Institut für Landwirtschaft
KERNFORSCHUNGSANLAGE JÜLICH
des Landes Nordrhein-Westfalen-e.V.

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Jül – 185 – LW

November 1964

Berichte der Kernforschungsanlage Jülich – Nr. 185

Institut für Landwirtschaft - Jül - 185 - LW

DOK.: ELODEA DENSA
PHOSPHATE, INORGANIC
PHOTOPHOSPHORYLATION
PHOTOSYNTHESIS
DK.: 541.144.7 : 577.154.52 : 631.851 : 631.873.2

Zu beziehen durch: ZENTRALBIBLIOTHEK der Kernforschungsanlage Jülich
Jülich, Bundesrepublik Deutschland

INTRACELLULAR TRANSLOCATION OF ATP, ADP AND INORGANIC PHOSPHATE IN LEAF CELLS OF ELODEA DENSA IN RELATION TO PHOTOSYNTHESIS

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Received January 17, 1964

It is known, that in the leaf cell the incorporation of ^{32}P into organic compounds is stimulated by light (Simonis and Grube 1952, Simonis and Urbach 1963). This lends support to the commonly held view that photophosphorylation takes place in vivo as well as in vitro. However, it is not yet known whether high energy phosphate, presumably formed in the chloroplasts in the light, becomes directly available to the cytoplasm. In previous communications it has been found that some phosphate esters formed in the chloroplasts during photosynthesis are readily transferred to the cytoplasm, while others are retained in the chloroplasts (Heber and Willenbrink 1963). In this report information is presented concerning the translocation of ATP, ADP and inorganic phosphate from the chloroplasts to the cytoplasm and vice versa.

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Experimental: Shoots of Elodea densa (Planch.) Caspary, which had previously been kept in a phosphate-free medium for 20 hours, were transferred, either in the light or in the dark, for various lengths of time into a solution containing $^{32}PO_4^{---}$ (0.0125 µmole phosphate and 60 µmoles NaHCO$_3$ per 30 ml water, p$_H$ 7.5). The material was then killed by immersion in liquid air. After freeze-drying, chloroplasts were isolated by a non-aqueous procedure (Thalacker and Behrens 1959). From the fractions obtained soluble compounds were extracted and separated by twodimensional paper chromatography in isobutyric acid/N-NH$_3$/EDTA (Tyszkiewicz 1962) and n-butanol/propionic acid/water (47/23/30). The radioactivity of individual compounds was determined by counting the spots on t paper. From the values obtained the distribution of labelled material between chloroplasts and cytoplasm (defined here as the nonchloroplastic part of the protoplasm) was calculated. Details of the procedures used are communicated elsewhere (Heber, Pon and Heber, 1963, Heber and Willenbrink 1963). Inorganic phosphate was determined according to Fiske and Subbarow (1929) and ATP by an optical test, according to Adam (1962).

Results and Discussion: In the light and also in the dark the labelling of ATP and ADP with P^{32} proceeds more slowly in the chloroplasts than in the cytoplasm of the leaf cell, as is shown in a typical experiment in table 1. In the light this seems not in accord with the commonly held view, that the ATP consuming photosynthetic carbon cycle is operating exclusively in the chloroplasts. The latter view is supported by a mass of indirect evidence (Arnon 1960, Jagendorf 1962) and, recently, by kinetic data on the distribution of ^{14}C-labelled intermediates of the photosynthetic carbon cycle between chloroplasts and cytoplasm (Heber and Willenbrink 1963). In the dark the rates of incorporation of radioactivity are lower than in the light, but the ratio of labelling between chloroplasts and cytoplasm remains unaltered. The ratios of light to dark labelling range from 1 to 3 in our experiments.

Table 1. The labelling of ATP and ADP in chloroplasts and cytoplasm during feeding of leaf cells with $^{32}PO_4^{---}$ (10^3 counts/mg protein).

		ATP			ADP		
		30 sec	60 sec	180 sec	30 sec	60 sec	180 sec
light	chloroplasts	3.9	6.4	17.4	0.7	1.4	4.0
	cytoplasm	16.0	31.7	74.0	2.7	5.5	11.6
dark	chloroplasts	2.8	3.2	6.2	0.6	0.7	1.3
	cytoplasm	10.2	17.6	18.2	2.2	3.4	3.1

From these results the suspicion arises, that the data are not representative of the in vivo situation and that secondary translocation may have occured during the preparation of the material. However, this possibility is ruled out by ^{14}C-experiments, in which no secondary translocation could be found (Heber and Willenbrink 1963). Moreover, in double labelling experiments with ^{14}C and ^{32}P 90 % of the ^{14}C-labelled sugardiphosphates was found after 30 seconds in the chloroplasts, while only 11 % of the ^{32}P-labelled sugardiphosphates could be located there at the same time (unpublished results). These data speak directly against the occurence of secondary translocation processes in our experiments. Therefore, another explanation of the unexpected intracellular distribution of labelled ATP and ADP has to be sought.

The slow labelling of ATP and ADP in the chloroplasts as compared with that in the cytoplasm could easily be explained, if radioactive phosphate enters the chloroplasts very slowly. If this were the case, then the specific activity of inorganic phosphate should be higher in the cytoplasm than in the chloroplasts. Unfortunately direct estimations of the specific activity of inorganic phosphate are only possible in the non-aqueous chloroplasts, while estimations in the nonchloroplastic residue yield no useful information, since very "hot" phosphate in the cell walls and "cold" phosphate in the vacuole overshadow the situation in the cytoplasm. Therefore attempts have been made to determine the specific activity of inorganic phosphate in the cytoplasm indirectly. If equilibrium of the β- and γ-phosphate groups of ATP with inorganic phosphate is assumed, then the specific activity of the inorganic phosphate must be half that of the ATP, which can be determined directly. Since the phosphate groups of ATP are derived from inorganic phosphate, a lower value for the specific activity of inorganic phosphate is not possible, while, in the case that there is no equilibrium, higher values must result. The α-phosphate group of ATP is known to equilibrate only slowly with inorganic phosphate (Weichart 1961, Bieleski and Laties 1963).

Values of the specific activity of inorganic phosphate in the chloroplasts and in the cytoplasm are listed in table 2. It must be emphasized, that the calculated values (cytoplasm) are to be considered as minimum values, since an equilibrium between phosphate and ATP may not have been reached in the cytoplasm owing to the continuous uptake of labelled phosphate from the surrounding medium. Therefore, the in vivo ratios of the specific activities of cytoplasmic and chloroplastic phosphate are very probably higher than presented in table 2. Since a rapid intracellular exchange of phosphate would result in about equal specific activities of phosphate over the entire protoplasm, the considerable differences observed in the specific activities of chloroplastic and cytoplasmic phosphate prove directly, that the translocation of inorganic phosphate from the cytoplasm to the chloroplasts proceeds slowly and that the half time of equilibration must be of the order of minutes, not of seconds. This should be noted, if the phosphate metabolism of chloroplasts is investigated in the intact cell with the aid of labelled phosphate.

Table 2. The specific activity of inorganic phosphate in chloroplasts, as determined experimentally, and in the cytoplasm, as calculated from the specific activity of cytoplasmic ATP.

		30 sec	60 sec	180 sec
		10^5 counts/μmole phosphate		
light	chloroplasts	1.6	3.1	9.5
	cytoplasm	14.2	15.1	31.5
dark	chloroplasts	0.2	0.5	1.1
	cytoplasm	4.3	4.8	5.4
light	ratio $\frac{\text{cytoplasm}}{\text{chloroplasts}}$	9	5	3
dark	ratio $\frac{\text{cytoplasm}}{\text{chloroplasts}}$	21	10	5

From a comparison of the specific activity of chloroplastic ATP with that of chloroplastic phosphate, direct information on the translocation of ATP within the cell can be obtained. Pool sizes of ATP in the chloroplasts were between 2 to $4 \cdot 10^{-3}$ μmoles per mg protein (19 experiments), while values of inorganic phosphate ranging from 0.4 to $1.6 \cdot 10^{-1}$ μmoles per mg protein have been observed (24 experiments). In table 3 some values of the specific activity of ATP in chloroplasts and cytoplasm are listed. In every case the specific activity of the chloroplastic ATP is severalfold higher than that of the inorganic phosphate in the chloroplasts (compare with table 2). This means, that only part of the chloroplastic ATP can have been formed from the weakly labelled chloroplastic phosphate pool; the other part must have originated from the heavier labelled cytoplasmic phosphate. Indeed in the dark experiments, the specific activity of the chloroplastic ATP is about twice as high as that of the cytoplasmic phosphate and equal to that of the cytoplasmic ATP, which should be expected if, in the dark, all chloroplastic ATP were of cytoplasmic origin. In the light the specific activity of chloroplastic ATP is considerably lower than that of cytoplasmic ATP due to the fact that ATP is also synthesized in the chloroplasts from ADP and weakly labelled phosphate by photophosphorylation. Synthesis of weakly labelled ATP in the chloroplasts and influx of heavier labelled ATP from the cytoplasm lead to a specific activity of ATP in the chloroplasts about intermediate between the specific activities of cytoplasmic ATP and chloroplastic phosphate (compare with table 2).

Table 3. The specific activity of chloroplastic and cytoplasmic ATP.

		30 sec	60 sec	180 sec
		10^5 counts/μmole ATP		
light	chloroplasts	14.0	15.7	34.3
	cytoplasm	28.4	30.2	63.0
dark	chloroplasts	8.3	9.6	18.5
	cytoplasm	8.6	9.6	10.8

These results are direct evidence of a very rapid exchange of ATP between chloroplasts and cytoplasm. If cytoplasmic ATP can enter the chloroplasts, it is plausible that chloroplastic ATP migrates also into the cytoplasm.

What has been derived here for ATP, is valid also for ADP, for which about the same relationships have been found as for ATP (cf. table 1).

Summarizing, it can be concluded from the results of these experiments, that there is a fast translocation of ATP and ADP between chloroplasts and cytoplasm of the leaf cell. On the other hand, inorganic phosphate is transferred only slowly from the cytoplasm to the chloroplasts. Since chloroplasts, during photosynthesis, lose phosphate due to the transport of phosphate esters into the cytoplasm (Heber and Willenbrink 1963), the existence of a concentration gradient of inorganic phosphate between cytoplasm and chloroplasts in the light is likely.

Further results and implications of our findings will be discussed in a subsequent paper.

Acknowledgments: This investigation was supported by the Deutsche Forschungsgemeinschaft. The authors are indebted to Prof. Dr. H. Ullrich and to Prof. Dr. W. Simonis for stimulating discussions and to Miss R. Sustmann for her able technical assistance.

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