ADENINE METABOLISM IN SACCHAROMYCES CEREVISIAE ADENASE FROM BAKERS' YEAST*

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Di Carlo, Schultz, and McManus (1951) provided evidence for the presence of adenase in the yeasts Candida (torula) utilis and Saccharomyces cerevisiae. Roush (1954) prepared extracts of adenine-induced C. utilis that contained an adenase but found that extensive fractionation was required to separate the adenase from accompanying guanase and uricase (Roush, 1958). Heppel, Hurwitz, and Horecker (1957) obtained a highly purified adenase from Azotobacter vinelandii but the adenase in the crude extracts was accompanied by adenosine deaminase and several fractionation steps were required to separate the two activities. This paper reports an induced adenase in S. cerevisiae. The crude enzyme is practically free of enzymes acting on many purine and pyrimidine compounds other than adenine and it is obtained easily from commercially available fresh or active dry bakers' yeast.

Materials and Methods

To study the metabolism of adenine as a nitrogen source, either 0.7g of fresh bakers' yeast (Fleischmann's) or 0.5 g of active dry yeast

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(generously supplied by the Red Star Yeast Co., Milwaukee) was aerated in 100 ml of a medium containing 10 mg of adenine, 1 g of glucose, and the citrate buffer and salt supplement of Schultz and Atkin (1947). One ml samples were removed at appropriate times, diluted with 9 ml of distilled water, and centrifuged. The sedimented yeast was heated for 10 min in a boiling water bath with 9 ml of water and centrifuged. The original supernatant liquids and the yeast extracts were analyzed for adenine and hypoxanthine using purified adenase from C. utilis and milk xanthine oxidase by the spectrophotometric methods of Kalckar (1947). The colorimetric method of Lowry et al. (1951) was used for protein determinations and to test for the presence of xanthine and uric acid in the yeast extracts and media.

Yeast extracts were obtained with a Hughes' (1951) press for studies of the kinetics of enzyme formation. Larger amounts of extract for fractionation experiments were obtained by shaking the yeast with glass beads; extracts were prepared in 0.1 M phosphate, 10^{-3}M EDTA buffer, pH 7.0. Dialysis of the ammonium sulfate fraction was performed at 4° for 6 hr with stirring; 5 changes of 0.01 M phosphate, 10^{-3}M EDTA buffer, pH 7.0, were used. Enzyme activities were measured by the method of Kalckar (1947) in pH 7 phosphate buffer except for the cation and anion inhibition studies which were carried out in 0.05 M acetate, pH 6.5.

Results and Discussion

Figure 1 shows that when the yeast was aerated in an adenine medium, the adenine started to disappear from the medium without an appreciable time lag, and the disappearance of adenine was accompanied by the production of hypoxanthine, most of which was excreted into the

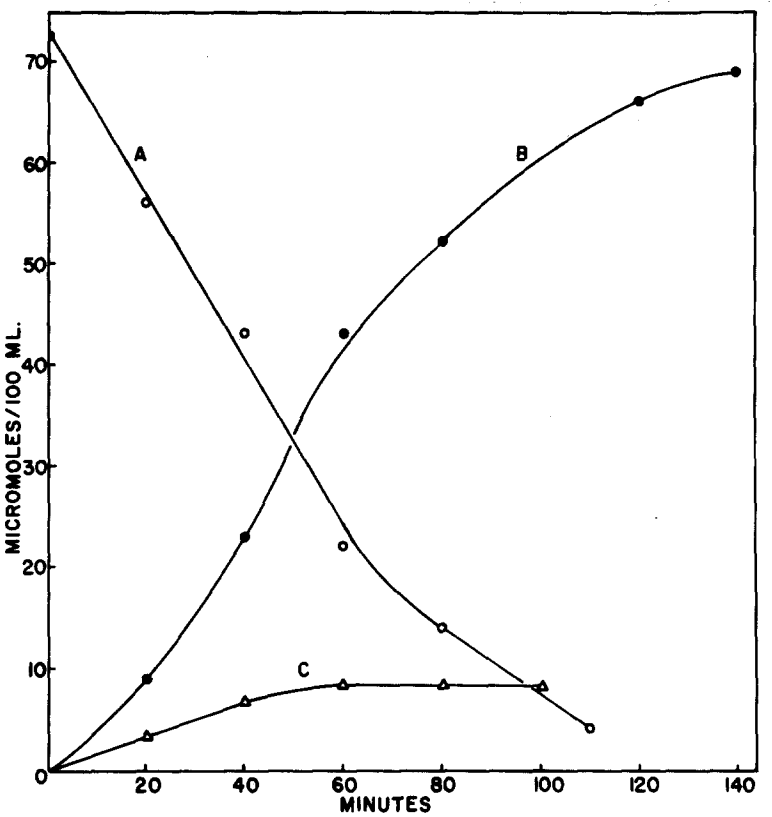

Fig. 1 Adenine metabolism in S. cerevisiae. A: adenine found in the medium; B: hypoxanthine found in the medium; C: hypoxanthine found in the cell extracts.

medium. After adenine uptake, ultraviolet absorption spectra of the medium and of the cell extracts were typical hypoxanthine spectra. No adenine was detected in the cell extracts nor was the presence of xanthine or uric acid detected in the medium with the Folin phenol reagent. In contrast, with C. utilis there is a time lag before purine uptake and the purine and its metabolic products are accumulated within the cell; no excretion of purines into the medium is noted (Roush, Questiaux, and Domnas, in press). Lahou (1958) found that S. cerevisiae metabolized guanine with the excretion of xanthine into the medium which is similar to the above results with adenine. However, in the case of guanine, an

equilibrium was attained between guanine and xanthine which is in contrast to the above finding that bakers' yeast affects a complete conversion of adenine to hypoxanthine under our conditions.

The metabolism of adenine by S. cerevisiae required a carbon source (glucose), it was inhibited by 10^{-3} M arsenate, but normal uptake was observed in the presence of both 10^{-3}M arsenate and 0.05 M phosphate. These results indicate that the uptake of adenine is a process involving active transport.

When extracts of the uninduced yeast were prepared and examined for adenase, no activity was detected. Aeration in the glucose medium without a nitrogen source resulted in an extract with an adenase specific activity of 1.3 μmoles/hr/mg protein; addition of adenine to the medium gave crude extracts with specific activities to 5.6 μmoles/hr/mg protein. Thus, adenase in an inducible enzyme in S. cerevisiae.

Upon ammonium sulfate fractionation and dialysis of a solution of the precipitate obtained between 0.5 and 0.8 saturation, the resulting solution had a specific activity of 8.8 μmoles/hr/mg protein and contained 59% of the adenase units of the crude extract. Optimum activity was near pH 7, maximum stability was near pH 6.5, and the enzyme was stable for 10 minutes at pH 6.5 and 40°. When 0.01 M fluoride was included in an assay solution, 70% inhibition resulted; 0.1 M chloride gave 55% inhibition. The adenase was strongly inhibited when 10^{-5} M zinc or cupric ions were present in the assay solution; manganous and cobaltous ion were less inhibitory, and 0.01 M magnesium ion gave only 10% inhibition. No change in absorbancy at the indicated wavelengths was observed when the crude extract or the purified adenase was incubated with the following compounds: deoxyadenosine, 3'-AMP, 5'-AMP, ATP,

DPN+, TPN+, and thymine, all at 265 mμ; guanine, 245 mμ; hypoxanthine, 250, 270, and 293 mμ; xanthine, 270 and 293 mμ; uric acid, 293 mμ; and uracil, 260 mμ. Cytosine, cytidine, and cytidylic acid gave slow increases in absorbancy at 265 mμ indicative of the presence of slight deaminase activities for these compounds. Adenosine (265 mμ) and guanosine (245 mμ) gave barely detectable rates of change that were less than 2% of the rates observed with adenine. It appears that the crude adenase obtained from adenine-induced bakers' yeast is similar in properties to the adenase from other species and is suitable for the determination of adenine in the presence of a number of other purines and pyrimidines and their derivatives.

References

Di Carlo, F. J., Schultz, A. S. and McManus, D. K., J. Biol. Chem., 189, 151 (1951).

Heppel, L. A., Hurwitz, J. and Horecker, B. L., J. Am. Chem. Soc., 79, 630 (1957).

Hughes, D. E., Brit. J. Expt. Pathol., 32, 97 (1951).

Kalckar, H. M., J. Biol. Chem., 167, 429, 445, 461 (1947).

Lahou, J., Biochim. et Biophys. Acta, 27, 371 (1958).

Lowry, O. H., Rosebrough, N. J., Farr A. L. and Randall, R. J., J. Biol. Chem., 193, 265 (1951).

Roush, A. H., Arch. Biochem. Biophys., 50, 510 (1954).

Roush, A. H., Abstracts of papers of the 133rd meeting of the American Chemical Society, Washington, 9C (1958).

Roush, A. H., Questiaux, L. M. and Domnas, A. J., J. Cell. Comp. Physiol., in press.

Schultz, A. S. and Atkin L., Arch. Biochem., 14, 369 (1947).