ENERGY CHARGE CONTROL OF THE CALVIN CYCLE ENZYME 3-PHOSPHOGLYCERIC ACID KINASE[1]

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Pea (Pisum sativum) leaf cytoplasmic and chloroplast 3-P-glyceric acid kinases are controlled by adenylate energy charge. In the light, when energy charge is high, the chloroplast enzyme will be stimulated in the direction of the Calvin cycle, and the glycolytic activity of the cytoplasmic kinase will be inhibited. In the dark when energy charge is lower, both enzymes may participate in the generation of ATP.

3-P-glyceric acid kinase (ATP:D-3-phosphoglycerate 1-phosphotransferase, EC 2.7.2.3) is present in cytoplasm and chloroplasts of higher plants (1, 2). The enzyme has been purified from chloroplast and cytoplasmic pea leaf fractions and some of its properties determined. In the ATP generating direction the cytoplasmic and chloroplast kinases are inhibited by AMP and ATP to the same extent, while in the ATP utilizing direction AMP, which is competitive with ATP, is a more effective inhibitor (with Ki, 0.035 for cytoplasmic and 0.053 mM for chloroplast kinase) than is ADP (Ki, 0.48 and 0.78 mM, respectively)(3). Because all three nucleotides, AMP, ADP and

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ATP, are present in the cell simultaneously, their cumulative effect on enzyme activity can best be demonstrated by energy charge experiments. Although Atkinson (4) suggested some years ago that energy charge control would be found ubiquitously in living organisms, there has not been any demonstration of such control for higher plant enzymes. We now wish to report that pea leaf cytoplasmic and chloroplastic 3-phosphoglyceric acid kinases are controlled by energy charge in both catalytic directions. Energy charge may, therefore, regulate the activity of the Calvin cycle and glycolysis in higher plants *in vivo*.

MATERIALS AND METHODS

3-P-glyceric acid kinases, purified from pea leaf chloroplast or cytoplasm, were used in these experiments. Enzyme activity, coupled to NADH formation or utilization, was followed using a Gilford 2400 recording spectrophotometer. The desired level of energy charge was generated using adenylate kinase as described by Klungsøyr, *et al.* (5); a mixture containing 20 μg adenylate kinase, 50 μmoles total ATP plus AMP (varied ATP:AMP ratios), and 75 μmoles $MgCl_2$ in a total volume of 1.35 ml was incubated at 25 C for 30 min. The final assay mixture contained, a) in the ATP utilizing assay: 10 μmoles 3-P-glyceric acid, 0.1 μmoles NADH, 21.1 μmoles $MgCl_2$, 7.14 μmoles total ATP, ADP and AMP, 2.3 μg adenylate kinase, 100 μg glyceraldehyde-3-P dehydrogenase, 50 μmoles Tris-HCl, pH 7.4, 0.25 mmoles NaCl, and 3-P-glyceric acid kinase in the total volume of 1 ml or, b) in the ATP generating assay: 1.0 μmole glyceraldehyde-3-P, 50 μmoles potassium phosphate, pH 7.4, 0.4 μmoles NAD^+, 13.1 μmoles

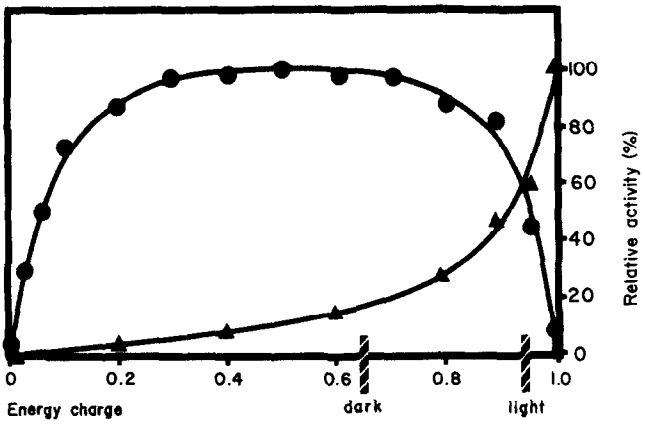

Fig. 1. Energy charge control of chloroplast 3-P-glyceric acid kinase in ATP generating (●) and ATP utilizing (▲) directions. The assay conditions are given in Methods. Similar results are obtained with either 7.14 or 3.57 μmoles total of adenine nucleotides in the assay mixture. Probable energy charge in the chloroplast in light and dark is indicated by the two vertical lines in the lower part of the figure. The dependence of the cytoplasmic enzyme on energy charge is essentially identical with that of the chloroplast enzyme.

$MgCl_2$, 7.14 μmoles total ATP, ADP and AMP, 2.3 μg adenylate kinase, 50 μg glyceraldehyde-3-P dehydrogenase, and 3-P-glyceric acid kinase in the total volume of 1 ml.

RESULTS AND DISCUSSION

The activity of the chloroplast pea leaf 3-P-glyceric acid kinase *in vitro* is clearly a function of energy charge (Fig. 1). The cytoplasmic kinase shows essentially identical dependence on energy charge (data not shown). In ATP utilizing direction the region of the greatest dependence of the pea leaf kinase activity is between energy charge 0.8 and 1.0. In ATP generating direction there are two regions of control at

either end of the energy charge range. In addition to the pea leaf enzymes, commercial (Sigma) yeast 3-P-glyceric acid kinase showed behavior comparable to that shown in Fig. 1. The reported inhibition of Hydrogenomonas facilis (6) and rabbit skeletal muscle (7) 3-P-glyceric acid kinases by ADP or AMP strongly suggests that these two kinases are also under energy charge control.

Heber and Santarius (8) have determined levels of ADP and ATP in Elodea chloroplasts in dark and light. Energy charge levels calculated from their data (assuming negligible levels of AMP) would be, in chloroplast in dark 0.67, in light 0.95, and in cytoplasm in dark 0.76 and in light 0.94. Energy charge levels in pea leaves will probably be approximately the same as in Elodea. Upon illumination, there will be an increase of about 0.28 in energy charge in chloroplast and 0.18 in cytoplasm. This increase is in the region where the direction of the 3-P-glyceric acid kinase reaction is controlled by energy charge, as shown in Fig. 1. Because of this relationship, both cytoplasmic and chloroplast kinases could participate significantly in regulation of carbohydrate metabolism in higher plants. The prevailing direction of the 3-P-glyceric acid kinase catalyzed reaction will be a function of energy levels in the cell compartments. Upon illumination, the chloroplast kinase will be stimulated in the Calvin cycle direction toward carbohydrate synthesis, while the increase of high energy nucleotides in cytoplasm will inhibit the glycolytic activity of the cytoplasmic kinase. In dark, both chloroplast and cytoplasmic kinases could participate in generation of ATP via a partial (in chloroplasts) or a full (in cytoplasm) glycolytic sequence.

In contrast to 3-P-glyceric acid kinase and two bacterial ribulose-5-P kinases (9, 10), pea leaf ribulose-5-P kinase is not affected by AMP or ADP and is totally insensitive to energy charge (11). It seems unlikely that other Calvin cycle enzymes, which do not utilize adenine nucleotides, will be controlled by energy charge in the pea plant. Energy charge apparently regulates the activity of the Calvin cycle in this plant only at the 3-P-glyceric acid kinase step. This may not be true for glycolysis, however, where in addition to 3-P-glyceric acid kinase, the possibility of energy charge control of hexokinase, phosphofructokinase and pyruvate kinase catalyzed reactions must be considered.

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