Acta Botanica Neerlandica **7** (1958) 489–502

SOME OBSERVATIONS ON PHOSPHATE METABOLISM IN THE INDUCTION PHASE OF PHOTOSYNTHESIS IN CHLORELLA

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(received March 31st, 1958)

INTRODUCTION

The special relations between phosphate metabolism and photosynthesis have been the subject of various investigations ever since EMERSON, STAUFFER and UMBREIT (1944) submitted as a hypothesis that radiant energy was converted by photosynthetic organisms first into phosphate bond energy, and that the various processes of reduction were driven by the chemical energy of high-energy phosphates. The formation of ATP in the light has been demonstrated indeed, and has been called photosynthetic phosphorylation. It is distinct from respiratory phosphorylation (ALLEN et al., 1957, KANDLER, 1955, 1957, WESSELS, 1957). With suitably prepared chloroplasts, ALLEN *et al.* (1957) obtained rates comparable to those of the maximal rate of photosynthesis.

With living cells, reports on changes in the concentration of various phosphate compounds at the transition from darkness to light and vice versa have been made by several authors, notably by KANDLER (1950, 1955), WASSINK and ROMBACH (1954), STREHLER (1953) and BRADLEY (1957). A conversion of orthophosphate in the light has been demonstrated beyond doubt. The time course of the ortho-P level is often irregular, showing oscillations which are approximately synchronous with various other induction phenomena of photosynthesis such as fluorescence, and redox potentials (WASSINK and SPRUIT, 1954). It was first thought, that this esterification reflected the formation of ATP (HOLZER, 1951). In experiments by STREHLER, however, the time course of the formation of ATP at the beginning of illumination was slower than the time course of orthophosphate conversion in the experiments by WASSINK and ROMBACH (1954); this prompted further investigation.

In the meantime, KANDLER (1957) has concluded from new experiments that the maximum rate of photosynthetic phosphorylation was very low in comparison with the rate of CO_2 reduction, and was light saturated at intensities, much lower than those required to saturate photosynthesis. According to the most recent scheme of

photosynthesis by the Berkeley group, at least one molecule of ATP is involved in the reduction of one molecule of CO_2, viz. in the reduction of phosphoglyceric acid to triose phosphate (CALVIN, 1957). KANDLER (1957), therefore, rejects this scheme as proposed by Calvin and coworkers. In view of this, the influence of light intensity on the induction curve of orthophosphate was more closely examined.

METHODS

Two strains of *Chlorella* were used in the present investigation, *Chlorella vulgaris*, strain A and a strain obtained from Myers, referred to as J.M. 1410. Inoculum from agar slants was given into 100 ml bottles containing about 50 ml nutrient solution, consisting of: 1.26 g KNO_3, 1.22 g KH_2PO_4, 2.46 g $MgSO_4.7$ aq., 0.03 g $FeSO_4$, 1.00 g Na-citrate, 15 g glucose, and water up to 1000 ml. These bottles were grown for 3–6 days in a light cabinet without aeration. Five ml of heavy algal suspension from these bottles were used as inoculum into 1 litre Erlenmeyer flasks, containing 500 ml of a nutrient solution containing: 1.00 g KNO_3, 0.135 g KH_2PO_4, 0.50 g $MgSO_4.7$ aq., 2 ml of an iron solution, 1 ml of spore elements solution A_4 and 0.1 ml of spore elements solution B_7 (cf. ARNON, 1938). The iron solution contained: 1.00 g $FeSO_4$, 2.00 g Na-citrate in 100 ml distilled water; solution A_4 contained: 2.86 g H_3BO_3, 1.81 g $MnCl_2.4$ aq., 0.222 g $ZnSO_4.7$ aq., 0.07 g $CuSO_4.5$ aq. in 1000 ml distilled water; solution B_7 contained: 259 mg $H_2MoO_4.4$ aq., 229.6 mg NH_4VO_3, 960.2 mg $Cr_2K_2(SO_4)_4.24$ aq., 447.8 mg $NiSO_4.6$ aq., 493.8 mg $Co(NO_3)_2.6$ aq., trace of TiO_2 in 10 litres of 0.1 n H_2SO_4. These cultures were given 12 hours of light daily. The flasks were continuously shaken and illuminated from below with light obtained from a bench of alternate blue and red fluorescent tubes. Ventilation was continuous with air containing 5 % CO_2. Temperature was not controlled closely, but remained about 25° C. After 3 to 4 days, cells were phosphate starved in a poorer medium, modified from KANDLER (1950), and containing: 0.4 g KNO_3, 0.1 g $Ca(NO_3)_2$, 0.1 g $MgSO_4.7$ aq., 1 drop of 5 % $FeSO_4$, distilled water 1 litre. This permitted a slow growth during 1 to 2 days, in which the orthophosphate reserves of the cells diminished gradually.

For an experiment, cells were harvested, washed once with distilled water, and there after suspended in distilled water. Densities varied from 30 to 60 mm^3 packed cells/ml. All experiments were carried out at room temperature.

Five ml cells were given into 100 ml Erlenmeyer bottles, painted black except for the bottom. These bottles were ventilated in darkness with common air, with air containing 5 % CO_2, or with CO_2-free air before exposure to light. After a dark period of 20 minutes the suspensions were illuminated from below with incandescent light. At first, 100 W bulbs were used, giving an intensity of about 75,000 ergs/cm^2 sec on the bottom of the flasks. Later on, when denser suspensions were used, a 150 W lamp with internal reflection was applied giving about 200,000 ergs/cm^2 sec on the bottom of the flasks, after having passed through 7 cm water. Lower light intensities were obtained by inserting wire filter combinations between the light source and the water filter. Transmissions could be varied from 1.7 to 56 % of total available light.

Extractions were made by adding equal amounts of 10 % TCA at room temperature to the suspensions, and lasted for 30–40 minutes. Phosphorus determinations were made according to MARTLAND and ROBISON (1926), readings being taken after 30 minutes, at 7000 A°.

EXPERIMENTAL RESULTS

a) Correlation between decrease in inorganic and increase in labile organic P.

The first series of experiments was made in order to find a possible correlation between a decrease in ortho-P and a rise in labile, TCA-soluble P. These experiments were made with suspensions of *Chlorella*

A, containing about 20 mm³ packed cells per ml. Gas phase was common air. After a short time in the dark, light was given for periods varying from 10 to 120 seconds. Orthophosphate generally reached a minimum between 30 and 40 seconds, whereupon in many cases a rise in ortho-P occurred. The rate of phosphorylation as a whole was very variable. The $\triangle_{10\ min}$ labile phosphate showed an increase, which was less subject to variation than the drop in ortho-P. In some experiments, therefore, the rise in labile P exceeded the fall in ortho-P, especially during exposures from 40 to 120 seconds. In these cases a decrease in stable or insoluble phosphates must have occurred. As will be shown in the following section, such effects can be due to lack of carbon dioxide (Table I).

TABLE I

Changes in TCA-soluble inorganic and ($\triangle_{10\ min}$) labile phosphates (μg P/ml), in suspensions of *Chlorella A* after short periods of illumination. Suspensions contained 20 mm³ packed cells/ml. Gas phase: air.

Light, seconds (a)	Decrease in orthophosphate (b)	Increase in ($\triangle_{10\ min}$) labile P (c)	Difference (b–c); (= increase in resistant or/and insoluble P (d)	Number of observations (e)
20	0.42 ± 0.04[5]	0.32 ± 0.04[5]	0.11 ± 0.04	27
40	0.60 ± 0.06[5]	0.34 ± 0.05	0.26 ± 0.06	27
60	0.62 ± 0.09[5]	0.37 ± 0.06	0.25 ± 0.10[5]	21
90	0.55 ± 0.14	0.51 ± 0.06	0.04 ± 0.12	16
120	0.54 ± 0.15	0.49 ± 0.06	0.05 ± 0.12	15

b) The influence of carbon dioxide

In order to obtain more reproducible results, the amount of CO_2 available to the cells was controlled by aerating *Chorella* J.M. 1410 suspensions for 20 minutes with either air + 5 % CO_2, or with CO_2-free air. The average results of a series of experiments are given in Table II. In the absence of CO_2, in the first 40 seconds of light,

TABLE II

Changes in TCA-soluble, inorganic and ($\triangle_{10\ min}$) labile phosphates (μg P/ml), in suspensions of *Chlorella* J.M. 1410, after short periods of illumination, as influenced by carbon dioxide. Suspensions contained 20 mm³ packed cells/ml.

Light, seconds (a)	Air + 5 % CO_2 (b)		CO_2-free air (c)		Difference (b–c) (d)		Number of observations (e)
	Ortho-P	$\triangle_{10\ min}$ P	Ortho-P	$\triangle_{10\ min}$ P	Ortho-P	$\triangle_{10\ min}$ P	
40	—0.76±0.06[5]	+0.25	—0.22±0.06	+0.24	0.53±0.07	0.01±0.07	25 10
120	—0.99±0.07		—0.56±0.10		0.43±0.07		15

phosphorylation is significantly slower than in its presence. The rise in $\triangle_{10\ min}$ labile P, however, is equal under both conditions. Between 40 and 120 seconds, the rates of phosphorylation do not differ significantly; the CO_2 containing suspensions in this stage are probably already close to the stationary level, whereas the CO_2-free ones gradually approach such a level.

A further analysis was made of the phenomena during the first 40 seconds of light.

Denser suspensions were used, containing about 60 mm^3 cells/ml, and higher light intensities (ca. 200,000 ergs/cm^2 sec) were given. After 0, 10, 20 and 40 seconds of light, cells were extracted twice in 5 % TCA (final concentration), for 60 minutes together. The combined extracts (15 ml) were treated with 100 mg of active carbon, and centrifuged. In the supernatant, ortho-P, $\triangle_{10\ min}$ phosphate, and, in several cases, total P were determined. The carbon with the adsorbed nucleotides was washed with distilled water, and hydrolysed for 15 minutes in 1 n HCl, after which labile P was determined. Fifteen minutes were taken instead of 7 or 10, since preliminary experiments had shown that the stability of the endgroups of ATP was increased by adsorption on active carbon.

The results of these experiments are given in Table III. They can be summarized as follows [1]).

1) Considering the general shape of the induction time curve, a transient minimum after about 10 seconds of illumination may, or may not be visible. This does not depend on CO_2 (see Fig. 1). The

TABLE III

Changes in some fractions of TCA-soluble phosphates in suspensions of *Chlorella* J.M. 1410, after short periods of illumination in the presence and absence of CO_2. Suspensions contained 60 mm^3 packed cells/ml. Data in μg P/ml, averaged from several experiments.

Light, seconds	Gas phase	Ortho-P	Labile, carbon adsorbed P (nucleotides)	Labile, not-adsorbed P	Not easily hydrolysed P
10	Air + 5 % CO_2	— 1.77	+ 0.41	+ 0.53	+ 0.96
20		— 1.45	+ 0.37	+ 0.23	+ 1.20
40		— 2.61	+ 0.18	+ 0.50	+ 2.50
10	CO_2-free air	— 1.74	+ 0.42	+ 0.37	+ 0.80
20		— 1.19	+ 0.44	+ 0.16	+ 0.21
40		— 1.04	+ 0.44	+ 0.20	— 0.22

reason for the variability of the material in this respect is not known. If present, the minimum occurs at about the same time as found by WASSINK and ROMBACH (1954). In KANDLER's most recent article, phosphorylation is assumed to follow a simple time course, without oscillations in phosphate level (1957). In our experiments the initial rate is distinctly higher than that observed by Kandler.

2) The rate at which ortho-P falls in the first 10 seconds of light is not dependent on carbon dioxide, nor is there any significant difference in one of the other fractions measured. This may point

[1]) The results of Tables I through III, relative to ortho-P, are represented schematically in fig. 2.

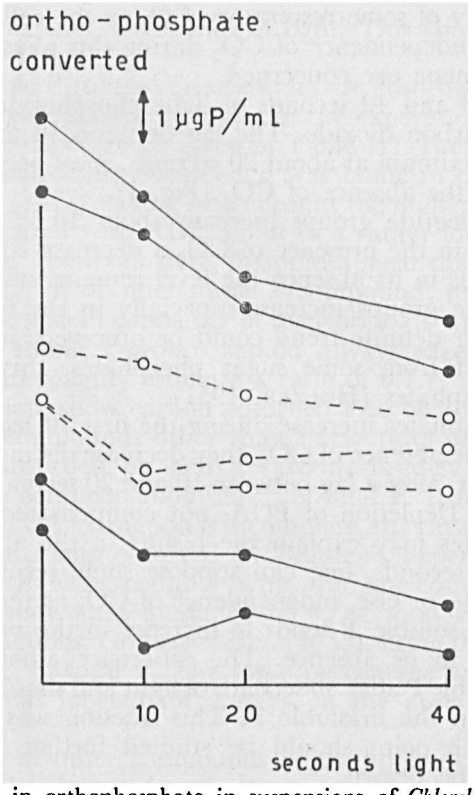

Fig. 1. Changes in orthophosphate in suspensions of *Chlorella* J.M. 1410 after illumination for 10–40 seconds. Some representative examples, density: 60 mm³ packed cells/ml; ● ---- ● gas phase air + 5 % CO_2, ○ ---- ○ gas phase CO_2-free air.

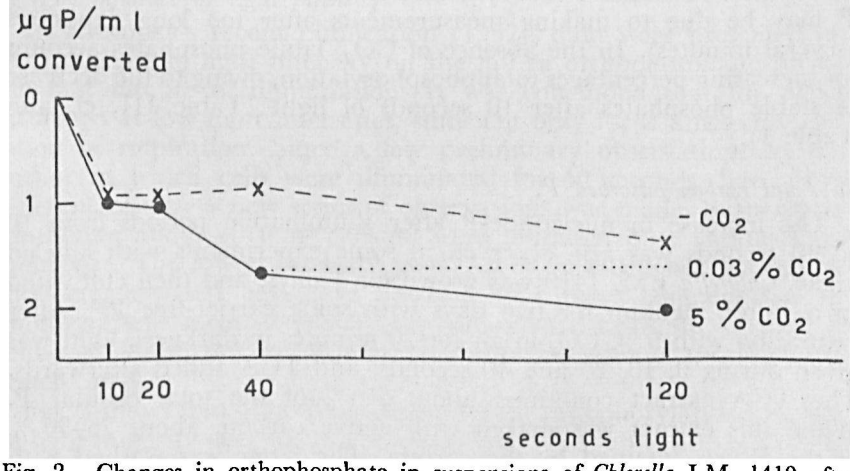

Fig. 2. Changes in orthophosphate in suspensions of *Chlorella* J.M. 1410 after short periods of illumination. Schematic representation of the influence of the CO_2 content of the air. Average rates for densities of 40 mm³ packed cells/ml.

to the availability of some respiratory CO_2 to the cells called "CO_2-free", or to the independence of CO_2 during this phase, as far as the observed phenomena are concerned.

3) Between 20 and 40 seconds of light phosphorylation is clearly dependent on carbon dioxide. The lag between 10 and 20 seconds, or a transient maximum at about 20 seconds, may occur both in the presence and in the absence of CO_2 (Fig. 1).

4) Labile nucleotide groups increase about 10 % in the first 10 seconds of light; in the presence of CO_2 a decrease is indicated after this time, whereas in its absence the level remains stationary.

5) Other labile groups increase especially in the first 10 seconds of light. No other definite trend could be observed, since variability was great. Apart from some sugar phosphates, this fraction may contain polyphosphates (HOLZER, 1951).

6) Stable phosphates increase during the first 10 seconds, irrespective of CO_2. In the absence of CO_2, they decrease distinctly afterwards. With CO_2 present, after a lag between 10 and 20 seconds they increase again definitely. Depletion of PGA, not compensated by formation of sugarphosphates may explain the results in the absence of CO_2. For the first 10 seconds, one can suppose some respiratory CO_2 to have been present, or else, independence of CO_2 as remarked sub *2*).

7) Total TCA-soluble P tends to increase in the presence of CO_2 and to decrease in its absence. The difference amounts to 1–2 % of total TCA-soluble P after 40 seconds of light and should be accounted for by changes in the insoluble P. This fraction was not analysed, however, and the point should be studied further before definite conclusions can be drawn.

It can further be remarked, that after 10 seconds of light, in the presence of 5 % CO_2, labile phosphates account for at least 50 % of total phosphorylation, against for only about 25 % after 40 seconds. KANDLER's (1957) failure to observe an increase in labile P may be due to making measurements after too long exposures (several minutes). In the absence of CO_2, labile phosphates account for increasing percentages total phosphorylation, owing to the decrease of stable phosphates after 10 seconds of light (Table III, cf. also Table I).

c) Total carbon adsorbed P.

The increase in nucleotide-P after illumination periods from 10 to 40 seconds was also observed in some experiments with labeled algae. *Chlorella* J.M. 1410 was grown for 4 days, and then cultivated in a P-free medium for two days with some carrier-free P^{32}. After saturation with 5 % CO_2 in air for 20 minutes in darkness, light was given during 0, 10, 20 and 40 seconds, and TCA added afterwards. The TCA extract contained about 20 % of the total cellular P. When this extract was shaken with active carbon, about 25–30 % of the P was retained by the carbon. The latter was washed with distilled water and 96 % ethanol, dried and counted. Counts were about 5 % increased after 10–20 seconds of illumination, and some-

what lower again after 40 seconds of light. This confirms the results given in Table III.

Since also stable P groups of nucleotides are adsorbed on the carbon, the relative increase should be lower in the experiments with labeled cells, than in the experiments given in Table III. It can be estimated, that less than half of the adsorbed P was labile in our experiments. BRADLEY's data (1957), taking into account the higher specific activity of ATP as compared with ADP, point to a rather low ratio of ATP/ADP in darkness. From our data, it would appear that also important amounts of AMP, or of other stable P-compounds must be adsorbed by carbon. (In a system consisting of nucleotide di- and triphosphates the ratio labile/stable P-groups should always exceed unity. From our data, we can roughly estimate a ratio of 0.7.)

The present data show carbon adsorbed P to be about 5 % of total cellular P. No compounds other than nucleotides are known to the author to be adsorbed on active carbon. Bradley's data indicate ATP + ADP to comprise about 25 % of total P. It must be assumed that, either our TCA extraction is incomplete, that insufficient labeling of all constituents has occurred in Bradley's experiments, or that other compounds were counted with the nucleotides by Bradley. Bradley's data show that about 60–65 % of total P was soluble in his ethanol extractions. GOODMAN *et al.* (1955), using the same method, give about 50 % of total P. The same percentage was found by the author, using cells labeled for 2 days, in the absence of further P supply.

The presence of other compounds in the spots of ATP and ADP is indicated by Bradley, and may also account for the high percentage of nucleotide-P, and for the relatively small changes in nucleotide-P found by this author in the first 45 seconds of illumination.

d) The influence of light intensity

In KANDLER's recent publication (1957), data were presented on the influence of light intensity on the fall in orthophosphate during the first minute of illumination. It was concluded that the effect saturated at low light intensities, sufficient only for 2 times compensation of respiration. Since a few preliminary observations by the author in which cells were illuminated for 40 seconds, had given different results, a new series of observations was made. Suspensions containing about 40 mm^3 cells/ml were illuminated at various light intensities. The lowest intensity used (1.7 % of full light), was about 3500 ergs/cm^2 sec, and gave about 3 to 4 times compensation in photosynthesis measurements, in which about the same amount of cells was illuminated per cm^2 of the bottom surface of the Warburg vessels used.

Fig. 3 shows that after 40 seconds of light, phosphorylation depends on light intensity over the full range of intensities, up to about 200,000 ergs/cm^2 sec. With 10 seconds of illumination the picture is different: here, phosphorylation is nearly light saturated at the lowest intensity used.

At this low light intensity, no further phosphorylation occurs between 10 and 40 seconds of light, as is shown by the coincidence of the dashes and circles in Fig. 3 at this light intensity.

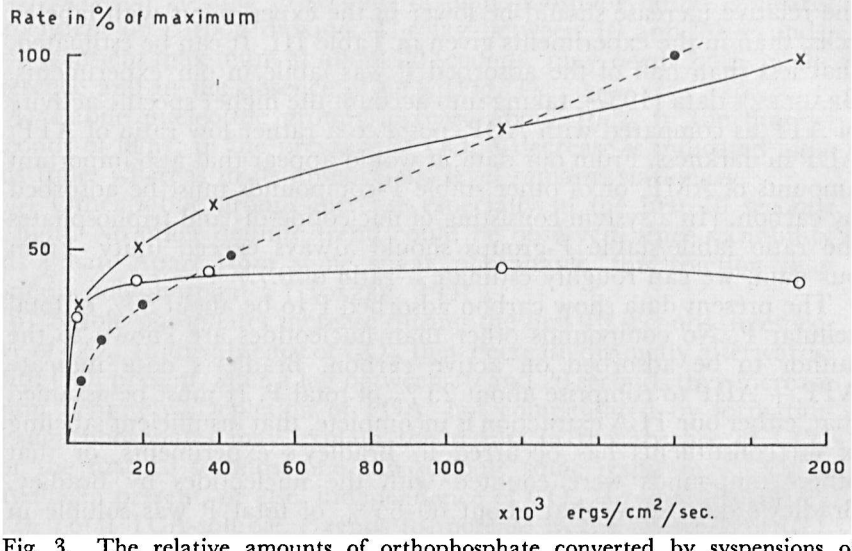

Fig. 3. The relative amounts of orthophosphate converted by syspensions of *Chlorella* J.M. 1410 after short exposures to light of different intensities. ○ —— ○: after 10 seconds, × —— ×: after 40 seconds of illumination. Data in percent of the phosphorylation, observed after 40 seconds at highest light intensity. Average results of several experiments. Gas phase air + 5 % CO_2; suspensions containing 40 mm³ packed cells per ml, and 8 mm³/cm² illuminated surface. ● ---- ●: Photosynthesis of suspensions measured in Warburg vessels with about the same amount of cells per cm² illuminated surface.

A few observations were also made with cells, kept free from CO_2. Phosphorylation, both after 10 and 40 seconds, was not different at 9 and at 100 % of light. In these experiments, the minimum at 10 seconds happened to be quite pronounced, resulting in 3 out of 4 cases in a higher P level after 40 seconds, compared with the dark control (Fig. 4).

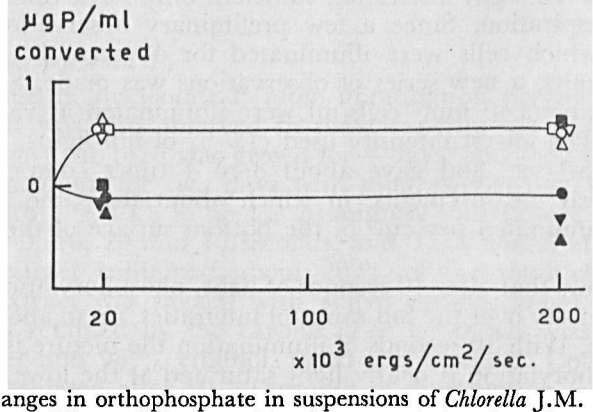

Fig. 4. Changes in orthophosphate in suspensions of *Chlorella* J.M. 1410 in CO_2-free air, at two different light intensities. Open symbols: after 10 seconds of light. Corresponding closed symbols: after 40 seconds of light.

e) Stage of development of the cells

A series of observation was made in which the influence of the developmental stage of the cells on the phosphate induction phenomena was investigated. TAMIYA and coworkers (1953) have presented a series of data, indicating that the rate of photosynthesis and of polyphosphate accumulation vary widely from young (dark, freshly divided) cells to mature cells (light cells). A synchronous culture of *Chlorella* J.M. 1410 was obtained, using a constant density continuous culture apparatus as developed by BONGERS (1958).

Cells were grown at about 30° C, as compared to 15° in Tamiya's experiments. Growth was quicker and the cycle operated quicker accordingly. A 24 hour cycle, with only 10 hours of light maintained a slow, but synchronous growth.

Measurements were made in air, and the decrease in orthophosphate was measured after 40 and 120 seconds. It appeared that the induction phenomena were much less pronounced in "dark" cells than in "light" cells, having received light from 4 to 9 hours before harvest. Data are contained in Table IV.

TABLE IV

Changes in orthophosphate in suspensions of *Chlorella* J.M. 1410, after short periods of illumination in air, as influenced by the stage of development of the cells. Suspensions contained 20 mm³ packed cells/ml. Data in μg P/ml.

Light, seconds	Cells from darkness (freshly divided)			Cells from light (growing cells)		
	CO_2-free air	Air	Air + 5 % CO_2	CO_2-free air	Air	Air + 5 % CO_2
40	0.10	0.28	0.62	0.26	0.94	0.96
120	0.44	0.38	0.95	0.70	0.57	1.04
Number of expts.	7	12	7	7	17	7

f) The influence of the phosphate level

A limiting factor for light phosphorylation by P-starved cells might be the amount of orthophosphate available. Table V represents the amount of orthophosphate converted in 10 and 40 seconds of light in the presence of CO_2. The data are taken from many observations throughout this investigation, all calculated to a density of 40 mm³ cells/ml. At very low levels, < 7 μg P/ml, phosphorylation tends to be low.

In Kandler's experiments, cells very low in ortho-P have been used, this may explain the low rates of phosphorylation found by this author. Dark values between 20 and 40 μg PO_4/5 ml, or 1.3–2.6 μg P/ml, containing 5–10 mg dry cells/ml are given. This density can be estimated to represent about 20–40 mm³ cells/ml.

TABLE V

Changes in orthophosphate in suspensions of *Chlorella* J.M. 1410, after short periods of light. The influence of the amount of orthophosphate present at the beginning of illumination. Gas phase: air + 5 % CO_2. Data in μg P/ml suspension, calculated to a density of 40 mm^3 cells/ml.

Light, seconds	Dark level: < 7 μg P/ml	n	Dark level: > 7 μg P/ml	n
10	— 0.61 ± 0.06	13	— 1.09 ± 0.12	32
40	— 1.32 ± 0.12	17	— 1.90 ± 0.12	45

g) The influence of DNP

In another series of experiments the influence of dinitrophenol on the phenomena under discussion was followed. Suspensions of *Chlorella* J.M. 1410 were used, containing ± 40 mm^3 cells/ml, and DNP in a concentration of 4×10^{-5} molar, at a pH of about 5.

At high light intensities, phosphorylation after 10 seconds of light was, on an average, equal in poisoned and non-poisoned cells, but cases of inhibition were observed next to cases of stimulation. A similar picture was observed after 40 seconds of light. In 8 experiments inhibition was seen only on 2 occasions, stimulation in the other cases was from 25 to 200 %.

In the dark pre-incubation time, a very pronounced dephosphorylation had occurred in the DNP containing suspensions, resulting in a higher ortho-P level. It seems that low activity of the controls is found in those suspensions, where DNP caused strong dephos-

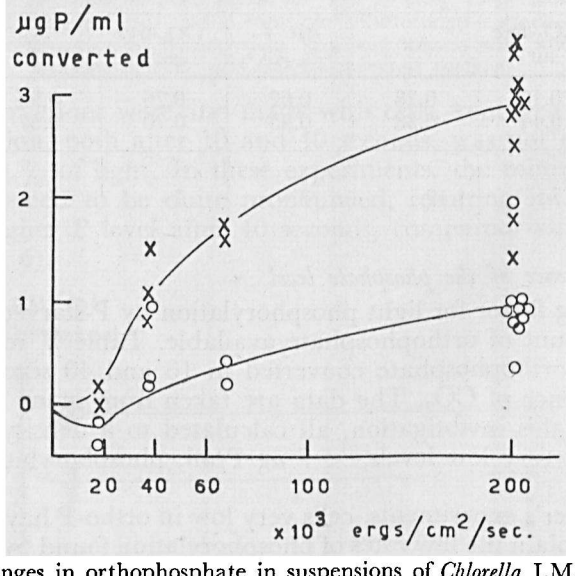

Fig. 5. Changes in orthophosphate in suspensions of *Chlorella* J.M. 1410, in the presence of DNP, at different light intensities. Density: 40 mm^3 packed cells/ml; DNP: 4×10^{-5} Molar; gas phase: air + 5 % CO_2; pH 5.0.
○ —— ○: after 10 seconds of light.
× —— ×: after 40 seconds of light.

phorylation. The light phosphorylation in the presence of DNP, however, is not visibly dependent on the degree of dark dephosphorylation.

For an explanation, it can be supposed that the ratio of phosphate acceptors to phosphorylated compounds is subject to variation. Cells in a state of high phosphorylation will react with a relatively large increase in orthophosphate on addition of DNP. These cells will then have more acceptor available than the non-poisoned controls. In the latter cells, light induced phosphorylation will be limited by lack of acceptor. Cells in a state of low phosphorylation will release relatively less phosphate under the influence of DNP; the amounts of acceptor which differ less in poisoned and control cells in this case, and an inhibitory influence of DNP can become apparent.

It had been found formerly (WINTERMANS, 1955), that the inhibitory action of DNP decreases with increasing light intensity. It was therefore of interest, to compare inhibition at different light intensities. The results are given in Fig. 5. It again appears, that inhibition is especially strong at low light intensities, both for 10 and for 40 seconds exposures. The difference with the comparable curves of Fig. 3 for not poisoned cells is apparent. Photosynthesis was measured on several occasions at a light intensity of 40.000 ergs/cm^2 sec, with approximately the same amount of cells per cm^2 of exposed vessel surface. Inhibition was from 40–60 % at this intensity.

DISCUSSION

The results obtained indicate that phosphorylation, in the first moments of illumination, as indicated by a drop in the orthophosphate level, reflects at least two processes. During the first seconds (maximally ten), phosphorylations resulting in the formation of both labile and stable phosphate compounds are predominant. Part of the labile compounds may be adsorbed on active carbon, and hence belong to the nucleotides, as far as present information goes. This phase is light saturated at low intensities, and is not influenced by carbon dioxide.

In the second phase, between 10 and 40 seconds of light, there is no further increase in labile phosphates. Carbon dioxide and light intensity determine the rate of phosphorylation. With CO_2 present, the decrease in orthophosphate depends on light intensity, stable compounds are formed. In the absence of CO_2, stable phosphates decrease, and orthophosphate rises again slightly; light saturation is at low intensity.

A third phase can be distinguished from 40–120 seconds of light, but this phase was not extensively analysed. Orthophosphate decreases, both in the presence and in the absence of CO_2. In common air, CO_2 exhaustion after about 40 seconds may result in a rise in ortho-P, since the level is higher when no CO_2 is present. See Fig. 2. CO_2 exhaustion also results in an increase in labile phosphates, and a decrease in stable compounds (cf. Table I).

The initial rate of phosphorylation depends on orthophosphate only when its concentration is very low. In most cases, this rate seems to

depend on the amount of some acceptor, or acceptors. The lag period after about 10 seconds of light may arise when the acceptors are exhausted after the first phase of phosphorylation. The necessary transformations before phosphorylation is resumed take less time when CO_2 is present than when it is absent.

The initial rate of phosphorylation was found to increase after anaerobic incubation (KANDLER, 1957; WASSINK and ROMBACH, 1954) and after addition of KCN in dark (KANDLER, 1957). To this can be added, that it was now found, that also after addition of DNP, at sufficiently high light intensity, phosphorylation was often stimulated. All these treatments result in dephosphorylation during the dark period, and it can be supposed that stimulation can occur because the competitive oxidative phosphorylation is absent and more acceptors are available. Only two cases of inhibition were found in 8 experiments with DNP. In these experiments dephosphorylation had been minimal.

In the experiments of BRADLEY (1957), the drop in ortho-P at the transition dark-light in N_2 was considerably larger than in air containing 5 % CO_2. In our opinion this must be ascribed to the effect of anaerobiosis rather than to the effect of carbon dioxide.

In the present investigation the highest rates were obtained in the first ten seconds of illumination. From the position of the lag period, it can be concluded that higher rates could probably be found by further reduction of the time of illumination. The distribution of phosphate over various types of compounds after 10 seconds of light, must be taken as an indication of secondary transformations. A better insight into the nature of the first phosphorylation product could be obtained also here by the use of still shorter periods of illumination.

In order to compare the observed rates with other data from literature, we may assume a dry weight percentage of 25, and a chlorophyll content of 5 % of dry weight. Forty mm^3 cells are then equivalent to 10 mg dry cells, and 0.5 mg of chlorophyll.

In the present experiments, we found the maximum (initial) rate about 1 μg P/40 mm^3/10 sec. (cf. Table III), or 0,2 μ Mole/40 mm^3/min. Such a rate can be attained at a low light intensity, say sufficient for a rate of photosynthesis equal to about five times respiration. This would mean about 200 mm^3 O_2/40 mm^3/hr, or 0.3 μg Atom O/40 mm^3/min; P/O = 0.65. At higher light intensity, this ratio becomes smaller. A P/O ratio in photosynthesis can become meaningful, if phosphorylation is compared, not with the oxygen produced, but with recombination of photoproducts. With respect to oxygen, an induction loss, equivalent to some 10 seconds of light has been found in *Chlorella* by HILL and WHITTINGHAM (1953). This loss might be ascribed to recombination of the primary photoproducts in this phase, with accompanying photosynthetic phosphorylation, as observed here. The accumulation of stable compounds, in a later stage of the induction, however, is positively, although not stoechiometrically, correlated with the amount of oxygen evolved i.e. with the rate of photosynthesis.

Kandler's results, although generally lower, are in the same order of magnitude.

ARNON and coworkers (1957), working with chloroplasts and added cofactors, now have achieved photosynthetic phosphorylation amounting to 400 μ Moles P/hour/mg chlorophyll, or 3.3 μ Moles P/40 mm^3/min., i.e. 16 times the rate found with living cells. Photosynthesis amounted to 200 μ Moles O_2/hr/mg chlorophyll, being about 55 cell volumes/hour. P/O = 1.0.

The latter results give support to a conception in which phosphorylation and photosynthesis bear a stoechiometric relationship. The data given here point to a special and significant contribution of light phosphorylation at low light intensities, for which a further indication can be seen in the fact that DNP inhibits phosphorylation at low light intensities, but is even stimulating at high light intensity. A sigmoid rate/intensity curve results (see Fig. 5), comparable to a similar sigmoid curve found earlier for photosynthesis in the presence of DNP (WINTERMANS, 1955).

SUMMARY

Orthophosphate decreases in *Chlorella* in the first minutes of illumination. At least two steps can be distinguished.

The highest rate of phosphorylation is found immediately at the onset of illumination. This phase is completed in at most 10 seconds. Both labile and stable compounds are formed. This phase is independent of CO_2, and is saturated at low light intensity (about 5 times compensation). It may reflect, at least partly, the light phosphorylation proper, and is probably limited by the available amount of acceptor.

In the second phase, stable phosphates are formed. The rate depends on light intensity and carbon dioxide. This phase is thought to represent the accumulation of products of photosynthesis.

The first and second phase are sometimes separated by a lag period of about 10 seconds.

DNP is inhibitory at low light intensity only. This is similar to its influence on photosynthesis. Sigmoid rate intensity curves are found in both cases.

It is concluded that photosynthetic phosphorylation is especially significant at low light intensities.

ACKNOWLEDGEMENTS

The author wishes to express his gratitude to Prof. E. C. WASSINK for continuous interest and stimulating criticism, and to Dr. W. LINDEMAN for his useful remarks during the preparation of the manuscript.

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