CATALYSIS OF PHOTOPHOSPHORYLATION BY ALLAGOCHROME[1]

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Several compounds capable of undergoing either reduction or a reversible oxidation-reduction catalyze photophosphorylation by chloroplasts. Of these, the pyridine nucleotides, flavins and ubiquinones (Bishop, 1959) are found in green leaves. Recently Habermann (1960) isolated a blue-green pigment-protein complex from sunflower leaves which is capable of undergoing a reversible oxidation-reduction. This complex has since been isolated from leaves of several other species and from sunflower seeds. The pigment has been named allagochrome (meaning "changeable pigment") because of its reversible changes in color from blue-green to yellow on reduction and to red in acid solution. It is reduced in vitro by ascorbate, borohydride, dithionite and reduced cytochrome c The reduced form is autoxidizeable. These properties suggested that allagochrome may function as a terminal oxidase in intact tissue. There is evidence that allagochrome is the terminal oxidase of cyanide resistant plant respiration (Habermann, in press). Its ease of oxidation and reduction led us to expect that this pigment could

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catalyze an oxidative photophosphorylation similar to that catalyzed by flavin mononucleotide or menadione. The results reported here confirm these expectations.

Materials and Methods: Spinach chloroplasts were prepared by grinding chilled leaves in a mortar with sand in ice cold buffer (0.35 M sucrose, 0.05 M trishyroxy aminomethane at pH 7.8), filtering the brei, centrifuging at 200 x g for 1 min., centrifuging the supernatant at 1000 x g for 7 min., resuspending the sedimented chloroplasts and resedimenting at 1000 x g for 7 min. The reaction mixture consisted of chloroplasts, radioactive KH_2PO_4 (8 μMoles) $MgCl_2$ (5 μMoles) and ADP* (5 μMoles). Total volume of the reaction was 1.0 ml. The catalyst was either 0.03 μM PMS or allagochrome at concentrations stated. The reactions were run for 5 to 10 minutes at 15°C. Incident illumination from incandescent bulbs was approximately 1500 fc. at the bottoms of experimental vessels. Phosphate uptake was determined by a modification (use of separatory funnels) of the isobutanol benzene extraction method of Nielson and Lehninger (1955) in which ATP^{32} remaining in the water was counted.

Allagochrome was prepared as previously described (Habermann, 1960) through the first acetone precipitation step. Impurities precipitated by 50% acetone were removed by centrifugation. Acetone was then added to 90% by volume. The resulting blue-green precipitate was collected by centrifugation, acetone removed by evacuation, the precipitate dissolved in water and dialyzed against cold water for 2 to 3 hours. Preparations were further purified by continuous flow paper

* Abbreviations used: Adenosine di- and tri-phosphate: ADP and ATP; phenazine methosulfate; PMS; inorganic phosphate: P_i.

electrophoresis. Pigment concentrations were determined from optical density at 675 mμ. The standard curve of optical density vs. weight was obtained by drying and weighing aliquots of known optical density. The molecular weight of allagochrome determined by a diffusion technique (Northrup and Anson, 1929) is approximately 50,000.

Results and Discussion: Preparations from either seeds or leaves were active in these experiments (Table I). With 0.0024 μM crude seed allagochrome a rate of 86 μMoles P_i esterified per mg. chlorophyll per hour was observed. Jagendorf (1957) reported that 0.004 μMoles PMS per ml. were required for a rate of 90. With freshly prepared pigments (cf. Fig. 1) about 0.0012 μM pigment were required to achieve this rate. Thus, on a molar basis allagochrome appears to be as effective or more effective than PMS, the best catalyst known up to this time.

TABLE I

Rate of esterification of P_i by chloroplasts using allagochrome as the catalyst of photophosphorylation

Catalyst*	Rates (μMoles P_i per mg chlorophyll per hr)		
	with 0.1 ml catalyst	with 0.2 ml catalyst	with 0.3 ml catalyst
Crude seed allagochrome	51	86	146
Crude leaf allagochrome	63	89	101
Electrophoretically purified leaf allagochrome	--	32	53
Control rate with 0.03 μM PMS		372	
Control rate (no added catalyst)		7	

*Concentrations of preparations were as follows: crude seed allagochrome 0.61 mg. per ml.; crude leaf allagochrome, 0.90 mg. per ml.; purified leaf allagochrome, 0.35 mg. per ml.

Fig. 1 shows the effect of increasing concentrations of allagochrome on the rate of photophosphorylation. A maximum rate of 158 μMoles P_i esterified per mg. chlorophyll per hour was attained with 0.024 μMole

allagochrome per ml. reaction mixture. 0.03 μMole PMS gave a rate of 250 in this experiment. The fall in rates at high allagochrome concentrations was probably a consequence of strong light absorption by allagochrome which lowered the intensity of light impinging upon the chloroplasts.

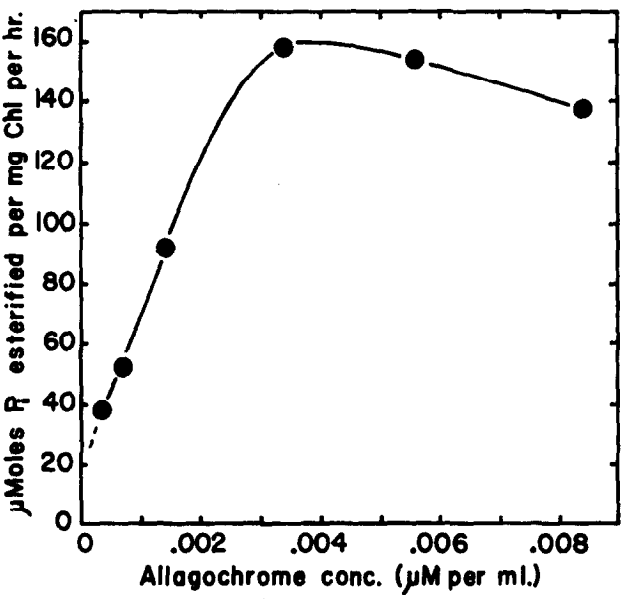

Figure 1

Effect of concentration of seed allagochrome on rate of photophosphorylation.

Preliminary experiments with O^{18} in which simultaneous uptake and production of oxygen were determined by methods described by Brown et al. (1952) showed that there was a high rate of oxygen uptake and equivalent oxygen production during photophosphorylation. Running the experiment in a system flushed with nitrogen, a condition which slightly increases the rate of phosphorylation with phenazine methosulfate, greatly reduced the rate of photophosphorylation with allagochrome.

PMS (or pyocyanine) is known to catalyze a cyclic photophosphorylation in contrast with all other known catalysts of photophosphorylation which function in a system capable of oxygen exchange

(Nakamoto et al., 1959; Krall et al., in press). The preliminary results with the mass spectrometer and N_2 flushing indicate that allagochrome catalyzes the latter type, an oxidative or non-cyclic photophosphorylation.

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