Acta Bot. Neerl. 21 (1): 86-94. 1972

REACTIVATION OF PHOTOSYNTHESIS IN DEPENDENCE ON WAVELENGTH IN PHOSPHATE DEFICIENT LEMNA MINOR

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SUMMARY

In phosphate deficient *Lemna minor* photosynthesis is decreased. The plants show a rapid increase in photosynthesis upon P-supply, often not only at saturating light intensities, but also at rate limiting intensities. The reactivation at light limitation is larger in white light than in far red, and as far as monochromatic light has been studied, larger at 650 nm than at 700, 711 and 717 nm, which probably depends on a stronger inhibition of photosynthesis at shorter wavelengths. The results are interpreted as demonstrating an effect of the P-level on electron transport to and from photosystem I, in which the coupling to photophosphorylation and a switch from cyclic to pseudocyclic photophosphorylation may be involved.

1. INTRODUCTION

Both the inhibiting effect of phosphate deficiency on the photosynthesis of duckweed fronds and the recovery of inhibition observed after subsequent supply of phosphate have been described in previous work (LINDEMAN 1951, 1952). These effects, observed at saturation with light and CO_2, were interpreted in terms of demonstrating the degree of phosphate saturation of the Calvin cycle. Phosphate enters the Calvin cycle (BASSHAM & CALVIN 1957) in the phosphorylation by ATP[1] of ribulose monophosphate to ribulose diphosphate. ATP is formed in illuminated chloroplasts in the process of photophosphorylation, which is linked to photosynthetic electron transport (ARNON *et al.* 1961; ARNON 1966). According to current views (BOARDMAN 1968), electron transport in photosynthesis depends on two light reactions, brought about by two different photosystems, with different activation spectra. Following this concept, activation of the photosystems leads to the transfer of electrons from water to $NADP^+$. NADPH is reoxidized in the Calvin cycle in the reduction of phosphoglycerate, which reaction consumes ATP. In the light-induced formation of ATP two types of photophosphorylation are distinguished: the non-cyclic type, driven by the cooperating photosystems I and II, and the cyclic type, driven solely by the photosystem I. Photosystem I is considerably more active in red light between 700 and 720 nm than photosystem II, whereas the latter shows greater activity in red light of 650 nm.

[1] The following abbreviations are used: ADP and ATP: adenosine di- and triphosphate. $NADP^+$ and NADPH: the oxidized and reduced form of nicotinamid adenosine dinucleotide phosphate, respectively. PGA: phosphoglyceric acid. P_i: orthophosphate. PS: photosynthesis.

The question arose, whether the phosphate level of the plant might interfere with electron transport coupled to the two types of photophosphorylation. In case phosphate should affect the activity of one system more than that of the other, different effects of P-deficiency might be expected at those wavelengths where the activities of the two photosystems are appreciably different. Thus, in the present study photosynthesis has been measured in duckweed plants at low and at re-increased P-levels, in a range of light intensities, either in broad spectral regions (white light compared with far red), or in narrow bands (light of 650 nm compared with light of 700, 710 or 717 nm).

2. METHODS

Lemna minor plants were grown in axenic culture in a complete nutrient medium containing per liter destilled water 1g Ca $(NO_3)_2$. 4 H_2O, 0,25 g $MgSO_4$. $7H_2O$ and 0,136 g KH_2PO_4; added were 0,5 ml of a micronutrient solution and 25 ml of peat extract as described by LINDEMAN (1951, 1952). After sterilization and shortly before inoculation, 0,05 ml of a 10% $FeCl_3$ solution were added. From this complete (+P) medium plants were transferred to a P-deficient (−P) medium, in which KH_2PO_4 was replaced by KCl (0.074 g/l). 700 ml of the culture medium were given into 2-liter conical Fernbach vessels. The plants were kept at 23 °C and illuminated continuously by two 20W white fluorescent tubes and two 25W incandescent bulbs. The light intensity at the plant level was about 10.000 erg/cm² sec in the wavelength region of 400–700 nm. Air enriched with 4% CO_2 was bubbled through.

In the first series of experiments photosynthesis has been measured with a Wösthoff Ultragas-3 apparatus, which continuously measures the CO_2 content in a gas stream by recording the change in conductivity of a dilute NaOH solution caused by the absorption of CO_2. Only low CO_2 contents of about 0.3% were applied, in order to obtain sufficient accuracy. Applying a flow rate of 2.4 liter/hour, the CO_2 content did not decrease more than down to 0.12% at the highest rates of photosynthesis at high light intensity.

In the subsequent experiments photosynthesis has been measured with a katharometer of the type developed by PIETERS (1971). As this apparatus is adapted to differential measurements in gas composition, photosynthesis could be measured at high CO_2 content, generally at 5% in air. The flow through the assimilation vessel was 4 l/h.

In photosynthesis measurements the plants received light from a 500 W tungsten projection lamp, mounted in a lamphouse of a Leitz Prado – 500 slideprojector, equipped with its aspheric condensor only. Light intensity was controlled by a variable voltage transformer. The plants, generally 300 fronds, densely packed, however without overlap of fronds, floated in an assimilation vessel of 5 cm diameter placed in a water bath of constant temperature. Before transfer to the assimilation vessel, the roots were cut with scissors at a few mm distance from the fronds, which facilitated an even arrangement of the fronds. In the first series of experiments the plants were illuminated from the bottom. Illumination from the top, however, appeared to yield higher rates of photosynthesis and was applied in the other experiments.

As another improvement, in these cases, the plants were placed on a small circular nylon gauze in order to keep them immobile. This serves the purpose of obtaining an illumination as equal as possible in the − P and the − P+ conditions.

In all these cases horizontal light beams were thrown by means of a mirror under an angle of 45° vertically upon the frond surface. The light intensity was measured with a thermopile.

Red light of wavelength 700 nm was obtained by combining the Röhm and Haas plastic filters nr. 501 S (red) and nr. 627 (blue), in a thickness of 3 mm each.

Monochromatic light was obtained with Balzer B 40 interference filters. These filters were mounted as the front wall of a cuvette, attached to the diaprojector, tap water running through the cuvette protecting the filters from overheating and deterioration.

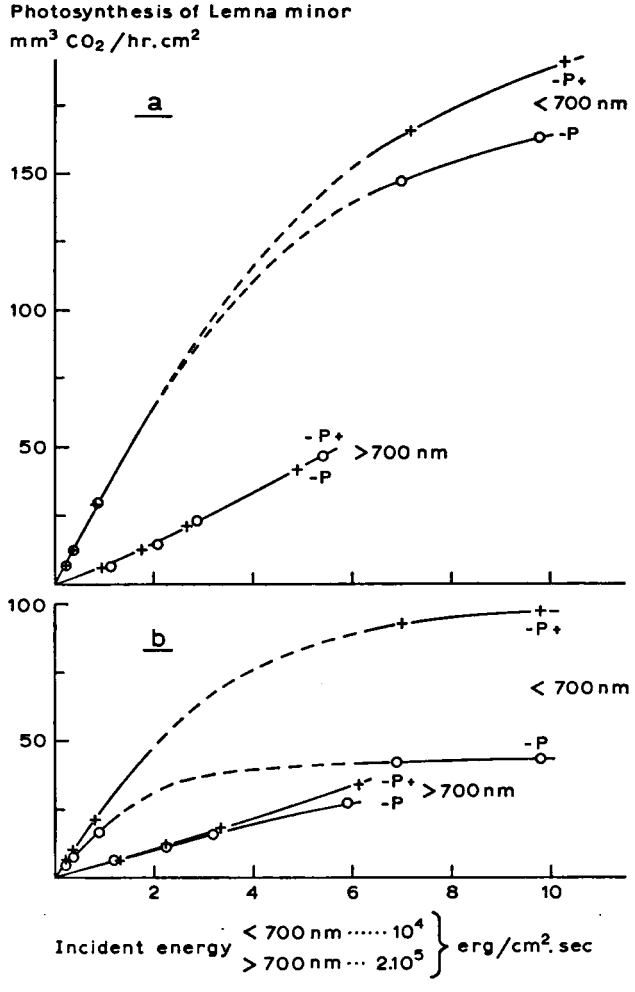

Figure 1. Effect of phosphate uptake on photosynthesis in P-deficient *Lemna minor* in white and in far red light.
O--O, −P : Photosynthesis of P-deficient plants.
+--+, −P+: Photosynthesis after P-uptake.
Photosynthesis measured at 30°C, in air with 5% CO_2.
P-uptake in the dark from 1 mM KH_2PO_4 solution during a. 165 min. b. 120 min.
a. −P+ effect at light saturation only.
b. −P+ effect both in the light limited and in the saturation range.

3. EXPERIMENTS

3.1. Broad spectral regions: "white" and "far red"

In the first series of experiments the effects on photosynthesis, brought about by phosphate supplied to P-deficient plants (the −P+ effect), were compared in

white light and in far red ($\lambda > 700$ nm). Some typical experiments are represented in *fig. 1*. In contrast with the author's previous work, the —P+ effect could not only be observed in white light at saturation intensities, but frequently also at light limitation, though generally in smaller percentage than at light saturation. Since the —P+ effect in the linear part of the photosynthesis *versus* light intensity curve does not fit in well with an interpretation in terms of saturating the Calvin cycle with phosphate, this effect is called "anomalous". The cases of anomalous behaviour became of interest with respect to its wavelength dependency.

In *table 1* the expts. 1, 2, 3, 6, 8, 10 and 11 and the expts. A-D are cases of anomalous —P+ effect. It is clear that the increase in percent of photosynthesis in white light is constantly larger than in far red light. Due to the poor absorption in the far red, it was difficult to obtain light saturation in the far red with the light sources available. Only in some cases, at low temperatures (9 to 12°), light saturation was reached with —P plants, but could not be reached with —P+ plants.

Table 1. Effect of phosphate on photosynthesis in white and far red light with *Lemna minor* in —P and in —P+ condition.
P-uptake in the dark in 0.1 mM KH_2PO_4 solution.
Initial CO_2 concentration in PS measurement 0.25–0.35% in expts. 1–11 and 5% in expts. A-E.
—P+ effect in percent increase of photosynthesis.

Expt. no.	Days of growth on —P medium	—P+ effect: PS light limited, white %	—P+ effect: PS light limited, far red %	—P+ effect: PS light saturated, white %	Duration of P-uptake hr	Temperature of PS measurement °C
7	9	0	0	57	1	12
8	10	35	10	47	1	18
10	10	40	20	52	1	12
6	13	40	32	55	1½	25
9	13	0	0	57	1	12
2	15	22	20	79	16	25
4	15	0	0	58	4	25
11	18	100	65	132	2	12
5	21	0	0	34	3½	25
3	22	160	50	150	16	25
1	27	57	10	81	16	25
E	3	0	0	15	3	30
B	4	15	12	80	4	30
C	6	42	0	123	2	30
A	7	20	5	50	2½	30
D	10	50	20	72	2	30
Average "Normal"		0	0	44		
"Anomalous"		53	22	84		

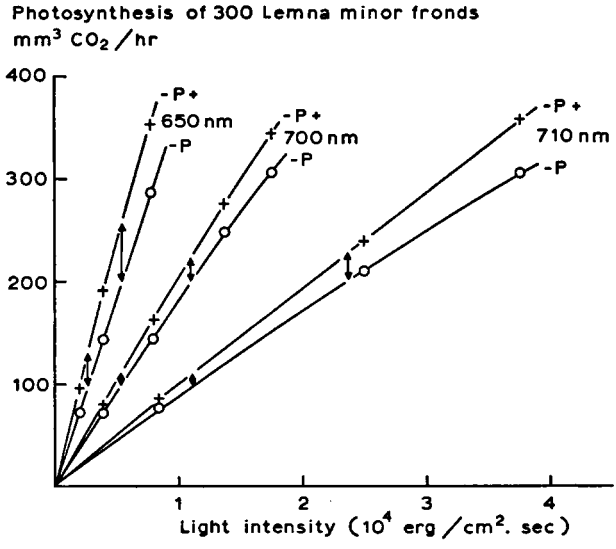

Figure 2. Effect of phosphate uptake on light limited photosynthesis of P-deficient *Lemna minor* in narrow red and far red wavelength regions.
O------O, −P : Photosynthesis of P-deficient plants.
+------+, −P+: Photosynthesis after P-uptake.
↕ : −P+ effect in mm^3CO_2/hr at two levels of light-limited photosynthesis.
Photosynthesis measured at 30°C, in air with 5% CO_2.
P-uptake during 16 hrs. in the dark from 1 mM KH_2PO_4 solution.

The reasons for either "normal" or "anomalous" −P+ behaviour are not clear.

Probably after a brief P-deficiency, characterized by a moderate −P+ effect at light saturation (e.g.) up to 60%, the effect generally is "normal", whereas in a later stage of P-deficiency the −P+ effect at light saturation is higher and, simultaneously, the anomalous behaviour seems to prevail.

3.2. Narrow bands of the 650 and of the 700–717 nm region

The anomalous −P+ effect was studied by means of irradiation in narrow bands around 650, 700, 710 and 717 nm, at intensities which are rate limiting for photosynthesis.

From the ascending, linear part of light *versus* PS curves of the type presented in *fig. 2* the data summarized in the *tables 2* and *3* have been obtained.

In the narrow spectral regions the effect of P− supply on light-limited PS is greater in red light around $\lambda = 650$ than in far red light around $\lambda = 700$, 710 or 717 nm. There is a tendency of the −P+ effect to decrease with increasing wavelength. The experiments of *table 2*, moreover, demonstrate a certain in-

fluence of the duration of P-deficiency on the degree of the −P+ effect: e.g. in expt. 22, after 10 days, it is 27% at 650 nm and 0% at 710 nm, whereas in expt. 24 after 17 days it amounts to 40% at 650 nm and 23% at 710 nm.

Table 2. Effect of phosphate on light limited photosynthesis at 650, 700 and 710 nm during proceeding P-deficiency in a *Lemna minor* culture.
Photosynthesis in air + 5% CO_2, illumination from the top.
P-uptake during 16 hr in the dark from 0.1 mM KH_2PO_4 solution.
−P+ effect in percent increase of photosynthesis.

Expt. no.	Days of growth on −P medium	−P+ effect 650 nm %	700 nm %	710 nm %	Ratio of incident energies at equal rates of PS: incid. en. 700 / incid. en. 650 +P	−P	−P+	incid. en. 710 / incid. en. 650 +P	−P	−P+
20	0	–	–	–	2,2	–	–	5,1	–	–
21	8	10	4	0	–	2,3	2,5	–	5,2	5,4
22	10	27	14	0	–	2,1	2,3	–	4,3	5,4
23	14	22	14	13	–	2,1	2,2	–	4,5	4,9
24	17	40	25	23	–	2,1	2,3	–	4,2	4,7

Table 3. Effect of phosphate on light limited photosynthesis at 650 and 717 nm with *Lemna minor* in −P and −P+ condition.
Photosynthesis in air +5% CO_2 at 30°C, illumination from the top.
P-uptake during 16 hr in the dark from 1 mM KH_2PO_4 solution.
−P+ effect in per cent increase of photosynthesis.

Expt. no.	Days of growth on −P medium	−P+ effect 650 nm %	717 nm %	Incident energy 717 nm: Incident energy 650 nm at equal rate of PS +P	−P	−P+
20	0	–	–	9,5	–	–
28	0	–	–	10,2	–	–
42	0	–	–	9,7	–	–
43	5	24	0	–	7,4	10,0
38	6	13	0	–	6,8	8,0
37	6	22	10	–	7,3	8,3
32	6	23	2	–	8,7	10,2
29	6	33	0	–	6,4	10,6
31	6	69	30	–	6,7	9,8
34	7	14	0	–	6,5	10,4
33	7	36	28	–	7,2	8,4
39	7	44	12	–	7,7	9,8
41	8	19	0	–	6,9	8,1
35	8	53	39	–	6,4	9,1
36	8	63	0	–	4,4	6,9
40	8	66	24	–	5,8	7,0
30	10	92	10	–	6,4	10,6
Average		41	11	9,8	6,8	9,1

4. DISCUSSION

According to measurements of activities ascribed to the two photosystems (JOLIOT *et al.* 1968; SAUER & PARK 1965), photosystem I has a relatively great activity at wavelengths between 700 and 720 nm as compared with photosystem II, owing to a considerably greater absorption of light in this spectral region. The difference in activity between the two photosystems, which depends on wavelength, can be described in terms of limiting processes: beyond 700 nm, light reaction II is rate limiting, at 650 nm light reaction I. In the "anomalous" experiments of the present study the gain in yield upon P-supply is smaller with far red than with red of 650 nm. Two alternative possibilities herefor exist viz.: a. P-deficiency depresses the rate of PS more at 650 nm than beyond 700 nm, and consequently, recovery upon P-supply is higher at 650 nm. b. P-deficiency has the same inhibiting effect at 650 nm and at wavelengths > 700 nm, but P-supply has a greater effect at 650 nm.

An answer is given by calculating from available data the ratio $\frac{\text{energy input far red}}{\text{energy input 650 nm}}$ for equal rates of PS for +P plants, for −P plants and for the corresponding −P+ plants. The results are given in the right columns of the *tables 2* and *3*.

In long wavelength red this ratio shifted to a lower value during P-deficiency. Supply of phosphate leads to an increase in the ratio, which is close to the value originally found in the +P plants or even surpasses this. This means that the efficiency of light energy conversion during P-deficiency in the light limited range of PS at 650 nm has decreased more than at $\lambda > 700$ nm. Upon P-supply, it shows a correspondingly larger increase at 650 nm than beyond 700 nm. At low P-level, the photosynthetic apparatus seems to make a more economic use of the available phosphate in the far red than at 650 nm. The same may hold true for far red as compared with the white light used in the experiments concerned.

Part of the depressive effect of P-deficiency on PS may be due to total inactivation of part of the photosynthetic apparatus. This, however, will result in the same degree of inhibition at all wavelengths concerned and in a corresponding equal −P+ effect.

Consequently, another explanation is required for the differences in anomalous −P+ effect at various wavelengths. This, based on the present knowledge of the mechanism of PS, for the moment only can be tentative. In terms of the chemical theories on oxidative phosphorylation (cf. SLATER 1966), the different effects might be connected to the linkage of photophosphorylation to the oxydation of a reduced compound C_{red} in the electron carrier chain between photosystems II and I. The concentration of C_{red} is submitted to the "push and pull" effect (KOK 1965), when the plant is illuminated either at 650 nm or at $\lambda > 700$ nm. Then, in −P plants, the same low concentration of P_i at 650 nm may be rate limiting in comparison to a relatively high $[C_{red}]_{650}$, and at $\lambda > 700$ nm may be in excess in comparison to a relatively low $[C_{red}]_{700}$.

Another, more speculative way of explanation, which may hold aside of the

former, might be a switch from cyclic to pseudocyclic photophosphorylation in —P plants, based on the assumption that the cyclic process, with a phosphorylation site of its own (cf. ARNON 1966; VAN RENSEN 1971), has a lower affinity for P_i than has the pseudocyclic (= noncyclic) one. Other assumptions to be made are that cyclic or pseudocyclic photophosphorylation are necessary for the formation of additional ATP (see, however, TANNER *et al.* 1969; RAVEN 1970) and that the cyclic process operates with a better quantum yield than the pseudocyclic one does (cf. TANNER *et al.* 1968). At 650 nm, then a switch from the cyclic to the pseudocyclic pathway in —P plants should lead to a decrease in the efficiency of energy conversion, whilst the reverse switch in —P+ plants should lead to an increase in efficiency (as actually was observed). At $\lambda > 700$ nm this switch should not occur, or only to a minor degree. The reason may be that in the far red the excess absorption in photosystem I mobilizes all P_i available in cyclic photophosphorylation in order to supply sufficient additional ATP to CO_2-reduction, so that the switch to the pseudocyclic pathway is not necessary (cf. ARNON 1966), or only takes place to a smaller extent.

ACKNOWLEDGEMENT

The author wants to acknowledge the skilful technical assistance of Miss G. H. HEERINGA.

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