A.K. Mattoo and N.R. Porikh: Influence of sodium pyruvate on Neurospora fructose diphosphatase.

Fructose-I, 6-diphosphatase, which catalyzes an essential step in gluconeogenesis, has been shown to be present in most of the living tissues examined. However, very little information is available on this enzyme from N. crassa. Reinert and Marzluf (1 974 Neurospora Newsl. 21: 16) indicated that the Neurospora enzyme is derepressed when grown on ethanol and showed that the enzyme level in vivo might not be regulated by turnover. We hove obtained similar results. In the present communication we wish to show that cyrteine, cations and pyruvate might afford a control of the FDPase activity in Neurospora. Additionally, we suggest that pyruvate moy act as a "feed-forward" activator of the enzyme and, hence, of gluconeogenesis.

N. crassa was cultivated on Vogel's medium (100 ml in 250 ml erlenmeyer flasks) containing 2% sucrose as described previously (Mattoo and Shah 1974 Z. allge. Mikrobiol. 14: 581). 24-hr-old mycelia from this liquid culture were transferred into fresh medium containing one of the following carbon sources; glucose, sucrose, glycerol, or ethanol. After further incubation for 48 hr, mycelia were harvested by filtration and immediately frozen. Cell-free extracts (Mattoo and Rao 1974 Biochem. Biophys. Rer. Commun. 60: 1229) were passed through a Sephadex G-25 column and used as the enzyme in the experiments reported. The enzyme activity was highly labile, as also observed by Reinert and Marzluf, because of which values obtained from experiment to experiment varied enormously. The results reported here are averages from those triplicate assays whore variation was less than 5%. FDPase was estimated by measuring the release of orthophosphate by the method of Fiske and Subbarow (1925 J. Biol. Chem 66:375). The reaction mixture (final volume 1 ml) contained all or some of the following components: fructose 1, 6-diphosphate (FDP), Tris-HCL buffer (pH 7.5), EDTA, $MgCl_2$, and KCl. After incubation for 1 hr at 37° C, the enzyme reaction was terminated by adding 0.1 ml of ice cold 100% TCA. One unit of enzyme activity represents the liberation of 1 nmole of orthophosphate/min under the experimental condition.

The enzyme showed a peak of activity at pH 7.5 in Tris-HCl buffer and the velocity of the reaction was linearly dependent on the amount of enzyme protein and time of incubation. Maximum derepression of the enzyme occurred in cultures grown on glycerol, the derepressed level of the enzyme being one-and-a-holf timer that elaborated on ethanol. The enzyme was repressed in cultures grown on glucose or sucrose. Maximum enzyme activity of the enzyme was obtained when the reaction mixture contained EDTA, $MgCl_2$, KCl and FDP. The requirement for Mg^{2+} could not be met by Mn^{2+}, as found with the enzyme from Escherichia coli (Neu and Heppel 1964 Biochem. Biophyr. Res. Commun. 17: 215). However, unlike the E. coli and Pseudomonas (Fossitt and Bernstein 1963 J. Bacteriol. 86: 598) enzymes, cyrteine strongly inhibited the enzyme from Neurospora (Table 1). At 8 mM cyrteine the enzyme activity was lowered by 3- to 5-fold.

Our earlier (Mattoo and Rao) work indicated a role for pyruvate in regulating glycolysis by its inhibitory effect cm FDP-aldolase of Neurorpora. Potassium ions were shown to negate this effect. As potassium ions had stimulatory effect on FDPase also (Table 2), it was interesting to see whether or not pyruvate hod any effect on this enzyme in the absence and presence of cyrteine and potassium ions. Pyruvate was found to stimulate the enzyme and it effectively reversed the inhibitory action of cyrteine (Table 1). The presence of potassium ions and sodium pyruvate had an additive effect on the stimulation of the enzyme (Table 2).

Table 1. Inhibition of FDPase by cysteine and its reversal by pyruvate.

Addition to the reaction mixture*	Cultural Condition: Glucose grown	Glycerol grown	Ethanol grown
Nil	22**	5.5	36
Cyrteine (8 mM)	7	11	9
Pyruvote (0.5 mM)	36	68	40
Cyrteine (8 mM) + Pyruvote (0.5 mM)	14	40	34
Cyrteine (8 mM) + Pyruvote (1 mM)	17	42	38

*The reaction mixture contained FDP (0.5 mM), Tris-HCl buffer (pH 7.5, 40 mM), EDTA (2 mM), $MgCl_2$ (20 mM), KCl (100 mM) and enzyme protein (100 µg).
** FDPore activity in units/mg protein.

Table 2. FDPase activity of glycerol-grown culture in the presence and absence of potassium ions and sodium pyruvate

Addition to the reaction mixture*	FDP Concentration, mM: 0.5	1.0
Nil	19**	34
KCL (100 mM)	56	64
Pyruvate (1 mM)	40	68
KCL (100 mM) + Pyruvote (1 mM)	76	96

*The reaction mixture contained FDP (as indicated), Tris-HCl buffer (pH 7.5, 40 mM), EDTA (2 mM), $MgCl_2$ (20 mM) and enzyme protein (150 µg).
**FDPase activity in units/mg protein.

There results confirm the observation that gluconeogenic substrates derepress the formation of Neurospora FDPore and bring out the interesting observation of the reversal of cysteine inhibition of the enzyme by pyruvate. Potassium ions, which reverse the inhibitory effect of pyruvote on FDP-aldolase (Mattoo and Rao), complemented the stimulatory effect of pyruvote on FDPase. We, therefore, *suggest* that pyruvate might regulate *gluconeogenesis* by acting *as a* "feed-forward" *activator* of FDPore and keeping it in on active state. Intracellular levels of potassium ions, pyruvate and FDP at the site of the enzyme in vivo could therefore ploy an important role in the control of FDPore activity and of gluconeogenesis. ---We thank Professor V.V. Modi for his inter&. - - - Deportment of Microbiology, Faculty of Science, M.S. University of Baroda, Borodo 390002, India, (until March 1976, Department of Biochemistry, University of Adelaide, Adelaide, South Australia 5001).