Calcium effects in Neurospora crassa

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Abstract
Calcium effects in N. crassa
The resulting homogenate was shaken for 10 min and then centrifuged at 3,000 rpm for 15 min at 15°C. The aqueous phase was taken and an equal volume of phenol:chloroform:isoamyl alcohol; 49:49:2 was added. After shaking for 10 min, the mixture was centrifuged as above and the aqueous phase taken. Phenol was removed by four extractions with ether. To the RNA solution 2.5 volumes of cold absolute ethanol (-20°C) was added and the mixture was placed in a freezer at -80°C for at least 30 min. The RNA precipitate was collected by centrifugation, washed with cold absolute ethanol and then dried under a flow of N2 gas. The precipitate was dissolved in 5 ml of buffer containing 0.1 M sodium acetate (pH 5.0)/1 mM EDTA/1% SDS (buffer A).

The crude RNA extract was subjected to gel filtration through a Sephadex G-100 column (2x32 cm) equilibrated with buffer A and fractionated into 2 ml fractions. RNA fractions 6 to 11 were pooled and the solution was diluted to 4-fold with buffer A. About 450 times as much RNA was recovered using method II compared with that recovered with method I. The gel filtration is useful to remove small RNAs and to remove free radiisotope when labeling of RNA was performed. To the RNA solution 2.5 volumes of cold absolute ethanol was added and the resulting mixture was stored at -80°C for at least 30 min. The ethanol precipitate was dissolved in 10 ml of 0.2 M NaCl and 25 ml of cold absolute ethanol added. After collecting the precipitate by centrifugation, it was dissolved in 10 ml of 10 mM Tris (pH 7.5)/0.5 M KCl. The RNA solution was loaded to an oligo (dT)-cellulose column. Polyadenylated mRNA fraction eluted with 10 mM Tris (pH 7.5) was pooled and loaded to an oligo (dT)-cellulose column. Polyadenylated mRNA was purified by 2 or 3 cycles of oligo (dT)-cellulose chromatography. Total amounts of RNA isolated by method II were 670-fold larger than those by method I, and polyadenylated mRNAs isolated by method II were 150-fold greater in amounts than those by method I. The ratio of polyadenylated mRNA to total RNA was 1.0%; this value is very similar to those reported previously (M.C. Lucas et al. 1977, J. Bacteriol. 130:1192-1198.

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Table 1: Effect of Ca^+2 on the activities of extracellular amylase, FDP aldolase, isocitrate lyase and G6P dehydrogenase in N. crassa.

| Growth conditions | Extracellular amylase (U/50 ml) | FDP aldolase (U/mg protein) | Isocitrate lyase | G6P dehydrogenase (U/mg protein) |
|-------------------|---------------------------------|-----------------------------|----------------|----------------------------------|
| Calcium deficient | 150                             | 193                         | 799            | 397                              |
| Calcium optimal   | 280                             | 120                         | 440            | 322                              |
| Calcium supraoptimal (100 ug/ml) | 261 | 126 | 381 | 348 |
| Calcium supraoptimal (1000 ug/ml) | 339 | 129 | 327 | 359 |

The mechanism of the effect of calcium on enzymes is not known. Whether calcium influences the activity of enzyme(s) or changes the rate of synthesis or changes the level of cyclic AMP which subsequently alters the level or activity of enzyme(s) is not known. Calcium mediated activation of amylase has been reported by Takegi et al. (1971 In: The Enzymes (P.D. Boyer, Ed.) Academic Press 5:235). In other studies in a number of instances, antagonistic regulatory roles of Ca^+2 and cyclic AMP have been suggested (M.J. Berridge 1975 Adv. Cyclic Nucleotide Res. 6:1-98; Rasmussen and Goodman 1977 Physiol. Rev. 57:421-509). Earlier, Flavell and Woodward had demonstrated that isocitrate lyase is subjected to catabolite repression (Flavell and Woodward 1971 J. Bacteriol. 105:200). In the present studies, if calcium changes the level of cyclic AMP at all, then the possibility exists it can affect the synthesis of isocitrate lyase.

Calcium did not affect the activity of G6P dehydrogenase when added in the growth medium. G6P dehydrogenase is known to provide reducing power for the biosynthesis of lipids. The level of G6P dehydrogenase was not found to be affected whereas the level of carotenoids was significantly reduced under supraoptimal as compared to calcium deficient and calcium optimal conditions (Table 2.). This suggests that the effect of calcium may be on one of the enzymes(s) of a carotenogenic pathway. In order to ascertain if there was accumulation of any intermediate(s) in calcium supraoptimal conditions, thin layer chromatography of extracts of carotene from the Ca^+2 supraoptimal and calcium deficient cultures was carried out and the plates were then checked for fluorescence under UV light. The extract of calcium supraoptimal grown culture showed a distinct band which was not so prominent in the calcium deficient culture.

Table 2: Effect of Ca^+2 on carotene production in N. crassa.

| Growth conditions | Carotenoids (ug/g mat wet weight) |
|-------------------|----------------------------------|
| Calcium deficient | 25                               |
| Calcium optimal   | 34                               |
| Calcium supraoptimal (100 ug/ml) | 6     |

Data obtained in this study indicate the influence of Ca^+2 in the growth medium on some enzymes of carbohydrate metabolism and the production of carotenoids in N. crassa. These studies on the regulatory effects of calcium on biochemical changes could be useful in boosting primary metabolism which can then trigger secondary metabolism. The desired products of secondary metabolism can potentially be increased by maintaining a suitable concentration of Ca^+2 in the growth medium. - - - Dept. of Microbiology, Faculty of Science, M.S. University of Baroda, Baroda 390002, India.