EVIDENCE AGAINST THE FORMATION OF QUINONE METHINE INTERMEDIATES IN OXIDATIVE PHOSPHORYLATION

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The purpose of this communication is to question the hypothesis that quinone methines are intermediates in oxidative phosphorylation.

The mechanism of oxidative phosphorylation and its specific role in electron transport has been the centre of much interest in the last few years (Lehninger and Wadkins, 1962; Racker, 1961; Vilkas and Lederer, 1962). It has been shown that many of the coenzymes involved in electron transport are quinone derivatives and the formation of quinone phosphates has been suggested as one of the steps in oxidative phosphorylation in the cell (Brodie, 1961; Arnon, 1961). The most recent hypothesis (Chmielewska, 1960; Vilkas and Lederer 1962) to account for the actual phosphorylation step involves the reversible formation of a quinone methine intermediate, as shown below

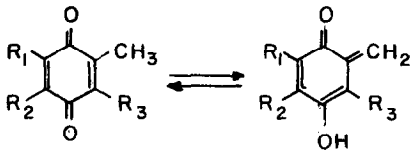
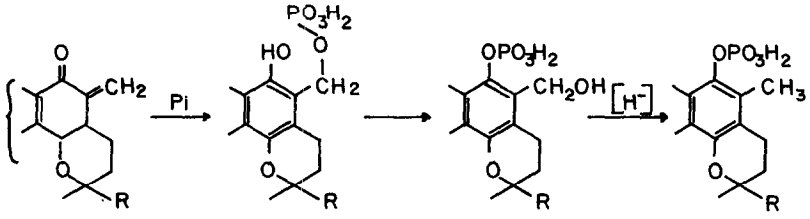


Fig. 1

Vilkas and Lederer 1962, suggested that phosphorylation occurs initially at the methine group, followed by transfer of phosphate to the hydroxyl group:



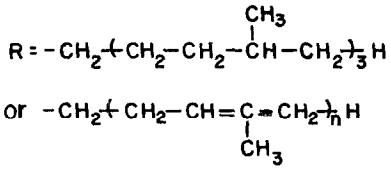


Fig. 2

Chemical support for this mechanism has been provided by the synthesis of phosphomethyl derivatives of quinone by Folker's group (Wagner et al, 1963). Although the synthesis of such compounds supports the quinone methine hypothesis it by no means proves that such compounds are intermediates in oxidative phosphorylation. However, evidence has recently been put forward (Scott,1965) to show that certain quinone derivatives related to coenzyme Q and vitamin K_1 can undergo isotopic hydrogen exchange in alkaline solution. Such exchange would be expected if the equilibrium shown in figure 1 were to take place in solvents containing exchangeable deuterium, and could be considered to be strong supporting evidence for the reversible formation of quinone methine intermediates. In this communication we bring forward evidence against the occurrence of hydrogen exchange in quinones.

The evidence for hydrogen exchange is based on the observation than when duroquinone and trimethyl (3-methyl-2-butenyl)-1,4 benzoquinone are allowed to stand in basic (NaOD) methanol-d (CH_3OD) solutions the nuclear magnetic resonance (NMR) peaks due to certain protons in the

molecules lose intensity, indicating that some of these protons have been replaced by deuterium which does not contribute to the intensity of the signal. We have three independant proofs that hydrogen isotope exchange does not in fact occur in duroquinone.

(1) We have observed that although the methyl peak of duroquinone is broadened and loses height following the addition of base to the solutions, the total area remains effectively unchanged as shown by integration of the area under the recorded absorption line. This shows that none of the protons contributing to the area of the NMR peak has been replaced by deuterium. The extent of broadening of the methyl peak appears to be related to the concentration of base in solution, stronger base giving greater broadening.

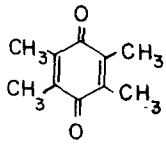
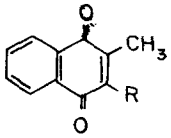
(2) On neutralization of a basic solution of duroquinone, peaks which were broadened in alkali are restored completely to their original height and intensity. The spectral changes on successive addition of NaOH and HCl, are the same in both normal and deuterated solvents, indicating that these changes are not connected with deuterium exchange, and that such exchange does not occur in these conditions.

(3) A solution of e.g. duroquinone in methanol-d treated with NaOD showed a very broadened resonance peak due to the methyl groups. After neutralization with DCl, the quinone was recovered by evaporation of the solvent and sublimation in vacuo. When redissolved in methanol-d the recovered quinone gave a NMR spectra identical with that of normal duroquinone in normal methanol, with no loss of intensity or broadening of the methyl resonance.

The nature of the chemical processes responsible for the spectral changes is not known. In a concentrated ethanol solution of duroquinone (tetramethyl benzoquinone) addition of KOH results in the formation of a dimer of unknown structure (Smith, 1944; Cameron, 1964). The precise details of the reactions occurring in alkali are however

not relevant to the conclusion that hydrogen isotope exchange does not occur. In vitamin K_1 (20) hydrogen isotope exchange was not observed, consistent with the negative results of Scott (1965) for 2,2,5,7,8 pentamethyl 6-chromanyl phosphate. The lack of hydrogen exchange in the latter compounds is a particularly strong point against the hypothesis of quinone methine formation, since calculations of Lederer (1965) suggest that in these molecules the methine group will be stabilized by the cyclic side-chain.

We have not examined trimethyl (3-methyl-2-butenyl)-1,4 benzoquinone, the other compound stated to undergo hydrogen isotope exchange under basic conditions. Scott states that in methanol-d containing 0.1M NaOD the peak due to the methyl group on the ring lost about 95% of its intensity in 20 minutes whilst a control lost about 20% of its intensity in the same time. If the loss of intensity on addition of base to this compound depends on the concentration of base, as we have found for duroquinone, it may be that the difference between the control and the experiment can be explained by a small difference in the base concentration. We conclude that there is as

	Solvent	Temp.	Reaction Time
	Methanol-d Substrate 0.02N	23°	30 min.
	Methylene chloride containing 0.4M CH_3COOD and 2.4×10^{-3}M $HClO_4$	4 - 5°	4 hrs.

R = $-CH_2CH{=}\underset{\displaystyle CH_3}{\underset{|}{C}}CH_2(-CH_2CH_2\underset{\displaystyle CH_3}{\underset{|}{C}}HCH_2)_3-H$

yet no evidence from hydrogen isotope exchange to support the hypothesis of reversible quinone methine formation in oxidative phosphorylation.

The experimental conditions are listed below.

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