ADENINE NUCLEOTIDE TRANSLOCATION ACROSS THE MEMBRANE OF ISOLATED ACETABULARIA CHLOROPLASTS

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Santarius and Heber (1965) have shown that the ATP concentration is increased, while ADP and AMP concentrations are decreased in both, the chloroplast and cytoplasmic compartments of whole cells of higher plants, when previously darkened leaves are illuminated. These concentration changes occured simultaneously in both intracellular compartments and were rapidly reversed when the plants were returned to the dark. As the increase in ATP concentration within the chloroplasts is a consequence of photophosphorylation, the authors concluded that the rise in ATP concentration within the cytoplasmic compartment must be due to a rapid translocation of adenine nucleotides across the chloroplast membrane. Keys (1968) observed a similar response to illumination in tobacco leaves. However, when the leaves were returned to the dark a rapid conversion of ATP to AMP occured only within the chloroplast compartment. He concluded from these results that there must be a limited permeability of the chloroplast membrane - at least to AMP.

Comparison of the results of nucleotide translocation experiments performed on isolated chloroplasts is equally inconclusive. Following preillumination, Jensen and Bassham (1968) found a decrease in ATP concentration

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in the chloroplasts as well as in the surrounding medium, when CO_2 was added. Robinson and Stocking (1968) observed a stimulation of photosynthetic O_2 evolution in response to exogenous ADP. These results favour the conclusion that the chloroplast membrane is freely permeable to adenine nucleotides. On the other hand, Walker (1965) obtained maximum stimulation of photophosphorylation by ADP only after disruption of the chloroplast envelope.

The most difficult problem in performing nucleotide translocation experiments is to obtain chloroplasts with structurally and biochemically intact envelopes. Homogenization of the plant material, which frequently results in damage to the chloroplasts, can be avoided by using the siphonal green alga Acetabularia as a source of chloroplasts. Chloroplasts can be isolated from this alga without homogenization of the cells (Schweiger, 1966). We have studied the translocation of adenine nucleotides in chloroplasts which have been prepared without homogenization from Acetabularia cells. Adenine nucleotides, labelled with ^{14}C in the base moiety, were used in order to measure directly the translocation of the whole adenine nucleotide molecule.

MATERIAL AND METHODS

Acetabularia mediterranea was grown following the method of Hämmerling (1963). The cell rhizoid was cut off, and the algal cytoplasm was extruded by gentle centrifugation of the plants (Schweiger, 1966). The cytoplasm was resuspended in a medium described by Jensen and Bassham (1966), and the chloroplasts were sedimented by centrifugation at 1,800 x *g* for 5 minutes (Schweiger et al., 1967). The chloroplasts were washed twice.

Photosynthetic CO_2 fixation by the chloroplasts was measured according to the method of Jensen and Bassham (1966). Chlorophyll was assayed by the method of Arnon (1949). The adenine nucleotide content of the chloroplasts was measured enzymatically in neutralized perchloric acid extracts accord-

ing to the methods described by Lamprecht and Trautschold (1962) and by Adam (1962). The translocation of adenine nucleotides into the chloroplasts was measured by a modification of the centrifugal filtration technique and by the back exchange technique of Pfaff (1965). The experiments were performed at 3°C in the dark.

RESULTS AND DISCUSSION

The content of endogenous adenine nucleotides of isolated washed Acetabularia chloroplasts was found to vary between 70 and 120 nmoles per mg chlorophyll. The variation may be due to fluctuations in chlorophyll content and/or to a fluctuation in the leakage of nucleotides during preparation. Examination of the washed chloroplast fraction with a phase contrast microscope showed fairly clean organelles. The envelopes were intact according to the criteria described by Leech (1966). The CO_2 fixation rate of the chloroplasts was 20 µmoles per mg chlorophyll per hour. This rate is at least 50 % of the CO_2 fixation rate of intact Acetabularia cells reported by Shephard et al. (1968). The CO_2 fixation rate is a sensitive biochemical indicator of the presence of intact chloroplast envelopes.

The average initial rate of uptake of ^{14}C-ATP by the chloroplasts was 5 nmoles per mg chlorophyll per minute as determined by the centrifugal filtration technique (Fig. 1). In some experiments higher values were measured, but they never exceeded 10 nmoles per mg chlorophyll per minute. The adenine nucleotide content of the chloroplasts was measured enzymatically after an incubation with and without exogenous ^{14}C-labelled ATP in order to determine, whether the uptake of ATP causes a net increase in endogenous nucleotide concentration. The results of this experiment clearly indicate that there is no change in the total endogenous adenine nucleotide concentration when the chloroplasts are incubated with ^{14}C-ATP, although within 5 minutes 42 % of the endogenous adenine nucleotides were labelled (Table 1).

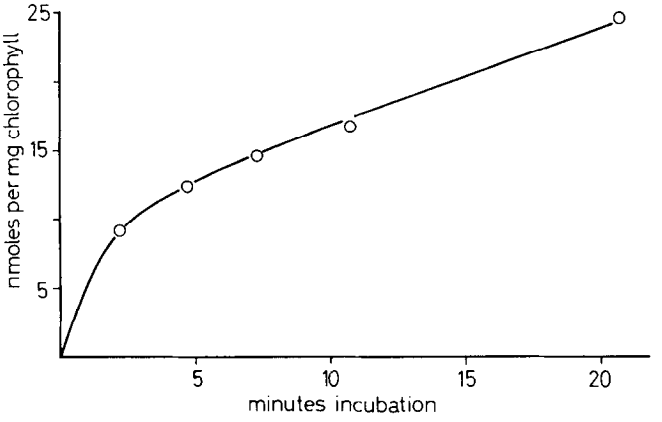

Fig. 1. Uptake of ^{14}C-labelled ATP by isolated Acetabularia chloroplasts. The chloroplasts were incubated in preparation medium containing 2 mM ^{14}C-labelled ATP. An aliquot (0.8 ml) of the suspension was layered onto 0.5 ml of silicon oil which in turn was layered above 0.2 ml of 13 % perchloric acid in small glass tubes. After different incubation times the chloroplasts were centrifuged through the silcon oil layer, and the reaction was stopped by the perchloric acid. ATP uptake was calculated from the radioactivity appearing in the perchloric acid extract. In a control experiment the amount of ^{14}C-ATP due to adhering medium was determined using high molecular weight ^{14}C-labelled polyoxyglucose which does not permeate into the chloroplast.

Table 1. Content of endogenous nucleotides as affected by the addition of 2 mM ^{14}C-labelled ATP. Incubation time 5 minutes. After this time 42 % of the endogenous adenine nucleotides were exchanged against labelled exogenous ATP.

	nmoles/mg chlorophyll			
	AMP	ADP	ATP	sum
minus ^{14}C-ATP	36	40	17	93
plus ^{14}C-ATP	35	37	20	92

Consequently endogenous nucleotides must have been exchanged against exogenous labelled ATP. A quite similar mechanism of adenine nucleotide exchange was found to occur in isolated mitochondria prepared from rat liver (Pfaff, 1965; Klingenberg and Pfaff, 1965; Pfaff et al., 1965), and from yeast (Ohnishi et al., 1967). The uptake of ^{14}C-labelled ATP does not

cause a striking increase in the concentration of ATP inside the chloroplast. The relative concentrations of the 3 adenine nucleotides within the chloroplast is assumed to be held constant by adenylate kinase.

The rate of translocation of ADP into mitochondria is much higher than the transport of ATP and AMP (Pfaff, 1965). Isolated Acetabularia chloroplasts differ from mitochondria under our conditions. The exchange rate is highest with exogenous ATP. It is about 1/3 slower with ADP and about 2/3 slower with AMP (Fig. 2). The exchange rate is even higher with ATP as compared to ADP at low exogenous nucleotide concentrations. The translocation of ATP is nearly saturated at 0.2 mM ATP, while about tenfold higher ADP concentrations are necessary for maximum ADP exchange rates.

The translocation of adenine nucleotides through the chloroplast membrane seems to be an enzyme mediated reaction rather than a physical process. This is concluded from the temperature dependence of the trans-

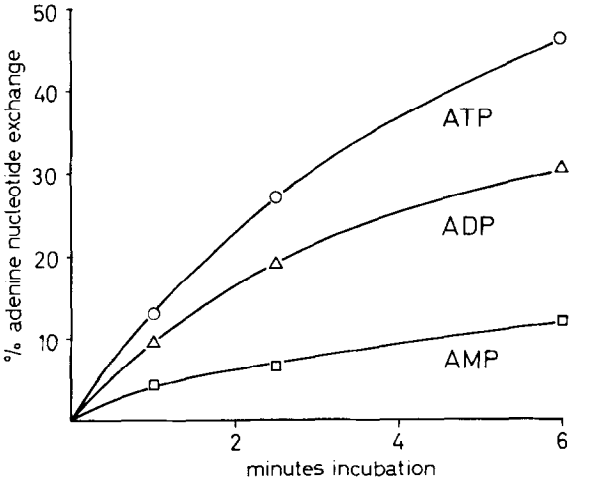

Fig. 2. Kinetics of the exchange of endogenous nucleotides against exogenous ATP, ADP, and AMP. Back exchange experiment: The chloroplasts were pre-incubated for 70 minutes in preparation medium containing 2 mM ^{14}C-labelled ADP. After this time the endogenous adenine nucleotides were found to be in equilibrium with the added ^{14}C-labelled ADP. After two washings with nucleotide free medium the chloroplasts were incubated with 2 mM unlabelled ATP, ADP, and AMP, and in a control experiment without any nucleotide. After different incubation periods the chloroplasts were quickly sedimented by centrifugation, and the exchange of adenine nucleotides was calculated from the radioactivity appearing in the medium und from the loss in activity in the chloroplast extract.

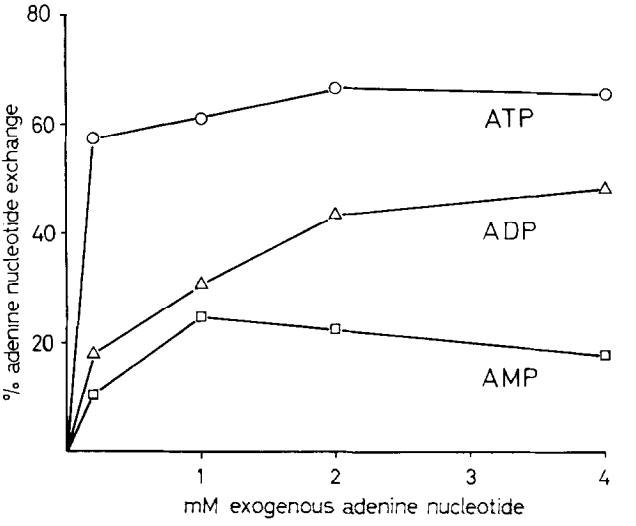

Fig. 3. Concentration dependence of adenine nucleotide exchange. Incubation time 5 minutes. Other experimental conditions as described in Fig. 2.

location process. Between 3 and 20°C a temperature factor of about 2.5 was found to occur in the exchange of ATP. The exchange rate of adenine nucleotides through the envelope of Acetabularia chloroplasts would not be expected to exceed 25 nmoles per mg chlorophyll per minute at the temperature at which the algae are normally cultured. This rate is too low to account for the rapid increase of the ATP concentration in the cytoplasm which is caused by a dark-to-light transition in higher plant cells, as measured by Santarius and Heber (1965).

This discrepancy might be explained by one or more of the following alternatives: (1) The properties of the chloroplast envelope in relation to nucleotide translocation might have been changed by the isolation procedure. (2) The properties of the envelope of algal chloroplasts might be different from those in higher plants. (3) The exchange of adenine nucleotides through the chloroplast membrane might be slower in Acetabularia than in the plants investigated by Santarius and Heber, because of the lower general metabolic activity of Acetabularia. (4) A high exchange

rate of adenine nucleotide molecules in Santarius and Heber's experiments might be simulated by a fast transphosphorylation.

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