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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) 5 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 catalizes the hydrolysis of polyphosphates to arthophosphate. 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 etal., 1975 Molekulyarnaya biologia 9: 594) is identical with the enzyme in unpurified preparation of the wild strain Abbott 4A by its properties (pH-dependence, effect of K^+ and Mg^{++} concentrations (Kulaev and Konoshenko, l97 Biokhimiya 36: 1175).

PP-ase synthesis is independent of the concentration of exogenous phosphate (limnov et al., I975 Biokhimiya 44: 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. Microbial. 35: 81; Nesmeyanova et al., 1975 Kokl. AN SSSR 224: 710; Maraeva 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 28610)a after treatment by mutagen;wild-type strain 74-OR8-la; 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).

TABLE 1

Electrophoretic mobility of phosphohydrolases in extracts of ad-6 (28610) a and 30,19-3

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
Polyphosphatase I	0.684±0.009	0.683±0.011
Polyphosphatase II	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 mdbility 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 mabilities of TPP-ase, PyroP-ase, ATP-ase, alkaline and acid phosphatases were identicalin 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 4O°C, polyphosphatase retained 95% of itsactivity in the mutant and 60% in strain ad-6 (26810) 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 comnon system for the regulation of the synthesis of phosphohydrolases. To study the activity of phosphahydrolases in conditions of repression and derepression, mycelium was harvested at the beginning of logarithmic growth, washed with cold distilled water andtransferred 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 derepres-sion of alkaline phosphatase and acid phasphatase.

TABLE 2

Effect of exogenic orthophosphate on activity of phosphohydrolases

	PP-ase		TPP-ase		Pyro-P-ase		Alkaline phospha-tase		Acid phos-phatase	
	+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-OR8-1a	93	78	1097	750	3411	2966	37	66	-	-
nuc-1	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 PyroP-ase. We assume therefore that synthesis of phosphohydrolases in eukaryotes is regulated otherwise than in prokaryotes. - - - Institute of Biochemistry and Physiology of Microorganisms, USSR Academy of Sciences, Pushchino, 142292, USSR.