AMP-SENSITIVE FRUCTOSE DIPHOSPHATASE IN HIGHER PLANTS: EFFECTS OF CARBON SOURCES ON THE LEVEL OF THE ENZYME

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The presence of a phosphatase (FDPase) which specifically dephosphorylates Fructose 1,6-diphosphate (FDP) to Fructose 6-phosphate (D-Fructose 1,6-diphosphate-1-phosphohydrolase, [E.C. 3.1.3.11]) has been demonstrated in many organisms. In yeast and in animal tissues FDPase has been intensively studied in relation to its gluconeogenetic function (Krebs, 1963), and several authors have reported that FDPase is an allosteric enzyme, regulated by AMP and inhibited by substrate (Taketa and Pogell, 1963; Mendicino and Vasarhely, 1963; Underwood and Newsholme, 1965). Gancedo et al. (1965) and Rosen, Rosen and Horecker (1965) have shown that the level of FDPase in yeast is considerably higher when lactate, ethanol or glycerol are the carbon source, than when glucose serves this purpose. In plants, FDPase activity has been often strictly linked with the photosynthetic activity of green tissues (Racker and Schroeder, 1958; Smillie, 1960; App and Jagendorf, 1964). The presence of a non-photosynthetic, specific FDPase, in connection with the reversal of glycolysis, has been suggested by Cocucci and Marrè (1963). Moreover, Bianchetti and Sartirana (1966) have pointed out that also in plant tissues a control on glycolysis is carried out through an allosteric regulation by 5'-AMP on FDPase, in addition to the control by ATP and citrate on phosphofructokinase. In this work the presence, in germinating wheat embryos, of a non-photosynthetic, AMP-sensitive FDPase is reported and the effects of

various carbon sources on its level given . FDPase level, which is very low in the isolated embryos grown with glucose, increases very much in the embryos grown in glycerol or in mineral medium. This increase is suppressed by either puromycin or actinomycin and is reversed by the presence of glucose in the medium.

Experimental and Results. Isolated wheat embryos were grown as previously described (Bianchetti and Sartirana, 1966). Free glucose and fructose were determined enzymically in neutralized perchloric extracts.

FDPase activity was assayed in the supernatant fraction of tissue homogenates in 50 mM Tris-HCl buffer plus 5 mM mercaptoethanol and 1 mM EDTA, pH 7.3, centrifuged at 20,000 xg and filtered through Sephadex G25, by fluorimetric or spectrophotometric measurement of the rate of NADP reduction. The composition of the reaction mixture was: 100 mM Tris-HCl buffer, pH 8.8, 3 mM $MgCl_2$, 1 mM EDTA, 10 mM cysteine, 0.2 mM NADP, 0.1 mM FDP and an excess of phosphoglucoisomerase and glucose 6-phosphate dehydrogenase. Almost no non-specific phosphatase activity on FDP was detected under these assay conditions and FDP was nearly completely converted to Fructose 6-phosphate. Non-purified FDPase from wheat embryos is characterized by these properties: pH optimum 8.6, apparent Km 2 μM, Mg^{++} requirement, protection by -SH compounds, inhibition by substrate (about 50% inhibition with 1mM FDP), sensitivity to 5'-AMP as negative modifier with m = 1.8 (Atkinson,1966). Further characterization is in progress.

FDPase development is very different for the embryos grown with different carbon sources. Fig. 1 shows that FDPase does not increase in the embryos grown in glucose, while it increases significantly in the embryos grown in glycerol and even more in those grown without any external carbon source. The increase of the enzyme is partially blocked by 30 μg./ml. actinomycin D and completely abolished by 0.2 mM puromycin.

From the 2^{nd} to the 90^{th} hour, the fresh weight of the embryos increases from 30 to 45 mg./10 embryos in mineral medium, from 30 to 60 mg./10 embryos in glycerol and from 30 to 100 mg./10 embryos in glucose.

Preceding the large increase in the enzyme level (embryos grown in mi-

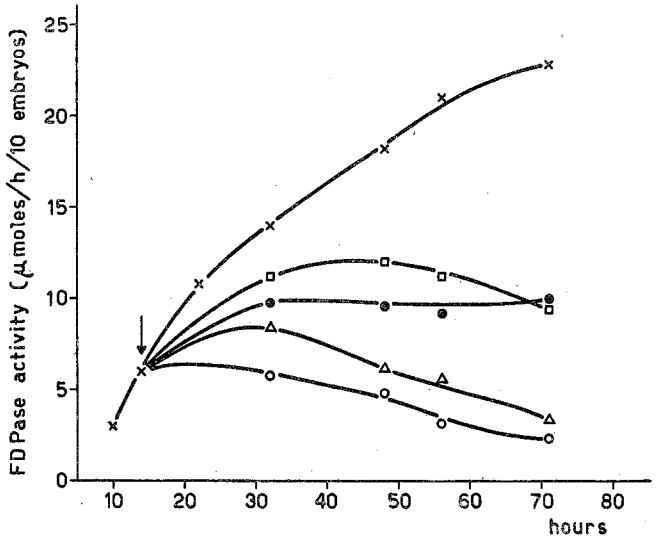

Fig. 1 - Effect of various compounds on the level of wheat embryo FDPase. (X) mineral medium; after 14 hours of germination (arrow) were added: (●) 0.5% glycerol; (Δ) 50 mM glucose; (O) 0.2 mM puromycin; (□) 80 μg./ml/ actinomycin D. FDPase activity is not expressed as specific activity because in the early germination no significant change in the amount of total protein (storage material) was observed.

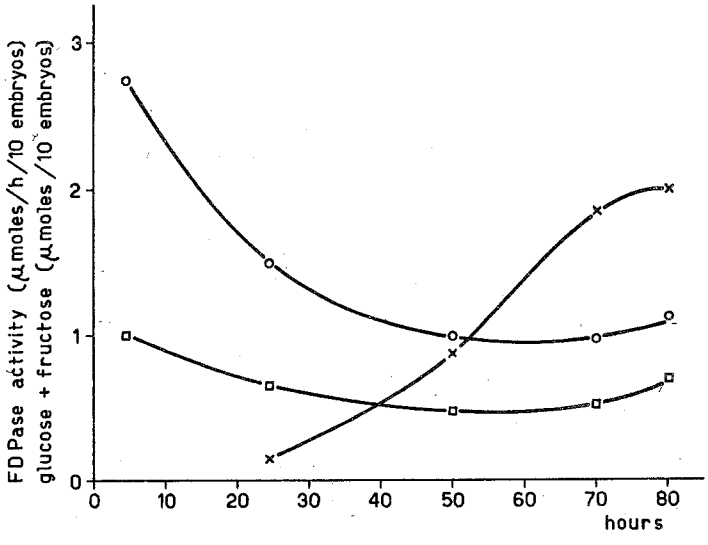

Fig. 2 - Embryos grown in mineral medium: FDPase level (X); free glucose + fructose (O); R.Q. (□).

neral medium), marked decreases in the level of free glucose plus fructose and in the R.Q. are observed (Fig. 2). The changes of the R.Q. from 1 to about 0.5 might indicate a change in the respiratory substrates, together with a partial conversion of more reduced substrates (lipids and/or proteins) to more oxidized substrates (sugars).

If now glucose or glycerol are supplied to these embryos (grown in the mineral medium, and showing a high enzyme level) a large increase in the amount of free glucose plus fructose is observed, while the level of the enzyme goes down to the values detectable in the embryos grown with these substrates (Fig. 3).

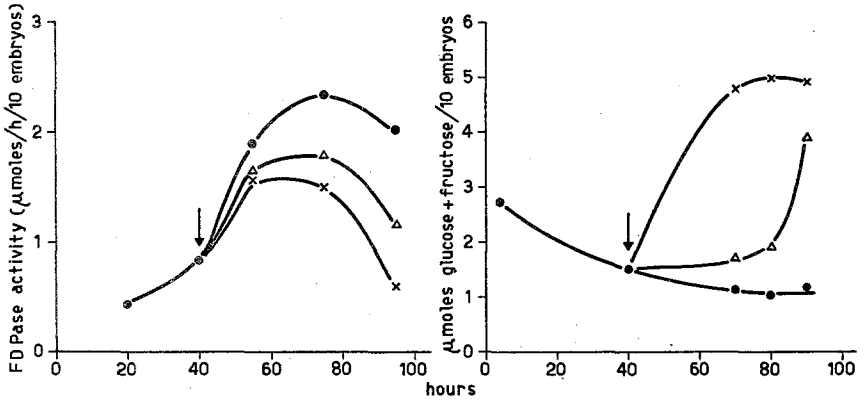

Fig. 3 - Effects of the addition of glucose (×) or glycerol (Δ) on FDPase level (left) and on the level of free glucose + fructose (right). Glucose (50 mM) or glycerol (0.5%) was added to the culture medium after 40 hours of germination (arrow). Mineral medium (●).

Discussion. Very different levels of AMP-sensitive FDPase are detectable in non-photosynthetic plant tissues. In this laboratory, various organs from germinating castor bean seeds, melon seeds and wheat seeds, and also etiolated pea internodes have been examined. The highest FDPase levels were detected in those tissues, such as castor bean endosperms and cotyledons, in which the reversal of glycolysis occurs to a considerable extent (Beevers, 1957). The increase of FDPase in the wheat embryos grown either in mineral medium or in glycerol and its repression by glucose coincides with a strong change in the ratio between the rates

of the opposite glycolytic flows. It is therefore legitimate to conclude that the presence and the level of an AMP-sensitive FDPase in higher plant tissues are connected with the extent to which, during the reversal of glycolysis, the thermodinamically irreversible phosphofructokinase reaction is bypassed. It is of interest that the FDPase activity induced in E. coli by growth on non-carbohydrate precursors is not inhibited by either AMP or high substrate concentrations (Gotto and Pogell, 1962).

The specific FDPase of streptomycin bleached strain of Euglena gracilis, presumably involved in gluconeogenesis, is inhibited by excess substrate but not by AMP (Taketa and Pogell, 1963). Sensitivity to AMP appears in yeast and is also observable in higher plants and in animal tissues in which, on the other hand, the inhibition by substrate may disappear (Pontremoli et al., 1965).

The repressive effect of glucose on the increase of the enzyme, which has been verified also in germinating castor bean cotyledons, is still obscure. Some cases of regulation by substrates on the synthesis of enzymes in higher plant tissues have been clearly demonstrated in this and in other laboratories (Marrè et al., 1965; Sartirana and Bianchetti, 1965; Glasziou et al., 1966).

Experiments with actinomycin D and puromycin, reported in this work, show that in wheat embryos the increase of FDPase is due to protein synthesis, but the mechanism by which glucose exerts the repression remains unknown. It is reasonable to think that FDPase level is strictly connected with the level of the intermediates of glycolysis.

Studies of the changes in the concentrations of these metabolites under the conditions which induce or repress the FDPase are in progress.

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