STUDIES ON PHOTOSYNTHETIC PHOSPHORYLATION

I. PHOTOSYNTHETIC PHOSPHORYLATION UNDER ANAEROBIC CONDITIONS

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In a previous paper[1] it has been postulated that vitamin K serves as the initial electron acceptor in photosynthesis. It was suggested that partial reoxidation of photochemically-reduced vitamin K via cytochrome *c* or cytochrome *f* generates high-energy phosphate bonds. The pyrophosphate bonds of ATP could provide the supplementary energy for the reduction of pyridine nucleotides, which requires an input of more than 42 kcal (the energy of one quantum of red light).

ARNON *et al.* [2-5] have reported that under anaerobic conditions in the absence of CO_2, chloroplasts supplemented with flavin mononucleotide (FMN), Mg ions and ascorbate, are able to convert light energy into the chemical energy of pyrophosphate bonds of ATP. In line with our hypothesis, this reaction was found to be stimulated by menadione (vitamin K_3).

ARNON *et al.* presented the following tentative scheme for photosynthetic phosphorylation.

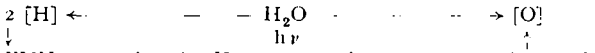

Fig. 1

This scheme implies that the recombination of the oxidized [O] and reduced [H] product of the photolysis of water proceeds via a series of electron carriers, *viz.* FMN, vitamin K, ascorbate and, possibly, components of a cytochrome system. The energy liberated thereby is stored in the pyrophosphate bonds of ATP.

In this paper some results of our experiments on anaerobic photosynthetic phosphorylation are reported, which indicate that FMN and vitamin K_3 are involved in separate pathways for photosynthetic phosphorylation, and support the idea that cytochromes serve as electron carriers in the "electron ladder' shown in Fig. 1. Results obtained under aerobic conditions will be presented in a forthcoming communication.

The ATP which was formed from ADP during photosynthetic phosphorylation was used to synthesize glucose-6-phosphate by adding hexokinase, glucose and $MgCl_2$. Measurements of phosphorylation by accumulation of glucose-6-phosphate and by disappearance of inorganic phosphate are in good agreement. For example, we have observed the formation of 10.8 μmoles of glucose-6-phosphate, while at the same time 10.5 μmoles of inorganic phosphate disappeared. Inorganic phosphate was determined by the method of LOHMANN AND JENDRASSIK[6].

References p. 100.

TABLE I

PHOTOSYNTHETIC PHOSPHORYLATION UNDER ANAEROBIC CONDITIONS

The reaction mixture included, in addition, 40 μmoles of Na and K phosphate buffer, pH 7.2, 10 μmoles of $MgCl_2$, 125 μmoles of glucose, 1 μmole of ADP, 15 K.M. units[7] of hexokinase, 1 ml of a suspension of chloroplasts in 0.1 *M* Tris buffer, pH 7.2, and deionized water to give a final volume of 3.0 ml. Chloroplasts were isolated from spinach leaves. The leaf blades were ground in 0.1 *M* Tris buffer, pH 7.2, containing 90 g glucose per litre. Whole chloroplasts were separated by centrifugation at 1000 *g* for 7 minutes. The chloroplasts were washed once with the same solution and resuspended in 0.1 *M* Tris buffer, pH 7.2. An aliquot of the suspension containing 1 mg of chlorophyll was used in each test. The reaction was carried out at 15°C in an illuminated Warburg respirometer with continuous shaking. Anaerobic conditions were maintained by filling the vessels with nitrogen and having chromous chloride in a side-arm. The reaction was terminated after one hour by adding 0.3 ml of 20% trichloroacetic acid to each vessel. Chloroplast debris was removed by centrifugation, the precipitate washed with 2% trichloroacetic acid, and the supernatant liquid and washings neutralized with KOH and made up to 10 ml.

Glucose-6-phosphate was determined by the increase in absorption at 340 mμ with TPN and glucose-6-phosphate dehydrogenase, prepared by the method of KORNBERG[8]. Hexokinase was prepared by the method of BERGER *et al.*[9], carried to step 5. Cytochrome *f* was isolated from parsley leaves according to the method of DAVENPORT AND HILL[10].

Cytochrome *c* was obtained from Boehringer, Mannheim; TPN, FMN and ADP were products of the Sigma Chemical Company.

Additions (μmoles)	*ATP (μmoles)*	*Additions (μmoles)*	*ATP (μmoles)*
0.1 FMN + 40 ascorbate	4.2	0.4 vit. K_3	3.7*
0.1 FMN	4.1	0.4 vit. K_3 + 0.2 cyt. *c*	3.9*
0.1 vit. K_3 + 40 ascorbate	5.9	0.4 FMN	1.8*
0.1 vit. K_3	5.5	0.4 FMN + 0.2 cyt. *c*	3.9*
40 ascorbate	0.7		
no addition	0.6	0.5 vit. K_3	4.5
0.1 FMN	4.3	0.5 vit. K_3 + 0.042 cyt. *f*	2.2
0.1 FMN + 0.2 vit. K_3	8.3	0.5 vit. K_3 + 0.014 cyt. *f*	2.6
0.2 vit. K_3	6.8	0.5 vit. K_3 + 0.0045 cyt. *f*	3.6
		0.5 vit. K_3 + 0.0015 cyt. *f*	4.2
0.04 vit. K_3	2.7	0.5 FMN	3.2
0.2 vit. K_3	5.1	0.5 FMN + 0.0045 cyt. *f*	3.0
1 vit. K_3	9.6	0.5 FMN + 0.014 cyt. *f*.	2.9
0.1 FMN	4.2	0.5 vit. K_3	4.6*
1 FMN	6.4	0.5 vit. K_3 + 0.007 cyt. *f*	3.1*
1 vit. K_3	9.9	0.5 FMN	2.2*
1 vit. K_3 + 1 FMN	8.8	0.5 FMN + 0.007 cyt. *f*	2.2*
0.5 vit. K_3	8.3	0.5 FMN	4.2
0.5 vit. K_3 + 3 KCN	9.8	0.5 FMN + 10 TPN	0.9
0.5 vit. K_3 + 9 KCN	14.3	0.5 FMN + 10 TPNH	4.1
0.5 vit. K_3 + 30 KCN	17.9	0.5 vit. K_3	5.7
0.5 vit. K_3 + 90 KCN	4.4	0.5 vit. K_3 + 10 TPN	1.1
		0.5 vit. K_3 + 10 TPNH	5.8
0.5 FMN	5.3		
0.5 FMN + 3 KCN	2.3		
0.5 FMN + 30 KCN	1.2	0.5 vit. K_3	9.4
		0.5 vit. K_3 + 40 ascorbate	10.3
0.5 vit. K_3	6.9	0.5 vit. K_3	6.5
0.5 vit. K_3 + 0.2 cyt. *c*	7.5	0.04 vit. K_3 + 40 ascorbate (aerobic conditions)	13.7
0.5 vit. K_3 + 0.5 cyt. *c*	7.9		
0.5 FMN	4.5	0.04 vit. K_3 (aerobic conditions)	6.3
0.5 FMN + 0.2 cyt. *c*	6.2		
0.5 FMN + 0.5 cyt. *c*	9.3		

* Chloroplasts washed once with Tris buffer containing glucose, and once with Tris buffer.

References p. 100.

Table I shows that ascorbate, in contrast with phosphorylation under aerobic conditions, does not significantly stimulate anaerobic photosynthetic phosphorylation. Phosphorylation is enhanced when the concentration of FMN or menadione is increased. However, menadione is more effective in promoting anaerobic photosynthetic phosphorylation than FMN, especially when the chloroplasts are washed more than once. The stimulating effect of KCN on ATP synthesis in the presence of menadione, and its inhibitory action on phosphorylation when menadione has been replaced by FMN, suggest that vitamin K_3 and FMN are involved in separate pathways for photosynthetic phosphorylation. Further support for this idea is found in the effect of cytochrome *c*, which stimulates phosphorylation with FMN (especially when the chloroplasts are washed more than once), but scarcely affects phosphorylation with menadione. The inhibition by cytochrome *f*, particularly in the presence of vitamin K_3, seems to suggest that cytochrome *f* does not serve as an electron carrier in photosynthetic phosphorylation. The experiments of LUNDEGARDH[11] and DUYSENS[12], however, indicate that in algae cytochrome *f* becomes oxidized in the light and reduced in the dark. Cytochrome *f* is widely distributed in higher plants and algae[10]. It is bound in the chloroplast structure firmly enough to withstand extraction by aqueous solvents. The inhibition of photosynthetic phosphorylation by cytochrome *f* could be explained by assuming that the oxidation of external cytochrome *f* is not as tightly coupled with phosphorylation as is the oxidation of the cytochrome *f* which is an integral part of the electron transport system.

The results of our experiments suggest that *in vitro* the generation of ATP in chloroplasts can be coupled to two separate electron transport chains, *viz.* FMN, cytochrome *c*, cytochrome oxidase(?), oxygen (or $[O]$), and menadione, cytochrome *f*, $[O]$ (Fig. 2).

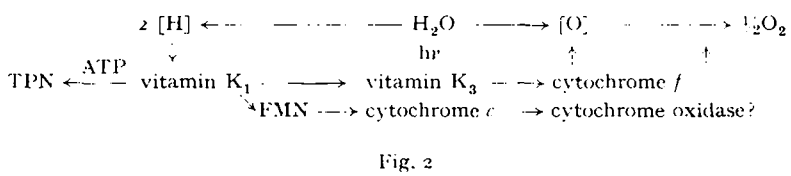

Fig. 2

The oxidation of cytochrome *f* by $[O]$ may be catalyzed by something like the photooxidase of photosynthetic bacteria[13]. It is worth mentioning that this enzyme was found to be KCN-insensitive. The inhibition of phosphorylation by TPN and DPN is in accordance with the assumption that the reduction of pyridine nucleotides is accomplished with the aid of ATP.

A comparison of the rate of ATP synthesis, either aerobically or anaerobically in the presence of KCN (the amounts of ATP generated under these conditions are nearly the same; the maximum rate equals about 30 μmoles of ATP per hour), with the rate of oxygen evolution in the Hill reaction (with benzoquinone as hydrogen acceptor), shows that per ½ mole of oxygen about 1 mole of ATP is formed. (P:O ratio 0.7–0.9). However, it is possible that menadione promotes electron transport from vitamin K_1 to cytochrome *f* via a pathway which by-passes a phosphorylative mechanism, so that *in vitro* phosphorylation is only coupled with the oxidation of the cytochromes. *In vivo*, on the other hand, the reduction of cytochrome *f* by vitamin K_1 may also be associated with phosphorylation.

References p. 100.

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SUMMARY

The photochemical esterification of inorganic phosphate into adenosine triphosphate by chloroplasts was investigated under anaerobic conditions in the presence of either vitamin K_3 or flavin mononucleotide. Evidence is presented in support of the conclusion that vitamin K_3 and flavin mononucleotide are involved in separate pathways for photosynthetic phosphorylation. A tentative scheme for the generation of adenosine triphosphate in chloroplasts, consistent with this conclusion, is given.

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PHOSPHAT-AUSTAUSCH ZWISCHEN ATP UND AD^{32}P DURCH HOCHGEREINIGTE AKTOMYOSIN-PRÄPARATE UND GEWASCHENE MUSKELFIBRILLEN

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I

Es herrscht weitgehende Übereinstimmung darüber, dass die Energie für den Kontraktionszyklus aller Muskeln durch Spaltung der endständigen Bindung der Triphosphat-Kette der Nukleosid-tri-Phosphate — besonders des ATP — geliefert wird. Es bestehen gegensätzliche Meinungen darüber, ob diese Energie in der Kontraktionsphase (vergl. WEBER[1,2], HILL[3] und DUBUISSON[4]) oder aber in der Erschlaffungsphase (KUHN[6],

Literatur S. 109.