JOURNAL OF BACTERIOLOGY, June 1986, p. 763–768
0021-9193/86/060763-06$02.00/0
Copyright © 1986, American Society for Microbiology
Vol. 166, No. 3

Adenosine Accumulation in *Saccharomyces cerevisiae* Cultured in Medium Containing Low Levels of Adenine

HOWARD M. LATEN,* PETER J. VALENTINE, AND CAROL A. VAN KAST
Department of Biology, Loyola University of Chicago, Chicago, Illinois 60626

Received 14 November 1985/Accepted 17 March 1986

By monitoring the in vivo incorporation of low concentrations of radiolabeled adenine into acid-soluble compounds, we observed the unusual accumulation of two nucleosides in *Saccharomyces cerevisiae* that were previously considered products of nucleotide degradation. Under the culture conditions used in the present study, radiolabeled adenosine was the major acid-soluble intracellular derivative, and radiolabeled inosine was initially detected as the second most prevalent derivative in a mutant lacking adenine aminohydrolase. The use of yeast mutants defective in the conversion of adenine to hypoxanthine or to AMP renders very unlikely the possibility that the presence of adenosine and inosine is attributable to nucleotide degradation. These data can be explained by postulating the existence of two enzyme activities not previously reported in *S. cerevisiae*. The first of these activities transfers ribose to the purine ring and may be attributable to purine nucleoside phosphorylase (EC 2.4.2.1) or adenosine phosphorylase (EC 2.4.2.-). The second enzyme converts adenosine to inosine and in all likelihood is adenosine aminohydrolase (EC 3.5.4.4).

Yeast and other organisms rely on purine salvage enzymes to recover and recycle intact purine rings (1, 4, 6, 7, 11–15, 17–19, 21–31, 33–39). While the elucidation of purine biosynthetic pathways in microorganisms has profited from the relatively easy isolation of auxotrophic mutants (16, 19), the reconstruction of metabolic pathways has relied heavily on assaying enzyme activities in vitro (19), isolating mutants resistant to purine analogs (6, 19, 27, 36, 37), or tracing the paths of radiolabeled purine derivatives or precursors (3, 4, 8–10, 19, 25, 27). By making a trivial modification in the medium used to study the incorporation of radiolabeled adenine into acid-soluble derivatives in *Saccharomyces cerevisiae*, we deduced the existence of two enzyme activities: one catalyzes the direct synthesis of adenosine from adenine and presumably ribose, and another deaminates adenosine.

Two enzymes reportedly capable of catalyzing the first reaction are purine nucleoside phosphorylase (EC 2.4.2.1) and adenosine phosphorylase (EC 2.4.2.-). The former has been well characterized in a number of organisms, including mammals and bacteria (2, 12, 17, 19, 20, 23, 24, 27, 28, 30, 33, 39), whereas the latter is presently known to occur only in bacteria and parasites (7, 11, 13, 15, 21, 31, 34). Responsibility for the second reaction is generally attributed to adenosine aminohydrolase (EC 3.5.4.4), which converts adenosine to inosine and has also been well characterized in a wide range of organisms (2, 20, 22, 23, 35, 38). In humans, a defect in purine nucleoside phosphorylase is associated with a type of T-cell immunodeficiency, and the absence of adenosine aminohydrolase is linked to a form of severe combined immunodeficiency disease (2, 20).

In yeasts, purine nucleoside phosphorylase has been detected in *S. cerevisiae* (12), and the existence of adenosine aminohydrolase in *Schizosaccharomyces pombe* has been inferred from purine utilization studies (26). A nonspecific nucleoside deaminase from *Aspergillus oryzae* can deaminate adenine, adenosine, and AMP (22, 35).

MATERIALS AND METHODS

[8-^{14}C]adenine (53 mCi/mmol) was purchased from New England Nuclear Corp. Nonradioactive purine bases, nucleosides, and nucleotides were purchased from Sigma Chemical Co. All chemicals were reagent grade, and those used for high-pressure liquid chromatography were redistilled and filtered prior to use.

The genotypes and corresponding enzyme deficiencies of the *S. cerevisiae* strains used in this study are listed in Table 1. Cells were grown in liquid YPD medium (32) overnight in a shaking incubator at 30°C. The preculture (0.5 ml) was added to 50 ml of synthetic dextrose medium (32) containing 10 μCi of [8-^{14}C]adenine (53 mCi/mmol). The final adenine concentration was 0.38 μM. Samples (8 ml) were removed at 0, 5, 15, 30, and 240 min and maintained between 0° and 4°C while they were centrifuged, washed, and mechanically lysed with glass beads in a Bead Beater (Biospec Products). The cell-free lysates were acidified with perchloric acid and neutralized as described by Burridge et al. (4). The lysates were centrifuged, and the supernatants were frozen at −20°C until further use.

The supernatants were combined with standards and loaded onto a Brownlee RP-15 high-pressure liquid chroma-

TABLE 1. Description of *S. cerevisiae* strains

Strain[a]	Genotype[b]	Source
370	**a**	Our collection
1520	**a** *ade4-su aptl*	Woods et al. (37)
1540	**a** *ade4-su aahl*	Woods et al. (37)
1530	**a** *ade4-su aptl aahl*	Woods et al. (37)

[a] Our designations.
[b] *ade4-su*, Reduced amidophosphoribosyltransferase activity; *aptl*, no adenine phosphoribosyltransferase activity; *aahl*, no adenine aminohydrolase activity.

* Corresponding author.

TABLE 2. Incorporation of ^{14}C into acid-soluble derivatives

Strain	cpm. at time (min) of collection (% total extracted radioactivity)				
	0	5	15	30	240
Wild type	14,990 (75)	83,820 (78)	260,560 (85)	488,080 (74)	942,535 (42)
apt1	17,760 (71)	68,280 (76)	178,680 (92)	373,580 (86)	847,027 (39)
aah1	40,850 (72)	75,620 (74)	97,980 (80)	404,140 (53)	707,382 (33)
apt1/aah1	6,624 (100)	5,700 (77)	6,520 (71)	10,260 (73)	2,722 (14)

tography column (4.6 by 150 mm). The samples were eluted with 0.01 M KH_2PO_4 in 14% methanol. Fractions were collected directly in scintillation vials containing 10 ml of scintillation cocktail and counted in a Packard 300C Liquid Scintillation System.

RESULTS

Table 2 summarizes the incorporation of ^{14}C into acid-soluble derivatives for each time point. Uptake of labeled adenine by the wild-type strain and the two single mutants was generally comparable through 4 h. Adenine is actively transported across the yeast cell membrane by adenine permease (5). However, despite the fact that the *aah1-apt1* double mutant proliferated as well as the strains carrying either mutation alone (on synthetic dextrose medium), the uptake of labeled adenine by the double mutant ranged from about 30% of the other strains at 5 min to less than 1% at 240 min. In all cases, the transfer of label into acid-precipitable material was relatively low for the first 30 min after inoculation of the labeled minimal medium, with between 53 and 86% of the label remaining in the acid-soluble pool. By 4 h, acid-precipitable derivatives accounted for more than half of the incorporated radioactivity. In the double mutant only 14% of the incorporated label remained in the acid-soluble pool.

The most dramatic finding was the virtual absence of imported adenine (Fig. 1 and 2). In all cases, the major acid-soluble radioactive product was adenosine. This was the case even when cells were harvested immediately after the radiolabeled medium was inoculated (data not shown).

In wild-type cells (Fig. 1), hypoxanthine, inosine, and a third unidentified product appeared within 5 min of the introduction of labeled adenine (Fig. 1a). AMP was barely detectable at 5 min but was clearly present after 15 min in the radiolabeled medium (Fig. 1b). Inosine appeared as a shoulder on the unidentified peak in Fig. 1b, but by 30 min (Fig. 1c) both the unidentified peak and AMP had disappeared, leaving only hypoxanthine, inosine, adenosine, and a barely detectable level of adenine. After 4 h (Fig. 1d) significant levels of radiolabeled AMP and IMP had accumulated, along with hypoxanthine and two unidentified derivatives.

Results of the incorporation of radiolabeled adenine into the mutant lacking adenine aminohydrolase (*aah1*) are summarized in Fig. 2. As in the wild-type cells, adenosine was by far the most prevalent radioactive product at all four time points. In contrast to the wild-type cells, however, inosine and not hypoxanthine was the second most prevalent product after 5 min (Fig. 2a). After 15 min, the ratio of inosine to hypoxanthine approached 1:1 (Fig. 2b) and remained roughly the same for 30 min (Fig. 2c). In addition, significant levels of radioabeled IMP were detected after 30 min (Fig. 2c). IMP and AMP constituted about 10 and 30%, respectively, of the acid-soluble radioactive derivatives.

Labeling of the mutant *apt1*, which lacked adenine phosphoribosyltransferase, resulted in a pattern of products similar to those observed for the *aah1* mutant (data not shown). The major product at all four time points was adenosine. Both hypoxanthine and inosine were present in all samples, inosine initially at slightly higher levels. In the double mutant, low levels of adenosine, hypoxanthine, and inosine were detected at all four time points (data not shown). Adenine constituted approximately 15% of the total acid-soluble material after 5 min but was virtually absent at 15, 30, and 240 min.

DISCUSSION

The pattern of labeled adenine derivatives in the present study was in sharp contrast to previous studies in which adenine metabolism and incorporation into nucleic acids were monitored in yeast (3, 4, 8–10, 26). Under our conditions, most of the adenine was immediately converted to other purine derivatives. Figures 1 and 2 indicate that the major product of adenine metabolism was adenosine. In addition, despite the presence of labeled acid-precipitable material—presumably RNA—we detected virtually no AMP, whether or not the strains possessed functional adenine phosphoribosyltransferase.

In previous studies, cells were grown in the presence of 50 to 100 μM adenine, and a significant portion of the purine was apparently converted to AMP by the action of adenine phosphoribosyltransferase (3, 4, 18, 37). Our observations suggest that at low adenine concentrations most of the adenine taken up by the cells is immediately converted to adenosine, with the remainder converted to AMP except in the *apt1* mutant strains. Furthermore, they suggest that at these concentrations any AMP, whether synthesized from adenine (by adenine phosphoribosyltransferase), from adenosine (by adenosine kinase), from hypoxanthine (by the sequential action of hypoxanthine-guanine phosphoribosyltransferase, adenylsuccinate synthetase, and adenylsuccinate lyase), or from GMP (from IMP, by the sequential action of IMP dehydrogenase and GMP synthetase) is immediately kinased and incorporated into RNA (Fig. 3). The observation that label was incorporated into RNA in strains lacking adenine phosphoribosyltransferase (Table 2) implies that alternate salvage pathways, such as the three suggested above, are functioning.

The most striking feature of the data represented in Fig. 1 and 2 is the virtual absence of adenine. Our results indicate that immediately following active transport (5), or perhaps concurrently, the majority of adenine molecules are converted into another derivative, namely adenosine. Since adenosine is also the major radiolabeled derivative in the *apt1* mutant, which cannot directly convert adenine into AMP, degradation of AMP to adenosine by AMP nucleotidase cannot account for the accumulation of the nucleoside, at least not in this mutant. The only previously characterized enzymes with the potential to catalyze the ribosylation of adenine are purine nucleoside phosphorylase (17, 27, 33, 39) and adenosine phosphorylase (11, 13, 21).

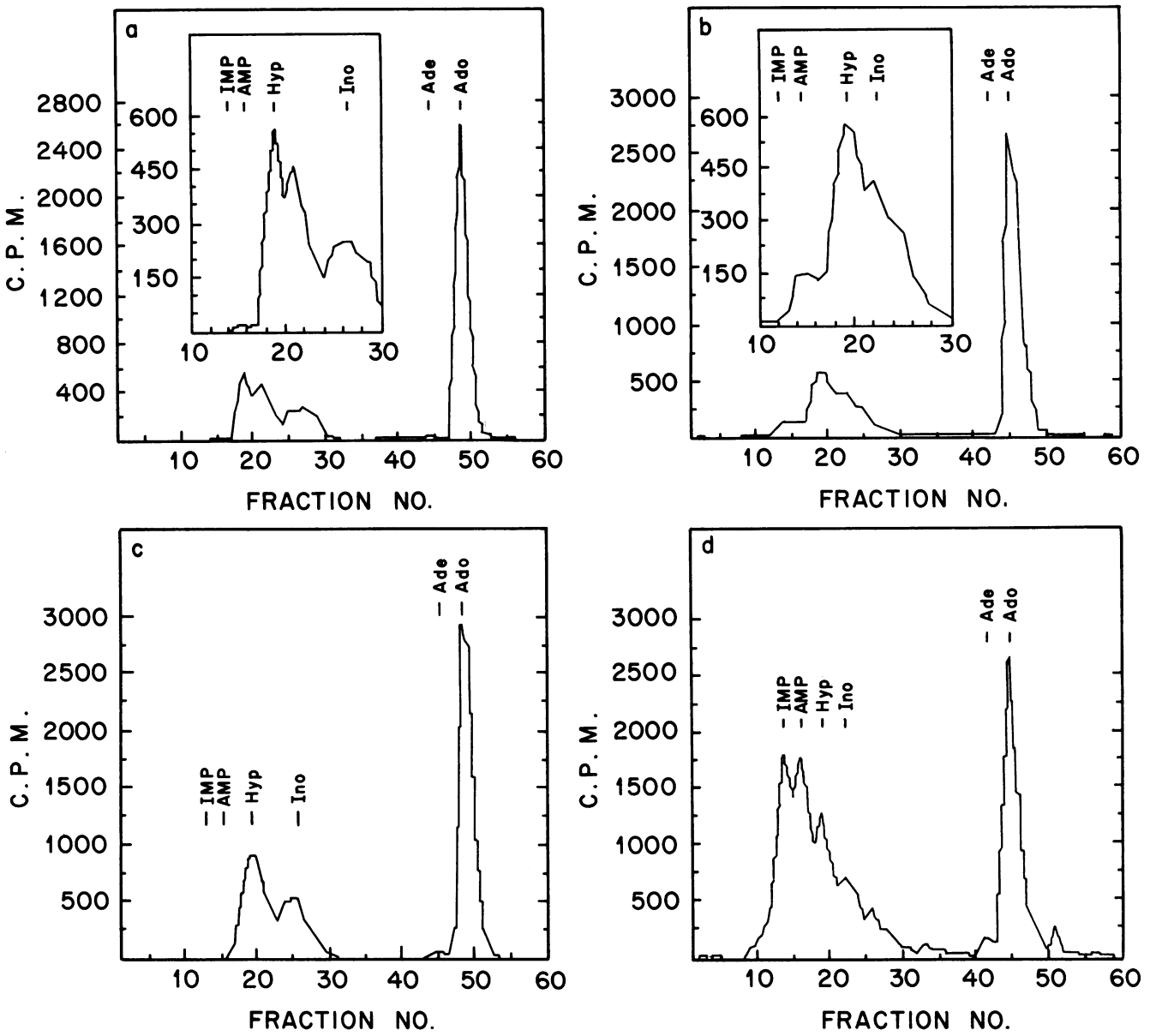

FIG. 1. High-pressure liquid chromatography profile of acid-soluble products derived from [^{14}C]adenine-labeled wild-type cells. (a) At 5 min; (b) 15 min; (c) 30 min; (d) 240 min. Samples were prepared as described in the text and combined with a standard solution containing adenine (Ade), adenosine (Ado), AMP, hypoxanthine (Hyp), inosine (Ino), and IMP. Between 9,000 and 13,000 cpm were loaded onto the column.

The latter has not been reported in yeast, and the former exhibits, at best, marginal preference for adenine (12).

Purine nucleoside phosphorylase catalyzes the reversible phosphorolysis of purine nucleosides (23, 24), although the significance of the reaction in the direction of nucleoside synthesis has been questioned (39). Most purine nucleoside phosphorylases, including that in *S. cerevisiae* (12), exhibit a strong preference for inosine and guanosine (23, 24, 39). The synthesis of adenosine from adenine and ribose-1-phosphate has been reported, however, in extracts from beef liver (17), rat liver and brain (39), calf spleen (39), human erythrocytes (39), and fish muscle (33), but the capacity of the phosphorylase to catalyze this reaction under physiological conditions, at least in mammalian cells, is disputed (39). An exception to this apparent discrimination against adenosine is exhibited by the purine nucleoside phosphorylase from *Salmonella typhimurium*. Purified extracts from *S. typhimurium* support the phosphorylation of inosine, guanosine, and adenosine with similar efficiencies (27).

Perhaps a more likely candidate for the enzyme whose activity we appear to have uncovered is adenosine phosphorylase. This enzyme, present in two species of *Bacillus* (15, 31, 34) and in mycoplasmas (11), parasitic protozoa (7, 13), and *Schistosoma mansoni* worms (21), exhibits a preference for adenosine. The enzyme has been shown to catalyze the reaction in the direction of nucleoside synthesis in *Trichomonas vaginalis* (13) and *Mycoplasma* spp. (11). The other major derivatives synthesized from adenine under our growth conditions were hypoxanthine and inosine. Previous studies in yeast (1, 8, 9, 26, 29, 37) and other organisms

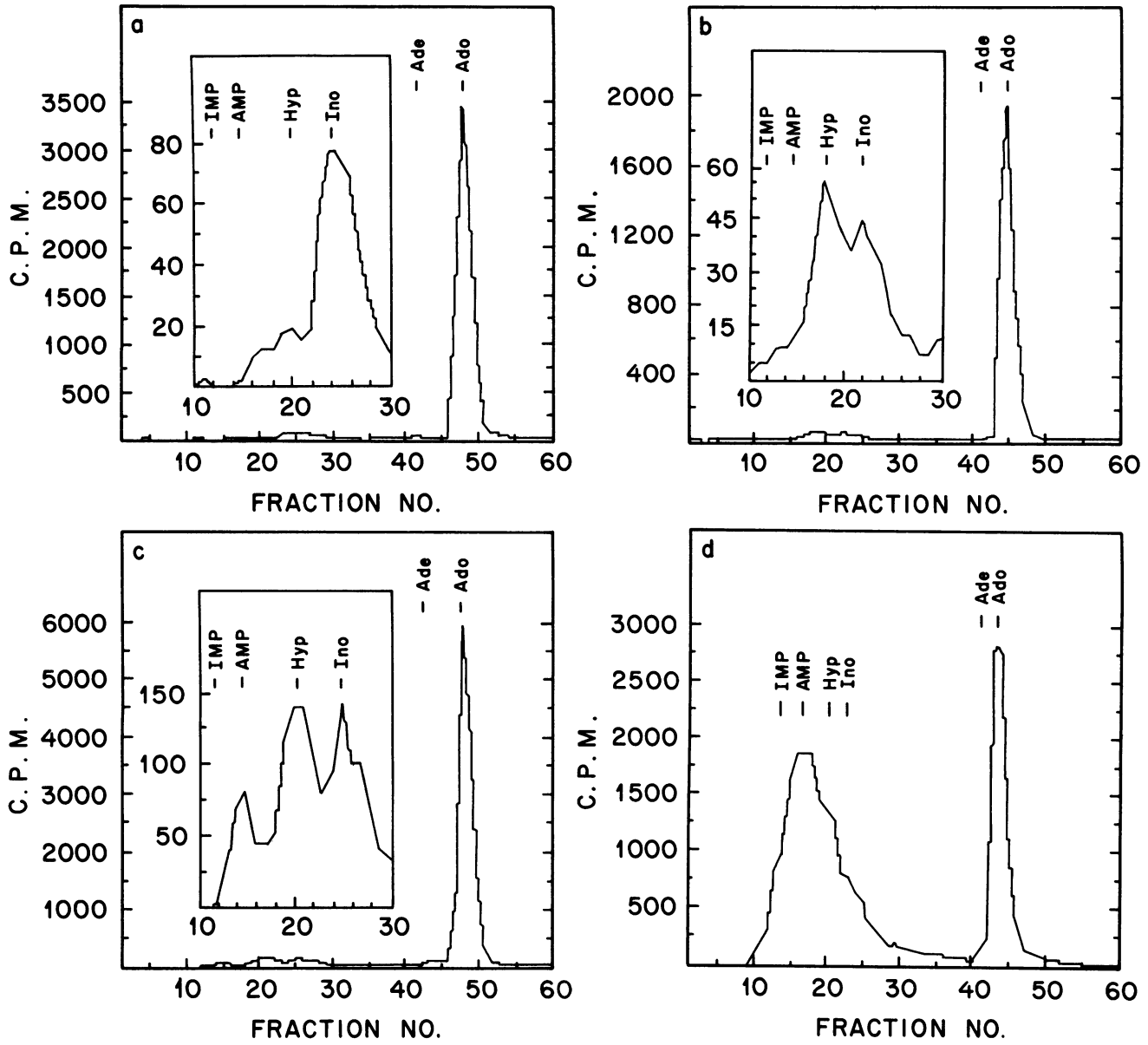

FIG. 2. High-pressure liquid chromatography profile of acid-soluble products derived from [^{14}C]adenine-labeled *aah1* cells. (a) At 5 min; (b) 15 min; (c) 30 min; (d) 240 min. Samples were prepared as described in the text and combined with a standard solution containing adenine (Ade), adenosine (Ado), AMP, hypoxanthine (Hyp), inosine (Ino), and IMP. Between 7,000 and 15,000 cpm were loaded onto the column.

(19, 23, 38) have suggested that adenine is deaminated to hypoxanthine by the action of adenine aminohydrolase. Based on this assumption, researchers have generally attributed the presence of inosine to the sequential action of hypoxanthine-guanine phosphoribosyltransferase and IMP nucleotidase. However, our results suggest that the presence of inosine can also result from the direct deamination of adenosine catalyzed by adenosine aminohydrolase (Fig. 2), an enzyme that has yet to be found in *S. cerevisiae*. The strongest argument for this possibility is that the level of radiolabeled inosine in our experiments was sevenfold that of hypoxanthine in the 5-min labeling of the adenine aminohydrolase-deficient mutant (Fig. 2a). The hypoxanthine that was present at increasing levels at 5, 15, and 30 min (Fig. 2) was most likely derived from the phosphorylation of inosine by the yeast purine nucleoside phosphorylase (12). A similar but less dramatic labeling pattern was found for the *apt1* mutant (data not shown).

Adenosine constituted greater than 90% of the acid-soluble derivatives in all strains for the first 30 min of labeling (Table 2; Fig. 1b and 2b). This suggests that in the two strains carrying functional adenine phosphoribosyltransferase, the transferase was either less actively expressed than the enzyme that ribosylated adenine or else it lacked access to the substrate. An alternative explanation is that relative kinetic parameters strongly favored the reaction catalyzed by the putative phosphorylase or "ribosyltransferase" over that of the phosphoribosyltransferase at low substrate levels.

Finally, a comparison between our results and those of previous studies involving the uptake and incorporation of adenine by *S. cerevisiae* (3, 4, 8–10, 25) suggests that the

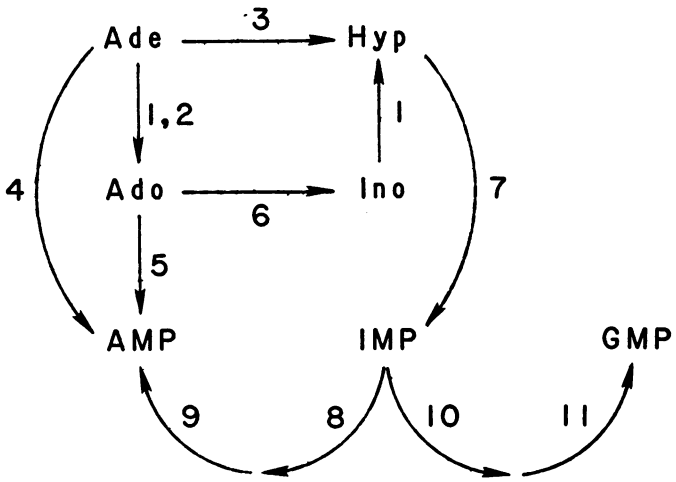

FIG. 3. Possible adenine salvage routes in *S. cerevisiae*. Ade, adenine, Ado, adenosine; hyp, hypoxanthine; ino, inosine; 1, Purine nucleoside phosphorylase; 2, adenosine phosphorylase; 3, adenine aminohydrolase; 4, adenine phosphoribosyltransferase; 5, adenosine kinase; 6, adenosine aminohydrolase; 7, hypoxanthine-guanine phosphoribosyltransferase; 8, adenylosuccinate synthetase; 9, adenylosuccinate lyase; 10, IMP dehydrogenase; 11, GMP synthetase.

high levels of adenine used in the latter either repressed or failed to induce the expression of the adenine ribosyltransferase. However, the nature of adenosine deaminase regulation cannot be deduced from these combined data, since virtually no adenosine was reported in previous purine uptake experiments.

ACKNOWLEDGMENTS

We thank R. A. Woods for the mutant yeast strains and for his valuable comments.

This work was supported in part by Public Health Service grant GM31023 from the National Institutes of Health.

LITERATURE CITED

1. **Abbondandolo, A., A. Weyer, H. Heslot, and M. Lambert.** 1971. Study of adenine aminohydrolase in the yeast, *Schizosaccharomyces pombe*. J. Bacteriol. **108**:959–963.
2. **Boss, G. R., and J. E. Seegmiller.** 1982. Genetic defects in human purine and pyrimidine metabolism. Annu. Rev. Genet. **16**:297–328.
3. **Burns, V. W.** 1964. Regulation and coordination of purine and pyrimidine biosynthesis in yeast. I. Regulation of purine biosynthesis and its relation to transient changes in intracellular nucleotide levels. Biophys. J. **4**:151–166.
4. **Burridge, P. W., R. A. Woods, and J. F. Henderson.** 1977. Purine metabolism in *Saccharomyces cerevisiae*. Can. J. Biochem. **55**:935–941.
5. **Cooper, T. G.** 1982. Transport in *Saccharomyces cerevisiae*, p. 399–461. *In* J. N. Strathern, E. W. Jones, and J. R. Broach (ed.), The molecular biology of the yeast *Saccharomyces*. Metabolism and gene expression. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
6. **DeGroodt, A., H. Heslot, L. Poirier, J. Pourquié, and M. Nagy.** 1969. La spécificité des phosphorylases des nucléotides puriques chez *Schizosaccharomyces pombe*. C.R. Acad. Sci. **D269**:1431–1433.
7. **Gutteridge, W. E., and M. J. Davies.** 1981. Properties of purine phosphoribosyltransferases of *Trypanosoma cruzi*. FEBS Lett. **127**:211–214.
8. **Halvorson, H.** 1958. Intracellular protein and nucleic acid turnover in resting yeast cells. Biochim. Biophys. Acta **27**:255–266.
9. **Halvorson, H.** 1958. Studies on protein and nucleic acid turnover in growing cultures of yeast. Biochim. Biophys. Acta **27**:267–276.
10. **Harris, G., and G. E. Neal.** 1960. Dynamic aspects of the nucleotide pool of brewer's yeast during growth. Biochim. Biophys. Acta **43**:197–205.
11. **Hatanaka, M., R. Del Guidice, and C. Long.** 1975. Adenine formation from adenosine by mycoplasmas: adenosine phosphorylase activity. Proc. Natl. Acad. Sci. USA **72**:1401–1405.
12. **Heppel, L. A., and R. J. Hilmoe.** 1952. Phosphorolysis and hydrolysis of purine ribosides by enzymes from yeast. J. Biol. Chem. **198**:683–694.
13. **Heyworth, P. G., W. E. Gutteridge, and C. D. Ginger.** 1982. Purine metabolism in *Trichomonas vaginalis*. FEBS Lett. **141**:106–110.
14. **Hochstadt-Ozer, J., and E. R. Stadtman.** 1971. The regulation of purine utilization in bacteria. II. Adenine phosphoribosyltransferase in isolated membrane preparations and its role in the transport of adenine across the membrane. J. Biol. Chem. **246**:5304–5311.
15. **Jensen, K. F.** 1978. Two purine nucleoside phosphorylases in *Bacillus subtilis*: purification and some properties of the adenosine-specific phosphorylase. Biochim. Biophys. Acta **525**:346–356.
16. **Jones, E. W., and G. R. Fink.** 1982. Regulation of amino acid and nucleotide biosynthesis in yeast, p. 181–299. *In* J. N. Strathern, E. W. Jones, and J. R. Broach (ed.), The molecular biology of the yeast *Saccharomyces*. Metabolism and gene expression. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
17. **Korn, E. D., and J. M. Buchanan.** 1955. Biosynthesis of purines. IV. Purification of liver nucleoside phosphorylase and demonstration of nucleoside synthesis from 4-amino-5-imidazolecarboxamide, adenine, and 2,6-diamino-purine. J. Biol. Chem. **217**:183–191.
18. **Kornberg, A., I. Lieberman, and E. S. Simms.** 1955. Enzymatic synthesis of purine nucleotides. J. Biol. Chem. **215**:417–427.
19. **Magasanik, B.** 1962. Biosynthesis of purine and pyrimidine nucleotides, p. 295–334. *In* I. C. Gunsalus, and R. Y. Stanier (ed.), The bacteria, vol. III. Academic Press, Inc., New York.
20. **Martin, D. W., Jr., and E. W. Gelfand.** 1981. Biochemistry of diseases of immunodevelopment. Annu. Rev. Biochem. **50**:845–877.
21. **Miech, R. P., A. W. Senft, and D. G. Senft.** 1975. Pathways of nucleotide metabolism in *Schistosoma mansoni* VI. Adenosine phosphorylase. Biochem. Pharmacol. **24**:407–411.
22. **Minato, M., and S. Fujii.** 1968. Adenosine deaminase from *Takadiastase*. J. Biochem. (Tokyo) **64**:815–826.
23. **Murray, A. W., D. C. Elliott, and M. R. Atkinson.** 1970. Nucleotide biosynthesis from preformed purines in mammalian cells: regulatory mechanisms and biological significance. Prog. Nucleic Acid Res. Mol. Biol. **10**:87–119.
24. **Parks, R. E., Jr., and R. P. Agarwal.** 1972. Purine nucleoside phosphorylase, p. 483–514. *In* P. D. Boyer (ed.), The enzymes, 3rd ed., vol. 7. Academic Press, Inc., New York.
25. **Pickering, W. R., and R. A. Woods.** 1972. The uptake and incorporation of purines by wild-type *Saccharomyces cerevisiae* and a mutant resistant to 4-aminopyrazolo(3,4-d)pyrimidine. Biochim. Biophys. Acta **26**:45–58.
26. **Pourquié, J., and H. Heslot.** 1971. Utilization and interconversions of purine derivatives in the fission yeast *Schizosaccharomyces pombe*. Genet. Res. **18**:33–44.
27. **Robertson, B. C., and P. A. Hoffee.** 1973. Purification and properties of purine nucleoside phosphorylase from *Salmonella typhimurium*. J. Biol. Chem. **248**:2040–2043.
28. **Robertson, B. C., P. Jargiello, J. Blank, and P. A. Hoffee.** 1970. Genetic regulation of ribonucleoside and deoxyribonucleoside catabolism in *Salmonella typhimurium*. J. Bacteriol. **102**:628–635.
29. **Roush, A. H., and M. Saeed.** 1960. Adenine metabolism in *Saccharomyces cerevisiae*: adenase from bakers' yeast.

Biochem. Biophys. Res. Commun. **2**:43–47.
30. **Schimandle, C. M., L. Tanigoshi, L. A. Mole, and I. W. Sherman.** 1985. Purine nucleoside phosphorylase of the malarial parasite, *Plasmodium lophurae*. J. Biol. Chem. **260**:4455–4460.
31. **Senesi, S., G. Falcone, U. Mura, F. Sgarrella, and P. L. Ipata.** 1976. A specific adenosine phosphorylase, distinct from purine nucleoside phosphorylase. FEBS Lett. **64**:353–357.
32. **Sherman, F., G. R. Fink, and C. W. Lawrence.** 1974. Methods in yeast genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
33. **Tarr, H. L. A.** 1958. Lingcod muscle purine nucleoside phosphorylase. Can. J. Biochem. Physiol. **36**:517–530.
34. **Tozzi, M. G., F. Sgarrella, and P. L. Ipata.** 1981. Induction and repression of enzymes involved in exogenous purine compound utilization in *Bacillus cereus*. Biochim. Biophys. Acta **678**:460–466.
35. **Wolfenden, R., T. K. Sharpless, and R. Allan.** 1967. Substrate specificity of adenosine deaminase. J. Biol. Chem. **242**:977–983.
36. **Woods, R. A., D. G. Roberts, T. Friedman, D. Jolly, and D. Filpula.** 1983. Hypoxanthine:guanine phosphoribosyltransferase mutants in *Saccharomyces cerevisiae*. Mol. Gen. Genet. **191**:407–413.
37. **Woods, R. A., D. G. Roberts, D. S. Stein, and D. Filpula.** 1984. Adenine phosphoribosyltransferase mutants in *Saccharomyces cerevisiae*. J. Gen. Microbiol. **130**:2629–2637.
38. **Zielke, C. L., and C. H. Suelter.** 1971. Purine, purine nucleoside, and purine nucleotide aminohydrolases, p. 47–78. *In* P. D. Boyer (ed.), The enzymes, 3rd ed., vol. 4. Academic Press, Inc., New York.
39. **Zimmerman, T. P., N. B. Gersten, A. F. Ross, and R. P. Miech.** 1971. Adenine as substrate for purine nucleoside phosphorylase. Can. J. Biochem. **49**:1050–1054.