Vitamin K as a cofactor of photosynthetic phosphorylation

The search for cofactors of photosynthetic phosphorylation (PSP)[1] has led to the identification of vitamin K compounds as important participants in this process (*cf.* footnote 7a in ref.[2]). Menadione, phthiocol, and vitamin K_5 (Table I) were found to stimulate PSP markedly. Since vitamin K is characteristically associated with green tissues[3], and was shown to be concentrated in chloroplasts[4], we conclude that it is a cofactor of photosynthetic phosphorylation *in vivo*.

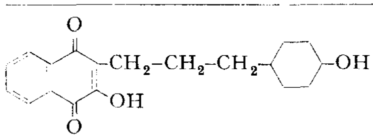
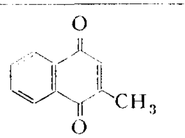
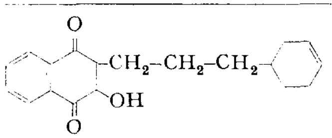
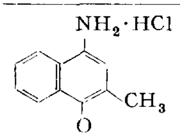
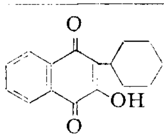
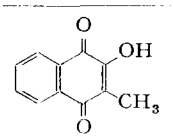
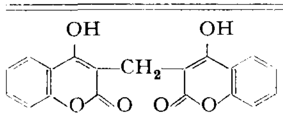
Previous experiments with vitamin K compounds, such as phthiocol and menadione, on the photosynthesis of whole cells[5] and on the Hill reaction[6] have demonstrated only an inhibitory action. These findings have led, on the one hand, to the conclusion that menadione and phthiocol "interact with the photochemical process by forming compounds with one or more heavy metal catalysts"[5], and, on the other, to a postulation, on theoretical grounds, that vitamin K "might play a part in the transfer of hydrogen in photosynthesis"[6]. The reported concentration of vitamin K in chloroplasts[4] has led during the past five years to a number of attempts in this laboratory to demonstrate a stimulating effect of this compound on the photochemical reactions of isolated chloroplasts. Only with the discovery of PSP[1,2] were we able to establish this effect.

In our PSP experiments menadione was used as the principal vitamin K compound. As in the presence of flavin mononucleotide (FMN)[1], both magnesium and ascorbate are required for anaerobic PSP with menadione. Under aerobic conditions there was very little stimulating effect of menadione. There was no phosphorylation in the dark in the reaction mixture containing menadione.

Table I shows the effect of various naphthoquinone derivatives on anaerobic PSP. The response of PSP to these naphthoquinone derivatives appears less specific than that of the blood-clotting

TABLE I

EFFECT OF VIT. K SUBSTANCES AND OTHER SUBSTITUTED NAPHTHOQUINONES ON PHOTOSYNTHETIC PHOSPHORYLATION

Naphthoquinone derivate (N.D.) added	P_i *esterified* 0.3 µm N.D.	P_i *esterified* 0.3 µm N.D. + 0.3 µm Menadione	*Naphthoquinone derivative (N.D.) added*	P_i *esterified* 0.3 µm N.D.	P_i *esterified* 0.3 µm N.D. + 0.3 µm Menadione
None	0.8	—	 A	19.3	10.5
 Menadione	19.3	18.8	 B	8.7	11.0
 K_5	19.7	19.7	 C (M-266)	0.5	2.8
 Phthiocol	19.1	19.0	 Dicumarol	0.3	4.8

reaction for which vitamin K is noted. For example, although the anti-haemorrhagic activity of phthiocol is only a small fraction of that of menadione[7] these two compounds were almost equally effective as cofactors of PSP. Those naphthoquinone derivatives which produced only a limited or no stimulation of PSP by themselves acted as inhibitors and depressed the activating effect of added menadione.

Green leaves are also known to be rich sources of riboflavin and its derivatives[7]. The problem arises whether FMN (which was previously found to be a cofactor of PSP[1]), and vitamin K are involved in separate pathways for PSP, or whether they form components of one chain. Fig. 1 indicates that the effects of FMN and vitamin K are interdependent. The effect produced by 0.1 μM of FMN depended on the amount of menadione added with it.

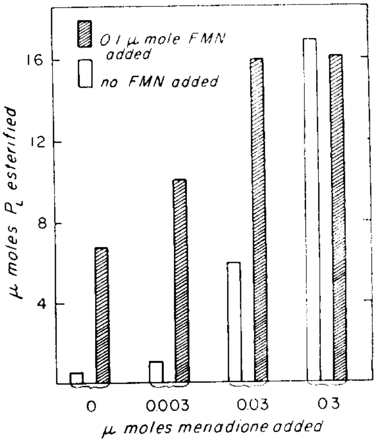
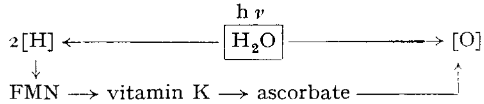


Fig. 1. Interdependence of FMN and menadione. Experimental conditions are given in Table I.

On the basis of the evidence now available, a tentative scheme for PSP is presented below



The light energy used for the photolysis of water is converted into the pyrophosphate bond energy of adenosine triphosphate during the transport of [H] to [O] *via* a series of electron carriers of which three, FMN, vitamin K, and ascorbate, have so far been identified. The identity of the electron carriers above ascorbate is unknown, but they may very likely prove to be components of a cytochrome system[8,9,10]. The relative positions assigned to FMN and vitamin K in the electron "ladder" are tentative, and based solely on published values of redox potentials[11]. It is possible that *in vivo* their positions are reversed. FMN can serve as the initial electron acceptor in the photolysis of water accomplished by one quantum of red light (44 Kcal.). Assuming an oxidation level of [O] equal to that of molecular oxygen at 0.2 atm., enough energy would then be available to maintain the ratio of [FMNW]/[FMNH] at approximately 5 at pH 7. Transfer of electrons from this reduction level to that of [O] would provide sufficient energy for the synthesis of at least three pyrophosphate bonds.

Within the last year vitamin K has been reported to be involved in oxidative phosphorylation in animal cells[12].

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