JOURNAL OF BACTERIOLOGY, Feb. 1972, p. 949-951
Vol. 109, No. 2

Printed in U.S.A.

p-Toluenethiol as an Initiator of Autolysis in Bakers' Yeast

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Received for publication 7 September 1971

Cysteine or dithiothreitol enhances the rate of autolysis in toluene-treated yeast. p-Toluenethiol alone is even more effective and is recommended for the isolation of β-fructofuranosidase. This suggests a more general application of p-toluenethiol in the isolation of enzymes from yeast.

The addition of organic solvents to initiate autolysis is an established technique for the isolation of enzymes from yeast. An example is the application of toluene in the preparation of β-fructofuranosidase (EC 3.2.1.26; reference 4). β-Fructofuranosidase is a cell wall enzyme which is fully assayable in the intact cell, and its release to the medium is dependent on the action of endogenous, wall-modifying enzymes (i.e. autolysis). A cytoplasmic invertase has been described for some yeast strains but represents no more than 4% of the activity of the β-fructofuranosidase in the cell wall (3). The wall-modifying enzymes are normally contained within the cytoplasm but become effective after membrane properties are altered by any one of a variety of treatments. A discussion of the early literature on autolysis and the several compounds that have been used as autolytic agents is contained in the monograph by Neuberg and Roberts (5).

During a study of the autolytic release of β-fructofuranosidase from yeast, we explored the role of sulfhydryl compounds as in vivo and in vitro modifiers of the wall structure, as first postulated by Nickerson and Falcone (6) and Duell et al. (2). We report now on the stimulating effects of cysteine and dithiothreitol on toluene-induced autolysis. Soejima and Shimura (7) introduced p-toluenethiol as a convenient reducing agent for the activation of papain (EC 3.4.4.10). The absence of denaturation in that instance, together with the obvious combination of chemical groups, suggested to us that p-toluenethiol might be a useful autolysis initiator.

Fresh bakers' yeast was obtained from the Red Star Yeast and Products Co. The cells were washed by centrifugation at 3,000 × *g* from 0.25 M sodium acetate-acetic acid (pH 6.0). This process was repeated two more times, and finally the yeast was resuspended in the same buffer to 10% (wet weight/volume). Samples were transferred to screw-capped test tubes at 30 C, and autolysis was initiated by the addition of toluene to each tube. Other additions were made as indicated. At zero time, and after prescribed intervals, 0.1 ml of each incubation mixture was removed and diluted with 0.9 ml of distilled water. A 10-μliter sample was assayed (1) for total β-fructofuranosidase concentration; the remainder was expressed through a membrane filter (0.22-μm pore size), and a 10-μliter sample of the filtrate was assayed for the concentration of cell-free (i.e., released) enzyme. The typical titer for total β-fructofuranosidase activity was 350 units/ml. A unit of enzyme is that amount which brings about the hydrolysis of 1 μmole of sucrose per min at 30 C, in the presence of 100 mM sucrose, 50 mM sodium acetate buffer (pH 5.0), and 2 mM ethylenediaminetetraacetic acid. Total enzyme activity did not change during the incubations reported here. Accordingly, values for the released enzyme at various intervals are expressed as a percentage of the total enzyme concentration at zero time.

The time-course for toluene-induced autolysis is shown in Fig. 1. The curve was drawn to the combined data of eight separate experiments and is indicative of the consistent behavior among different batches of yeast. There was a reproducible lag of 16 to 20 hr in the release of β-fructofuranosidase. Optimal conditions for this yeast are pH 6.0 and 30 C. At 22 C, the lag was more pronounced, and only 30% was solubilized in 100 hr. At 40 C, the lag was substantially reduced, but final yields were low.

The effects of cysteine and dithiothreitol are

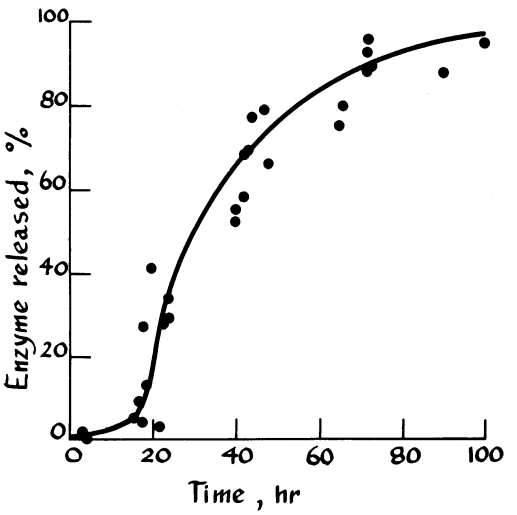

FIG. 1. *Release of β-fructofuranosidase from yeast upon treatment with toluene.*

itol with toluene. There was no inactivation of β-fructofuranosidase nor was there any apparent deleterious effect on the autolytic enzyme system. *p*-Toluenethiol is very sparingly soluble in aqueous salt solutions; it is quite soluble in absolute ethanol and is more conveniently handled as such. After application, it is possible to remove *p*-toluenethiol from the extract by adding toluene and separating the organic phase.

Recently, mechanical procedures involving high-pressure extrusion or high-frequency bombardment of fresh cell suspensions have been favored for extraction of yeast enzymes. However, for large batches, minimal equipment, and short operator contact time, the autolytic methods still have considerable attraction. *p*-Toluenethiol might find more general application in the autolytic extraction of yeast enzymes other than the example reported here.

TABLE 1. *Effect of sulfhydryl compounds on autolysis in three batches of yeast*[a]

Additions	Release (%) of β-fructofuranosidase after				
	4 hr	17 hr	21 hr	43 hr	70 hr
Batch A					
None	1		1	3	3
Toluene (0.2 ml)	1		38	80	92
Toluene (0.2 ml) + dithiothreitol (5 mg)	13		73	98	99
p-Toluenethiol (5 mg)	17		91	103	104
Batch B					
None		1		5	4
Toluene (0.2 ml)		9		58	93
Toluene (0.2 ml) + cysteine (4 mg)		24		85	99
Batch C					
None			2	3	2
Toluene (0.1 ml)			3	52	75
p-Toluenethiol (1 mg)			66	89	91

[a] Each incubation contained 2.0 ml of 10% yeast suspension in 0.25 M acetate buffer (*p*H 6.0), together with additions as indicated. Dithiothreitol, cysteine (free base), and *p*-toluenethiol (batch A) were weighed directly into the incubation tubes. In batch C, the *p*-toluenethiol was added as a 10% solution in absolute ethanol.

summarized in Table 1. The sulfhydryls were particularly effective in increasing the initial rate of release of β-fructofuranosidase and in removing the lag observed with toluene alone. These results are consistent with the finding (2) that the yeast cell wall is more susceptible to degradation by snail gut enzymes after pretreatment with sulfhydryl compounds. The usefulness of *p*-toluenethiol as an initiator of autolysis is demonstrated in Table 1. This compound appears to be even more effective than the combination of cysteine or dithiothre-

This investigation was supported by a grant from the Society of Sigma Xi.

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