyl phytyl quinone	$1x10^{-3}$	3.39	1.25	2.39	1.44	40	81
	$1x10^{-5}$	3.90	3.63	3.01	2.81	93	93
U_{phytyl}	$2x10^{-3}$	5.14	4.40	2.18	2.15	88	100
	$1x10^{-3}$	6.01	5.71	2.59	2.59	95	100
	$2x10^{-4}$	4.00	3.83	2.30	2.30	96	100
	$1x10^{-5}$	5.00	5.00	3.00	3.00	100	100
U_{50}	$1x10^{-4}$	3.52	3.41	2.71	2.70	96	100
	$1x10^{-5}$	2.96	2.96	2.60	2.60	100	100

* Per cent inorganic phosphate exchanged of control.

which cannot cyclize due to its saturated side chain, was added to irradiated mitochondria, no energetic restoration was observed.[3] According to Clark et al.,[4] the phosphoquinol could in the presence of ADP be oxidized to yield ATP and the starting quinone.

In our hypothetical mechanism and those of others the final reaction involves the oxidation of the quinol phosphate in the presence of a suitable acceptor, presumably ADP, to yield ATP and the original quinone. However, it would appear that all of these mechanisms require revision due to a discrepancy between these proposals and the existing data. The fact that P_i-ATP exchange occurs extremely rapid and equally well either in the presence or absence of substrate, as originally shown by Boyer et al.,[5] almost demands that the intermediate compound, X∼P does not undergo oxidation for exchange and thus would not require oxidation as one of the final steps in the generation of ATP (see Fig. 2). It would thus appear that the previously proposed mechanisms are incorrect. In Figure 4, a simplified mechanism is presented that perhaps more accurately depicts the chemical mechanisms concerned with mitochondrial oxidative phosphorylation if quinones do indeed play a direct role in ATP formation.

The starting quinone can be any fully substituted paraquinone possessing an isoprenoid side chain adjacent to one of the quinoid groups; i.e., vitamin K, ubiquinone and the group of oxidized E vitamins. Such a quinone could be reduced, presumably by one of the members of the respiratory chain (AH_2) and then oxidized presumably by another member of the chain (B_{ox}) to yield the oxonium derivative which would freely resonate to the carbonium ion. This electrophilic compound, presumably stabilized by the enzyme, in the presence of nucleophilic inorganic phosphate, could be converted to the phosphorylated hemi-ketal which then could donate the energized phosphate to ADP under proper conditions. The validity of the above proposal is currently being investigated through the aid of synthetic compounds and for the present, this scheme is accepted as a working hypothesis. A similar scheme has been proposed by others.[6,7]

Since we have been thinking about general reactions of quinones; i.e., reactions which not only apply to vitamin K but also ubiquinone, the possible role of ubiquinone in oxidative phosphorylation has also been investigated.[8] Intact rat mitochondria have been used which retained the internal ubi-

TABLE II
Effect of Benzoquinones on P_i^{32}-ATP Exchange Reaction

Compound	Molar Concentration	P_i Exchanged (μM.)		Activity (%)
		Normal	Quinone	
U_0	2.17×10^{-3}	0.90	0.008	0
	2.17×10^{-4}	0.90	0.008	0
	1.1×10^{-4}	1.34	0.046	4
	2.17×10^{-5}	0.90	0.65	70
	2.17×10^{-6}	0.90	0.82	91
U_5	2.17×10^{-3}	0.90	0.008	0
	1.1×10^{-3}	1.34	0.28	21
	2.17×10^{-4}	0.90	0.54	60
	5×10^{-5}	0.90	0.62	69
	2.17×10^{-5}	0.90	0.73	78
	2.17×10^{-6}	0.90	0.83	92
U_{phytyl}	2.17×10^{-3}	0.90	0.76	85
	2.17×10^{-4}	0.90	0.76	85
	2.17×10^{-5}	0.90	0.76	85
	2.17×10^{-6}	0.90	0.81	92
U_{50}	2.17×10^{-4}	1.34	1.20	90
	2.17×10^{-5}	1.34	1.20	90
	2.17×10^{-6}	1.34	1.29	92

quinone presumably intact. Varying concentrations of several homologues of ubiquinone were added separately and in combination to mitochondrial systems undergoing energy transformation and to systems carrying out P_i-ATP exchange. In Table I see that only ubiquinone$_0$ and trimethyl phytyl benzoquinone inhibit oxidation. Because of the greater amount of inhibition of ubiquinone$_0$ it would appear that the absence of a side chain on the quinone has a greater effect on mitochondrial oxidation than the substitution of methyl for methoxy groups. However, since phosphorylation was completely inhibited by ubiquinone$_0$ at this concentration, this conclusion is presented with reservations. It is also seen that ubiquinone$_0$, ubiquinone$_5$, trimethyl phytyl benzoquinone and ubiquinone-phytyl, in high concentration, inhibit phosphorylation associated with oxidation and the magnitude of inhibition is in that order.

These data suggest that the added ubiquinone homologues that inhibit the phosphorylative process may be acting as competitive inhibitors; i.e., by competing with the internal ubiquinone and for specific enzymes. Also, the decrease of inhibitory effect with increase in length of the isoprenoid side chain seems to indicate that the isoprenoid side chain is necessary for the proper function of ubiquinone.

In Table II we see that the P_i-ATP exchange reaction is inhibited by ubiquinone$_0$ and ubiquinone$_5$ but not substantially by ubiquinone$_{phytyl}$ or ubiquinone$_{50}$ and that this inhibition, like that observed with oxidative phosphorylation, is concentration dependent. In Figure 5 the graphic presentation of the data from Table I and II is shown.

In Table III it is seen that partial reversal of the inhibition is achieved when ubiquinone$_{phytyl}$ or ubiquinone$_{50}$ are added to the ubiquinone$_0$ and ubiquinone$_5$ inhibited P_i-ATP exchange systems. The restoration can be seen more clearly in Figure 6 which is a graphic reproduction of the data presented in Table III.

TABLE III
The Effect of U_{phytyl} or U_{50} on U_0 or U_5 Inhibited P_i-ATP Exchange

Molar Concentration Quinone(s)*	Activity† (%)					
	U_0	$U_0 + U_p$	$U_0 + U_{50}$	U_5	$U_5 + U_p$	$U_5 + U_{50}$
1.1×10^{-3}	...	...	...	21	44.5	...
2.17×10^{-4}	0	7	0	60	80	70
2.17×10^{-4}	4	17	14[a]	...	...	...
5×10^{-5}	...	...	...	69	86[b]‡	86[a]§
2.17×10^{-5}	70	74[b]	74[a]	...	...	...

* In all cases, the concentration of U_p or U_{50} added is the same as that of U_0 or U_5 unless otherwise specified.
† Percent inorganic phosphate exchanged of control.
§ a=U_{50}, 1×10^{-4} M.
‡ b=U_p, 1×10^{-4} M.

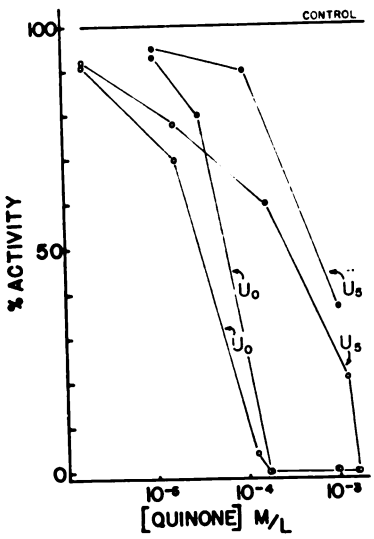

FIG. 5

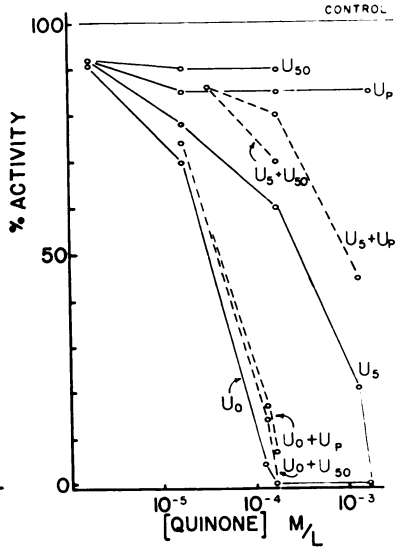

FIG. 6

SUMMARY

In conclusion, from the data presented it would appear that vitamin K and ubiquinone may participate in the transformation of energy during mitochondrial oxidation reduction reactions. Whether they are acting as cofactors and enter into the chemical reactions directly is a question that cannot yet be answered, at least, not by this investigator.

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