ENZYMATIC HYDROLYSIS OF CYCLIC 3', 5'-AMP AND CYCLIC 2', 3'-AMP IN EXTRACTS OF YEAST CELLS[1]

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Abstract: The enzyme capable of catalyzing the hydrolysis of cyclic 3', 5'-AMP in extracts of yeast cells (*Saccharomyces cerevisiae*) is distinguished from the enzyme which hydrolyzes cyclic 2', 3'-AMP with respect to pH optimum, metal-ion effects, and sedimentation behavior.

INTRODUCTION

In yeast cells evidence for the existence of cyclic 3',5'-AMP (Sy & Richter, 1972a), adenyl cyclase (Sy & Richter, 1972a) and a cyclic 3',5'-AMP specific binding protein (Sy & Richter, 1972b) has been presented. Since a decrased intracellular level of cyclic 3',5'-AMP in glucose-repressed yeast cells has also been noted (Sy & Richter, 1972a), cyclic 3'5'-AMP may play a similar regulatory role as a gene activator in catabolite-repression to that in *E. coli* (Zubay *et. al.* 1970). Although the presence of cyclic 3',5'-AMP phosphodiesterase activity has been demonstrated in crude extracts of *Saccharomyces carlsbergensis* (Speciali & van Wijk, 1971), it is not clear whether this phosphodiesterase is specific for cyclic 3',5'-AMP. Prelminary evidence indicated that crude extracts of *S. cerevisiae* contained an enzyme or enzymes showing activity toward both cyclic 3', 5'-AMP and cyclic 2',3'-AMP. This evidence together with the apparent lack of specificity of cyclic nucleotide phosphodiesterase of higher plants (Lin & Varner, 1972; Lin, 1973, 1974) makes it of interest to know the specificity of cyclic nucleotide phosphodiesterase from yeast cells.

MATERIALS AND METHODS

Freshly pressed baker's yeast (*S. cerevisiae*) from Budeveiser was stored at 4°C, for not more than a week, prior to use. Nucleotide and nucleoside derivatives, cysteine dithiothreitol and snake verom (*Ophiophagus hanna*, king cobra) were purchased from Sigma Chemical Co., Cyclic 3', 5'-AMP-[^{3}H] (16.3 Ci/μmole) was obtained from Schwarz BioRes. and purified by chromatography in a Dowex 50-H$^+$ column (Lin & Varner, 1972).

Assays of nuleotidase and cyclic nucleotide phosphodiesterase activities were carried out by the method described previously (Lin and Varner, 1972). The standard reaction mixture contained 0.1 M Tris-acetate buffer (pH8), 2mM substrate and a suitable dilution of enzyme solution (0.05-0.1ml) in a total volume of 0.5 ml, unless otherwise stated. The reaction was conduted at 37°C for 1-2 h. Pi released was assayed by the method of Fiske and SubbaRow (1925). For the study of metal-ion effects, 2mM cyclic 3', 5'-[^{3}H]-AMP (7×10^4 cpm/μmole) was used as substrate for the assay cyclic 3', 5'-AMP phosphodiesterase activity, and reaction products assayed by the method previonsly described (Lin, 1973). The amount of protein

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was determined according to Lowry *et al*, (1951) with bovine serum albumin as standard.

RESULTS AND DISCUSSIONS

For routine enzyme preparation, 100g of yeast was suspended and homogenized with 100ml of deionized water (300 g of glass beads) for 10 min at 4°C in Waring blender, and centrifuged at 10,000xg for 30 min. The supernatant was brought to 80% saturation by slowly adding solid ammonium sulfate (49.3 g/100ml fluid), and the resulting precipitate collected by centrifugation at 10,000xg for 30 min, then dissolved in and exhaustively dialyzed against 10mM Tris buffier (pH 8.0)

The enzyme preparation still contains alkaline nucleotidase activity; for 1 h of incubation at pH 8.0, 1.0 mg protein hydrolyzes 1.4 μmoles of 2′-AMP, 2.1 μmoles of 3′-AMP and 1.7 μmoles of 5′-AMP. Under the standard assay conditions, the phosphodiesterase reaction is the rate-determining step. The hydrolysis of cyclic AMP is linear up to 2 h of incubation with either cyclic 3′, 5′-AMP or cyclic 2′, 3′-AMP as substrate. The crude cyclic nucleotide phosphodiesterase hydrolyzes 0.12 μmole of cyclic 3′, 5′-AMP/h/mg protein and 0.16 μmole of cyclic 2′, 3′-AMP/h/mg protein. Because of the presence of strong nucleotidase activity as described above, it is not clear whether 5′-AMP is the exclusive product from the hydrolysis of cyclic 3′, 5′-AMP. It is also not clear whether 2′-AMP or 3′-AMP is the product from cyclic 2′, 3′-AMP. The specific activity of the present preparation of cyclic 3′, 5′-AMP phosphodiesterase is about two times of that report of *S. carlsbergensis* (Speciali and Aan Wijk, 1971).

The enzyme activities toward cyclic 3′, 5′-AMP and cyclic 2′, 3′-AMP are not sensitive to the presence of reducing reagent such as cysteine (0.1 mM) or dithiothreitol (0.05–0.1 mM). Pi, PPi or 5′AMP at the conentration of 4 mM result in 60% inhibition of both enzyme activities. The effect of pH on enzyme activity is shown in Fig. 1. Cyclic 3′, 5′-AMP phosphodiesterase shows maximal activity at pH 8.5 while cyclic 2′, 3′-AMP phosphodiesterase has optimal activity at pH 7.3 with only 20% activity remaining at pH 8.5. The pH optima for yeast cyclic nucleotide phosphodicaterases are quite similar to those for animal cyclic 3′, 5′-AMP phosphodiesterases (Robison *et al*, 1968), but different from that for cyclic nucleotide phosphodiesterase of higher-plants (Lin, 1974) which has an optimal activity between pH 5–6.

Like most animal cyclic 3′, 5′-AMP phosphodiesterases described in literature, yeast cyclic 3′, 5′-AMP phosphodiesterase requires Mn^{2+} (2.0 mM) and/or Zn^{2+} (0.2 mM) for full activity while cyclic 2′, 3′-AMP phosphodiesterase shows no additional divalent metal-ion requirements for maximal activity (Table I). Different metal-ion requirements and different EDTA effects have also been shown for rabbit brain cyclil 3′, 5′-AMP phosphodiesterase and cyclic 2′, 3′-AMP phosphodiesterase (Drummond and Perrtt-Yee, 1961; Drummond *et al*, 1962). Furthermore, sucrose density gradient centrifugtion (Fig. 2) clearly indictes that yeast cyclic 3′, 5′-AMP phosphodiesterase is different and separable from cyclic 2′, 3′-AMP phosphodiesterase.

Thus, I conclude that the enzyme capable of catalyzing the hydorlysis of cyclic 3′, 5′-AMP in yeast cells is different from that which hydrolyzes cyclic 2′, 3′-AMP

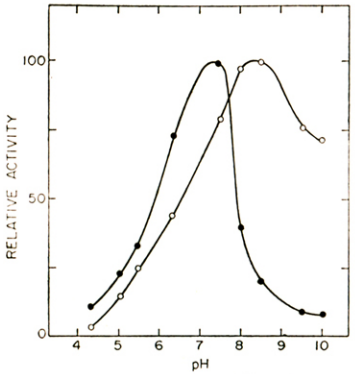

Fig. 1. Effect of pH on enzyme activity. The reaction mixtures were as described the standard assay. 2 mM $MnCl_2$ was included for the assay of cyclic 3′, 5′-AMP phosphodiesterase activity. 0.1 M K-acetate buffer was used for pH 4-6, while 0.1 M Tris-acetate buffer was used for pH 6-10. Substrates: cyclic 3′, 5′ -AMP (○) and cyclic 2′, 3′ -AMP (●).

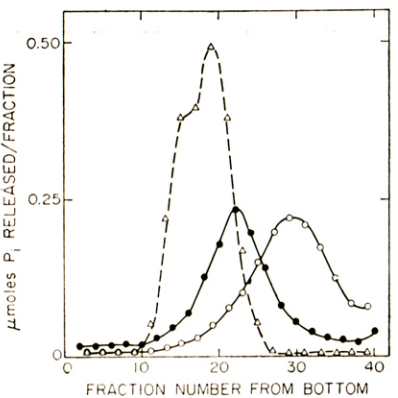

Fig. 2. The elution profiles of enzyme activities from sucrose density gradient centrifugation. 0.3 ml of enzyme solution containing 18 mg of protein was layered over a 4.5ml of 5-20% linear sucrose density gradient (10 mM Tris-acetate buffer, pH 8.0). Centrifugation was carried out at 35,000 rpm for 22 h at 2°C in SW-39 rotor. Upon completion of the run, 10-drop fractions were collected and enzyme activities assayed in alternate fractions throughout the gradient with two substrates for each set of gradients. Reactions were carried out at 37°C far 2h as described in Methods. Substrates used: cyclic 3′, 5′ -AMP (○), cyclic 2′, 3′ -AMP (●), and 2′ -AMP (△). 2 mM $MnCl_2$ was included in the assay of cyclic 3′, 5′ -AMP phosphodiesterase activity.

Table I Effects of Metal Ions and EDTA on Enzyme Activity

Addition	Concentration (mM)	Cyclic 3′,5′-AMP phosphodiesterase	Cyclic 2′, 3′-AMP phosphodiesterase
		Activity (%)*	
None	—	100	100
$MnCl_2$	0.1	388	—
	0.5	456	105
	2.0	655	115
	4.0	428	135
	10.0	285	—
$MgCl_2$	1.0	100	100
	5.0	140	110
	10.0	154	125
$ZnCl_2$	0.02	80	55
	0.2	440	71
	0.5	220	78
	2.0	55	—
KCl	2.0	135	125
EDTA	2.0	92	21

*With 0.8mg protein in the standard reaction mixture, 100% of cyclic 3′, 5′ -AMP phosphodiesterase activity represented as 1635 cpm of [³H]-adenosine formed at pH 8.5 (Tris-acetate buffer)/h from cyclic 3′, 5′-[³H]-AMP (7.10^4 cpm/μmole), while 0.24 μmole of Pi released at pH 7.3 (Tris-acetate buffer)/h from cyclic 2′, 3′ -AMP is expressed as 100% of cyclic 2′, 3′ -AMP phosphodiesterase activity.

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