Majmudar, G.H., A.M. Dandekar and A.K. Mattoo.

mediated control of polyphosphatase in Neurospora crassa

Considerable information is available on the accumulation of polyphosphates and on the enzymes metabolising them (Kulaev (1975) Rev. Physiol. Biochem. Pharmacol. 73: 131). However, very little is know about the effectors that regulate the level of polyphosphatases in vivo. We report here on the phosphate-mediated control of Neurospora polyphosphatase (EC 3.6.1 .11, polyphosphate phosphohydrolase).

Cultures were grown on synthetic medium (Mattoo et al. (1973) Indian J. Exp. Biol. 11: 511) with either 0.0025% or 0.0125% KH_2PO_4 concentration equivalent to 1.7 mg % or 8.7 mg % of phosphate respectively. Cell extracts for enzymic activity were prepared and stored as described previously (Mehta et al. (1972) Biochem. J. 130: 159). Standard reaction mixtures contained: tris-HCl buffer (pH 7.6), 100 mM; $MgCl_2$, 2 mM; β-mercaptoethanol, 1 mM; dialysed sodium polyphosphate (n̄20), 1 mg; and an appropriate aliquot of enzyme solution in a final volume of 5 ml. Incubation was carried out at 23° C for various time intervals and reactions terminated by the addition of ice-cold trichloroacetic acid (final concentration 15%). Orthophosphate released was determined by the method of Fiske and Subbarow (1925, J. Biol. Chem. 66: 375). One unit of enzyme activity corresponds to the release of 1 nmole of the product per min under the assay conditions. Only those values which showed a linear relationship with enzyme concentration and time of incubation were token into consideration. Each experiment was run in duplicate and repeated twice.

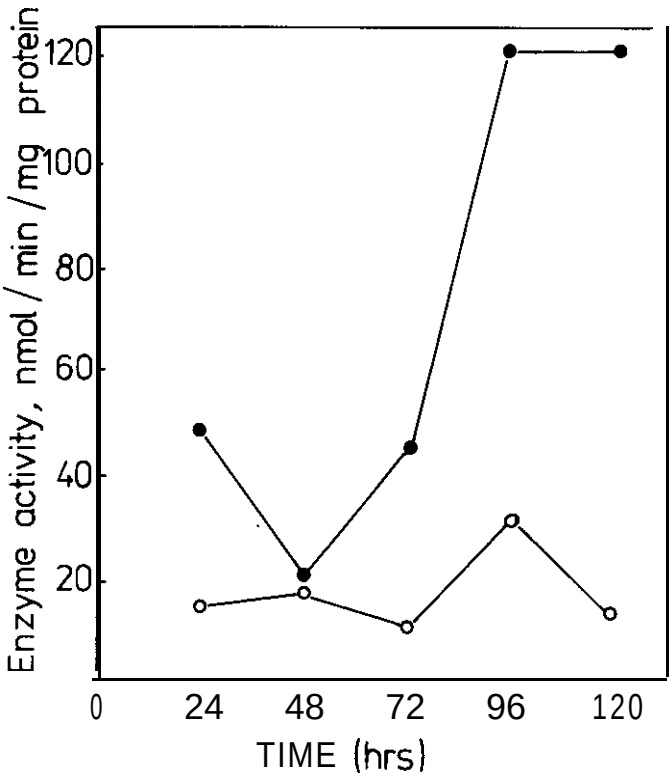

Figure 1. Effect of initial phosphate concentration in the culture medium on the levels of polyphosphatase during growth of N. crassa. (o-o), 8.7 mg % phosphate; (•-•), 1.7 mg % phosphate.

The level of intracellular polyphosphatase varied with the age of the culture and war found to be dependent upon the initial phosphate level in the culture medium (Fig. 1). Cultures grown on limiting phosphate concentration (1.7 mg %) elaborated a much higher level of polyphosphatase which reached a maximum by 96 hr and was d-fold higher than the enzyme level in the cultures grown on high phosphate (8.7 mg %)-containing medium.

In order to further examine the role of inorganic phosphate in modulating the intracellular level of polyphosphatore, experiments were designed to check the effect of transferring limiting phosphate-grown-cultures to fresh media containing either high or limiting phosphate on the levels of polyphosphatase. The results (data not shown) demonstrated a marked increase (4-fold) in the polyphosphatase level within 48 hr of transferring cultures grown on limiting-phosphate medium to phorphote-deficient medium. Repression of enzyme activity was evident in cultures that had grown previously in limiting phosphate medium but were transferred to the high phosphate medium. The increase in enzyme obtained with cultures grown on limiting phosphate medium was distinctive and may be related to a low intracellular level of orthophosphate in there cultures compared to those grown in high phosphate.

Inclusion of orthophosphate (0.25 mM) in the reaction assay mixture resulted in 50% inhibition of enzyme activity as was also shown by Afanasieva and Kulaev (1973, Biochim. Biophys. Ada 321: 336) for the polyphosphatase of Endomyces magnusi. The above results demonstrate the effect of phosphate in regulating the levels and activity of polyphosphatase in N. crassa which in turn may regulate the intracellular concentration of polyphosphates. Phosphate levels in the medium also control the levels and activity of nucleotide degrading enzymes including alkaline phosphatase in N. crassa (Mattoo and Shah (1974) Z. Allge. Microbiol. 14: 581).

We thank Dr. Kerstin Gezelius, Deportment of Plant Physiology, University of Umea (Sweden) for the gift of sodium polyphosphate and Prof. V.V. Modi for his continuing interest.

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