ROLE AND FORMATION OF THE ACID PHOSPHATASE IN YEAST*

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An acid phosphatase activity involved in the fermentation of hexosephosphates was identified in intact baker's yeast by Rothstein et al. (1949). Schmidt et al. (1956, 1962) have reported that the acid phosphatase of yeast is increased by growth in inorganic phosphate deficient media, as happens with the alkaline (but not the acid) phosphatase of E. coli (Horiuchi et al., 1959; Torriani, 1960; Hofsten, 1961). Evidence pointing to a location between the cell membrane and the cell wall, has been reported for the alkaline phosphatase of E. coli (Malamy, Horecker, 1961) and the acid phosphatase of yeast (Schmidt et al., 1962). The observations reported here indicate that an external location respect to the cell membrane, repression by Pi and a digestive role for esterified phosphate are indeed features of the acid, but not of the neutral phosphatase of baker's yeast.

As shown in Table I, the addition of Pi to a basal medium low in phosphate, markedly decreases the phosphatase

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activity of intact yeast, and its ability to ferment the carbohydrate moiety of glucose 1-phosphate (G1P). Significant growth was not detected with G1P as sole source of carbon and energy in conditions in which Pi was in excess. However, the enhancement of growth by addition of Pi to the basal medium plus glucose, was also obtained if G1P was substituted for Pi.

TABLE I

Influence of inorganic and esterified phosphates on the growth and formation of acid phosphatase in baker's yeast

Carbon source	Additions μmoles/ml	Relative growth	Phosphatase units per gram		Rate of fermentation of G1P as % of that of glucose
			external	internal	
Glucose	--	30	82	93	78
"	Pi, 0.1	47	37	74	
"	Pi, 5	100	3	37	6
"	G1P,5	118	6	45	
G1P	--	100	5		
"	Pi, 5	8			

S. cerevisiae (strain PM-1) was grown at 30º with agitation in a basal medium (Chung et al., 1954) without phosphates, with carbon sources and additions as indicated. Growth at 48 hours was estimated by the increase in optical density at 420 mμ. Cells were harvested and washed 3 times with distilled water. Fermentation was measured at pH 4 and 30º, manometrically, with N_2 as the gaseous phase, in a mixture containing 0.1 M substrate and 0.05 M phosphate. External phosphatase was estimated by incubating at 30º and pH 4, intact yeast with 0.05 M glucose 6-phosphate, 0.01 M $MgCl_2$ and 0.1 M acetate in a total volume of 0.1 ml. Reaction was stopped with 4.9 ml of cold distilled water and Pi determined after centrifugation (Fiske and Subbarow, 1925). Internal phosphatase was estimated at pH 6 in extracts from acid-treated cells (see Table II) in a mixture containing 0.05 M α-glycerophosphate, 0.01 M $MgCl_2$ and 0.1 M Tris-maleate. Pi was measured after desproteinization with 5% trichloroacetic acid. Phosphatase activity is expressed in international units (μmoles per minute).

The pH activity curve of α-glycerophosphate hydrolysis by yeast extracts, shows a second peak in the neutral range, which is not apparent in intact yeast, and corresponds to the α-glycerophosphatase described by Schäffner et al., (1938). The level of this neutral phosphatase was not markedly affected by the presence or absence of excess Pi in the medium, in contrast with the acid enzyme (Table I). A truly intracellular location is further indicated by the fact that it is not affected by acid treatment which destroys the acid enzyme, as shown in Table II.

TABLE II

Effect of acid treatment of intact yeast on phosphatase activity of the intact yeast and yeast extracts

	Intact yeast		Yeast extract	
	pH 4	pH 6	pH 4	pH 6
Before treatment	5.0	1.8	4.1	23.5
After treatment	0.2	--	0.7	22.5

S. cerevisiae was grown for 24 hours as indicated in Table I, in a medium containing yeast extract (0.3%) and glucose (2%), collected by centrifugation and washed 3 times with distilled water on the centrifuge. Acid treatment was made by suspending the cells in 0.1 N HCl (5% W/V) for 5 minutes. After neutralization with 0.1 N NaOH, cells were washed and finally packed on a porous disk. Homogenates were prepared by grinding yeast with 3 times its weight of alumina (Alcoa 301) in a cold mortar. After diluting with 0.005 M EDTA, the mixture was centrifuged in the cold at 8,000 x g for 10 minutes, and the supernatant used as crude extract. Phosphatase activity in intact yeast and yeast extracts was determined as in Table I with α-glycerophosphate as substrate.

These observations indicate that baker's yeast has at least two phosphomonoesterases with markedly different physiological significance. An ecto-phosphatase, with pH optimum in the range that tends to prevail in the media of fermenting yeast, which is repressible by excess of Pi in the growth medium and has as physiological role to increase the availability for growth of Pi out of phosphate esters that may be present in the medium. And an intracellular enzyme, with pH optimum in the physiological range of the yeast protoplasm, non-repressible and inactive on external substrate, that can only be involved in intermediary metabolism. The ability to grow with G1P as source of carbon in media low in Pi (Table I), is an indirect consequence of the physiological role of the ecto-phosphatase, since in these conditions glucose is made available to the cell together with inorganic phosphate. The repression of the ecto-phosphatase by excess Pi seems to be merely for economy sake, and it becomes harmful when the available source of carbon and energy is present in phosphorylated form. In these conditions the cell fails to grow through a misdirected repression.

The conclusion of a relationship between location and function is further supported by the fact that the 2-deoxyglucose 6-phosphatase of a yeast mutant that renders it resistant to inhibition by 2-deoxyglucose is intracellular (Heredia and Sols, unpublished work).

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