

Fructose-1, 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 (1974 *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 have 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 may 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. allg. 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. Res. 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 where 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-half times 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. Biophys. 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 on FDP-aldolase of *Neurospora*. 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 had 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            |
| Cysteine (8 mM)                   | 7                  | 11             | 9             |
| Pyruvate (0.5 mM)                 | 36                 | 68             | 40            |
| Cysteine (8 mM)                   |                    |                |               |
| +                                 |                    |                |               |
| Pyruvate (0.5 mM)                 | 14                 | 40             | 34            |
| Cysteine (8 mM)                   |                    |                |               |
| +                                 |                    |                |               |
| Pyruvate (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  $\mu$ g).

\*\* FDPase 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)                      |                       |     |
| +                                 |                       |     |
| Pyruvate (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  $\mu$ g).

\*\*FDPase activity in units/mg protein.

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