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Reçu le 15 janvier 1959

Influence of light on dark carboxylation reactions in etiolated barley leaves

Exposure of etiolated plants to white light results in rapid chlorophyll formation and visible greening within a few hours. Extensive investigations have shown no definite correlation between the development of chlorophyll and photosynthetic ability with increasing exposure to light[1–3]. SMITH[4] showed with etiolated barley leaves that the increasing O_2-evolving power resulted from both metabolic and photochemical reactions. TOLBERT AND GAILEY[5] found that when etiolated barley plants were placed in light, 2–3 h were required for the rate of chlorophyll synthesis to increase measurably and then a further 1–2 h of light were required before photosynthesis commenced, as measured by $^{14}CO_2$ fixation.

Large differences have been observed between green and etiolated barley leaves in the fixation of $^{14}CO_2$ by a number of substrates in crude homogenates (our unpublished data). For this reason changes in dark fixation of CO_2 with time of exposure of etiolated barley to white light were studied.

Week-old etiolated barley seedlings (*Hordeum vulgare*), var. Atlas, grown in sand with distilled water, were used in the experiments. The plants were illuminated in growth rooms at 22° with approximately 1000 ft. candles of continuous white light from 300-W "Champion" bulbs. Uniform samples (the leaf blade above the first sheath on each occasion) were taken from zero time up to 66 h after illumination started.

Samples of 0.5 g fresh weight of leaves were taken for chlorophyll analysis[6]. Samples of 5.0 g fresh weight were ground in a mortar with 5.0 ml 0.2 *M* Tris buffer, pH 8.0. The homogenate was strained through 2 layers of cheesecloth. Aliquots of 0.2 ml of this crude homogenate were added to each reaction mixture which also contained $H^{14}CO_3^-$. The mixture was incubated for 10 min at 37°; the reaction was stopped by adding 0.1 ml 1 *N* HCl which also expelled unreacted $H^{14}CO_3^-$. After centrifugation at 1000 × *g*, 0.2 ml of the supernatant was pipetted into a pyrex-glass planchet and dried under forced air at room temperature for approximately 1 h and counted with a thin-window Geiger-Müller tube. This method is the same as that reported previously from this laboratory[7].

Fig. 1 shows dark fixation of $^{14}CO_2$ by various substrates, and also the chlorophyll content, at various time intervals after the etiolated plants were first illuminated. Dark CO_2 fixation with G6P as a substrate and with ATP + Mg^{++} with no added

Abbreviations: Tris, tris(hydroxymethyl)aminomethane; ATP, adenosine triphosphate; G6P, glucose-6-phosphate; PEP, phosphoenolpyruvate; F6P, fructose-6-phosphate; R5P, ribose-5-phosphate; GSH, glutathione; RuDP, ribulose diphosphate; EDDHA, ethylenediamide *o*-hydroxyphenylacetic acid; TPN, triphosphopyridine nucleotide.

substrate slowly increased on illumination, and fixation by the PEP carboxylase (PEP + Mg) and PEP carboxykinase (PEP + ADP + Mn) enzyme systems slowly decreased linearly over the 66-h time period. CO_2 fixation with F6P and R5P as substrates increased linearly at a greater rate until a maximum was reached at which the rates of $^{14}CO_2$ fixation with these substrates leveled off. The chlorophyll content increased at first linearly on illumination, but reached a maximum later than $^{14}CO_2$ fixation with R5P and F6P. It thus seems that there was no consistent relationship between chlorophyll content and development of dark fixation of $^{14}CO_2$ during the illumination of the etiolated plants. This, however, conflicts with WARBURG[8], who reports "the establishment of the function of chlorophyll as a stochiometric, chemically reacting component of photosynthesis".

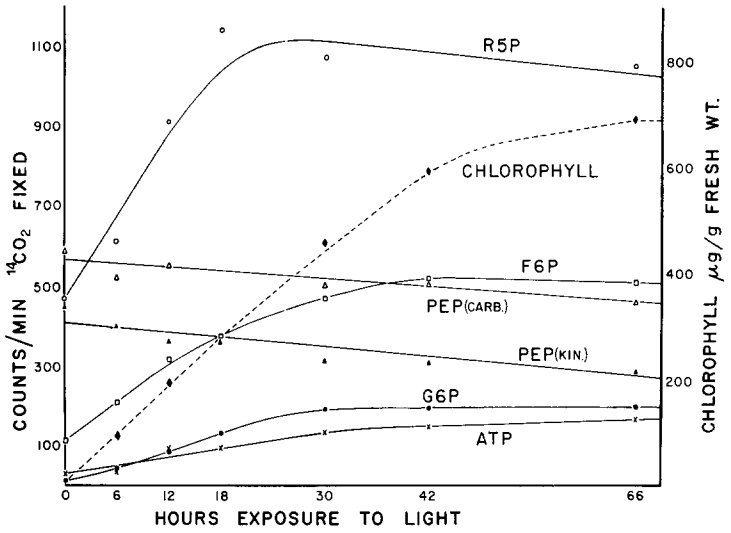

Fig. 1. $^{14}CO_2$ fixation into different substrates at varying intervals after exposure of etiolated barley seedlings to 1000 ft. candles white light. Experimental procedure in text. Reaction mixture (as designated in Table I): 120 μmoles Tris buffer pH 8.0; 5 μmoles $KH^{14}CO_3$ containing 34,600 counts/min as the $BaCO_3$ ppt. on Geiger-Müller counter; Mg^{++}, 20 μmoles; ATP, 4 μmoles; TPN, 0.02 μmole; PEP, 1 μmole; G6P, 4 μmoles; F6P, 4 μmoles; R5P, 2 μmoles. PEP(carb.) = PEP carboxylase; PEP(kin.) = PEP carboxykinase.

TABLE I

EFFECT OF CHELATE AND GLUTATHIONE ON DARK CARBOXYLATION REACTIONS IN GREEN AND ETIOLATED BARLEY LEAVES

Experimental technique described in text. Substrates and cofactors as in Fig. 1: EDDHA, 1 μmole; GSH, 1 μmole. ^{14}C, 425,000 counts/min added to each reaction mixture.

Reaction mixture	*Etiolated*			*Green (66 h light exposure)*		
	Control	*+ EDDHA*	*+ GSH*	*Control*	*+ EDDHA*	*+ GSH*
PEP + Mg^{++}	2400	2936	2348	2074	2636	2335
PEP + ATP + Mn^{++}	1414	1560	1293	1297	1436	1181
ATP + TPN + Mg^{++}	110	320	387	739	1004	872
G6P + TPN + ATP + Mg^{++}	243	488	704	860	1215	1195
F6P + TPN + ATP + Mg^{++}	967	1666	1780	2259	3233	2913
R5P + TPN + ATP + Mg^{++}	1903	3615	3766	2767	4044	3668

The addition of the chelate, (EDDHA), pH 8.0, to the reactions increased the activity of all these dark carboxylation enzymes (except possibly for PEP carboxykinase) throughout the illumination period (Table I). Addition of GSH resulted in a corresponding increase in dark carboxylation activity except for the PEP reactions.

The high initial rates of $^{14}CO_2$ fixation with R5P in our experiments are interesting since BASSHAM *et al.*[9] have shown in *Scenedesmus* that the "carboxylation reaction"[10] stops in the dark (after prior exposure to light) when the ribulose diphosphate concentration falls to zero. TOLBERT AND GAILEY[5] sprayed ribose on etiolated barley plants and found a stimulation in photosynthetic CO_2 fixation during the first hours of greening, but state that "the amount of RuDP present still appeared to be a rate limiting factor". These workers think that the synthesis of adequate amounts of enzyme to catalyze the regeneration of RuDP from the large pool of hexoses and sucrose present after 2 h of light (plants quite green), might be the limiting factor associated with the lag in photosynthesis in greening etiolates. However, the "carboxylation enzyme" must be present in considerable quantities since we find with etiolates approximately 1/2–2/3 of maximum activity of $^{14}CO_2$ fixation with R5P, assuming the phosphoriboisomerase and phosphoribulokinase enzymes[11] to be non rate-limiting. We find the rate of CO_2 fixation with R5P increases to a maximum after approximately 12–18 h exposure to white light.

The decrease in the PEP carboxylase and PEP carboxykinase reactions on light exposure seems to be correlated with TOLBERT AND GAILEY'S[5] observations that $^{14}CO_2$ in photosynthesis is first fixed in significant amounts into malate, aspartate and glutamate in greening etiolates, and that ^{14}C fixation into these compounds decreased on continued exposure to light. Green barley that had never been etiolated gave essentially the same results as etiolated plants that were exposed to light.

This study has indicated important changes in dark CO_2 fixation reactions during the greening of etiolated barley leaves.

DAVID O. HALL
RAY C. HUFFAKER
LELAND M. SHANNON
ARTHUR WALLACE

Department of Horticultural Science, University of California, Los Angeles, Calif. (U.S.A.)

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Received January 10th, 1959