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Abstract
Comparative study on some properties of polyphosphatasephosphohydrolase in Neurospora crassa strain ad-6 (28610) 5 and a leaky derivative.

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Comparative study on some properties of polyphosphatephosphohydrolase in Neurospora crassa strain ad-6 (28610) and a leaky derivative.

Previous work in our laboratory described a mutant of Neurospora crassa with an activity of polyphosphatephosphohydrolase (KF 3.6.1.11) about 30% that of the wild type. Polyphosphatephosphohydrolase catalyzes the hydrolysis of polyphosphates to arthroposphate. Our work has also shown the metabolic and topographic relation of this enzyme with the higher molecular weight polyphosphate fractions. The nature of the changes in its activity of both cultures was substantially lower at all developmental stages (Trilisenko et. al., 1980 Mikrobiologiya 49: 82).

Present literature contains data on polyphosphatephosphohydrolase (PP-ase) properties in N. crassa. It was shown that the purified PP-ase preparation of the strain ad-6(28610) a (Umnov et. al., 1975 Molekulyarnaya biologiya 9: 594) is identical with the enzyme in unpurified preparation of the wildstrain Abbott 4A by its properties (pH-dependence, effect of K+ and Mg++ concentrations (Kulaev and Konoshenko, 197 Biokhimiya 36: 1175).

PP-ase synthesis is independent of the concentration of exogenous phosphate (Ilimov et. al., 1974 Biokhimiya 39: 414). Synthesis of acid and alkaline phosphatase, nuclease, and high affinity phosphate permease are controlled by a common regulatory system in this fungus (Littlewood etc., 1975 Genetics 79: 419). In bacteria, the system controlling the synthesis of acid and alkaline phosphatases also regulates the synthesis of PP-ase and tripolyphosphatase (TPP-ase) (Harold, 1964 J. Gen. Microbiol. 35: 81; Nesmeyanova et. al., 1975 Kob. AN SSSR 224: 710; Miraeva et. al., Biokhimiya 44: 715).

It was of interest to determine whether eukaryotes, similarly to prokaryotes, are controlled by a common system regulating the synthesis of phosphohydrolases. The present work was devoted primarily to the study of PP-ase properties in a "leaky" mutant (strain 30.19-3), a mutant with a reduced level of the PP-ase activity, and in other strains. The following strains of Neurospora crassa were used: ad-6 (28610) a; mutant 30.19-3 with reduced PP-ase activity isolated from ad-6 mutagen; wild-type strain 74-OR8-la; 28610) a after treatment by Metzenberg, U. of Wisconsin and nuc-1 (the latter two were generously provided by Dr. R. L. Metzenberg, U. of Wisconsin Madison, USA). All the cultures were grown at 28°C on Fries medium plus adenine (50 mg/l) for ad-6 (28610) d and 30.19-3. To study PP-ase properties, cells were harvested at the phase of culture growth decrease.

In the mutant 30.19-3 and the parent strain, the dependence of the polyphosphatase activity in crude cell extracts on temperature of the incubation mixture and concentration of hydrogen ions was identical.

In both cultures in polyacrylamide gel electrophoresis showed two protein components with polyphosphatase activity. The electrophoretic mobility of these two protein components was the same in the mutant and parent strain (Table 1).

| Enzyme                | ad-6 (28610) a | 30,19-3        |
|-----------------------|----------------|---------------|
| Acid phosphatase      | 0.526±0.004    | 0.532±0.004   |
| ATP-ase               | 0.539±0.003    | 0.540±0.006   |
| Pyrophosphatase       | 0.554±0.006    | 0.550±0.019   |
| Tripolyphosphatase    | 0.595±0.01     | 0.595±0.008   |
| Pol yphosphatase      | 0.684±0.009    | 0.683±0.011   |
| Pol yphosphatase      | 0.727±0.006    | 0.724±0.013   |
| Alkaline phosphatase  | 0.763±0.01     | 0.758±0.004   |

A cell free extract of the wild-type strain contains one protein with polyphosphatase activity (Umnov et. al., 1974 Biokhimiya 39: 373). Alkaline phosphatase of E. coli may hydrolyze not only phosphomonoesters but polyphosphates as well. Therefore, one of the polyphosphatase components found by us might be any phosphohydrolase with a wide range of action. To check this, proteins of cell free extracts of both cultures were separated electrophoretically in polyacrylamide gel and stained for specific activities of TPP-ase, pyrophosphatase (PyroP-ase), adenosinetriphosphohydrolase (ATP-ase), alkaline and acid phosphatases.
As seen from Table 1, the electrophoretic mobility of none of the phosphohydrolases considered is equal to, or approximates that of either of the two protein components exhibiting the polyphosphatase activity. Evidently they are two isoforms of PP-ase. The existence of several PP-ase isoforms in fungi was shown earlier in *Endomyces magnusii* (Afanasieva *et al.*, 1975 *Biokhimiya* 41: 1078). Electrophoretic mobilities of TPP-ase, PyroP-ase, ATP-ase, alkaline and acid phosphatases were identical in both cultures (Table 1).

Therefore, electrophoretic activity, pH and temperature optima of the PP-ase activity are equal in the mutant 30,19-3 and parent strain. Thermostability of the enzyme was higher in the mutant than in strain ad-6 (28610) *a*, e.g. after 10 min incubation of crude cell extract at 40°C, polyphosphatase retained 95% of its activity in the mutant and 60% in strain ad-6 (28610) *a*. Also, the crude preparation of PP-ase of the mutant was more stable during storage.

As mentioned above, we determined if there is a common system for the regulation of the synthesis of phosphohydrolases. To study the activity of phosphohydrolases in conditions of repression and derepression, mycelium was harvested at the beginning of logarithmic growth, washed with cold distilled water and transferred to fresh Fries medium with and without phosphate. After incubation, the mycelium was harvested, and extracted. The activity of corresponding phosphohydrolases was determined in the cell-free extracts.

As seen from Table 2, in the presence of phosphate, TPP-ase and Pyro-P-ase, activities in mutant 30,19-3 and parent strain ad-6 (28610) *a* were the same. Activities of alkaline and acid phosphatases in the "leaky" mutant were less by 30% and 50-70% respectively, than in strain ad-6 (28610) *a*. In conditions of the phosphorus starvation, syntheses of TPP-ase, Pyro-P-ase and PP-ase by both cultures did not undergo derepression of alkaline phosphatase and acid phosphatase.

**TABLE 2**

| Activity of phosphohydrolases | PP-ase | TPP-ase | Pyro-P-ase | Alkaline phosphatase | Acid phosphatase |
|--------------------------------|--------|---------|------------|----------------------|------------------|
| +P -P | +P -P | +P -P | +P -P | +P -P | +P -P | +P -P | +P -P |
| ad-6 | 48 | 67 | 976 | 978 | 3178 | 2387 | 201 | 350 | 14.0 | 56.4 |
| 30,19-3 | 20 | 22 | 951 | 966 | 2854 | 2811 | 137 | 281 | 5.5 | 86.7 |
| 74-QRB-1a | 93 | 78 | 1097 | 750 | 3411 | 2966 | 37 | 66 | - | - |
| nucl | 66 | 69 | 1123 | 651 | 2594 | 2632 | 42 | 48 | - | - |

Activity is expressed in nM orthophosphate released by reaction in 1 min per 1 mg protein.

The results obtained show that the system controlling the synthesis of alkaline and acid phosphatase in *N. crassa* does not also regulate PP-ase, TPP-ase and Pyro-P-ase. We assume therefore that synthesis of phosphohydrolases in eukaryotes is regulated otherwise than in prokaryotes.

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Regulatory effect of inasitol on the synthesis of myo-inositol-1-phosphate synthase in *Neurospora crassa* strains

The inositol, synthesizing enzyme myo-inositol-1-phosphate synthase (MIPS, EC 5.5.1.4) converts glucose-6-phosphate into inositol-1-phosphate. It has been isolated in a highly purified form and its molecular properties have also been determined (Zsindely *et al*., 1977 *Acta* 11. Acad. Sci. hung. 28: 281; Aradi *et al*., 1980 *Neurospora* NEWSL. 27: 27). The production of a cross-reacting defective protein has been detected by immunological methods in inositol-requiring mutants which are characterised by lack enzyme activity (Zsindely *et al*., 1979 *Acta* biol. Acad. Sci. hung. 30: 141).

In the present experiments the biosynthesis of the enzyme and that of the defective protein were studied in various strains of *Neurospora crassa*. Enzyme activity was measured in crude extracts of cultures by analyzing quantitatively the production of inositol-1-phosphate as described by Barnet *et al.* (1970 *Biochem* 119: 183). The amount of proteins reacting with monovalent immune-sera produced against highly-purified enzyme was determined by rocket immunoelectrophoresis according to Laurel (1966 *Anal. Biochem* 15: 45 in a