THE ACCUMULATION OF GLYCINAMIDE RIBOTIDE BY ADE3 AND ADE8 MUTANTS OF SACCHAROMYCES CEREVISIAE

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SUMMARY

The purine intermediate GAR is present in cell free extracts of ade3 and ade8 mutants of yeast. It is also detectable following acid hydrolysis of extracts of ade6 and ade7 which accumulate FGAR and FGAM respectively. GAR accumulation is repressed by growing cells in high levels of adenine. Neither ade4 nor ade5 accumulate GAR and both prevent accumulation of GAR in ade3 and ade8 and FGAR in ade6. Since ade3 is known to be defective in folate metabolism these results indicate that ade8 is blocked in the conversion of GAR ⟶ FGAR.

Despite the extensive use of adenine-requiring mutants of yeast in genetic studies the correlations between metabolic and genetic lesions are incomplete. Mutants of the genes ade1 and ade2 are characterised by the presence of a red intracellular pigment and have been shown to accumulate CAIR and AIR respectively (Silver and Eaton, 1969). Accumulation of this pigment is prevented or reduced by mutation of genes which are epistatic to ade2 (Roman, 1956). Analysis of double mutants obtained in this way has resulted in the identification of six genes, ade3 to ade8, which are believed to act prior to ade2. Gene-enzyme correlations have been established for three of these. One, ade3, which causes a dual requirement for adenine

and histidine, is involved in the synthesis of the tetrahydrofolate cofactors which are required for two steps of purine biosynthesis; GAR⟶FGAR and AICAR⟷FAICAR (see Jones and Magasanik, 1967; Lazowska and Luzzati, 1970; Lomax, Gross and Woods, 1971). Both *ade4* and *ade5* are "*bona fide*" purine mutants and lack activity of PRPP amidotransferase and GAR synthetase respectively (Gross and Woods, 1971). Silver (1969) has shown that mutants of *ade6* and *ade7* can incorporate ^{14}C-formate into purine intermediates and must thus be blocked between FGAR and AIR. On the basis of this and other evidence she concluded that *ade6* accumulated FGAR and *ade7* FGAM.

The gene-reaction allocations established to date are shown in Figure 1.

This leaves *ade8* unallocated and one reaction, GAR⟶FGAR not accounted for. A mutant lacking GAR transformylase activity would be expected to accumulate GAR. The metabolic lesion in *ade3(his$^-$)* is thought to result in a deficiency of 5, 10 - methenyl tetrahydrofolate, the cofactor for GAR transformylase (Lomax, Gross and Woods, 1971). Thus *ade3(his$^-$)* mutants should accumulate GAR and can be used to test this hypothesis and the experimental procedures.

METHODS

Yeast strains. The mutants *ade3(his$^-$)*, *ade4*, *ade5*, *ade6*, *ade7* and *ade8* were from our laboratory stock collection. All were of the genotype *ade su-pur$^+$*. The double mutants were constructed and characterised by conventional genetic techniques.

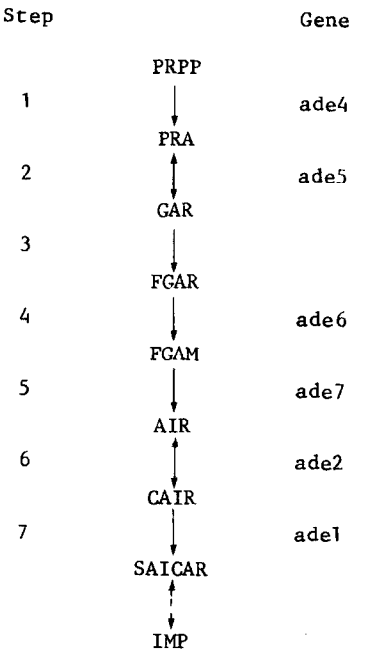

Figure 1. Purine biosynthesis in yeast; steps 1 to 7. Abbreviations: PRRP, 5-phosphoribosyl-1-pyrophosphate; PRA, 5-phosphoribosylamine; GAR, glycinamide ribotide; FGAR, formylglycinamide ribotide; FGAM, formylglycinamidine ribotide; AIR, aminoimidazole ribotide; CAIR, carboxyaminoimidazole ribotide; SAICAR, succinocarboxyaminoimidazole ribotide; IMP, inosine monophosphate.

Experimental procedures. Cultures for analysis were grown in yeast minimal medium containing 1 mM L-histidine and 40 μM adenine as described by Gross and Woods (1971).

Cultures were harvested by centrifugation at 4°C and resuspended at 10% w/v in 0.1 M Tris HCl (pH 8.1) containing 1 mM EDTA and 1 mM 2-mercaptoethanol. The cells were disrupted in a French pressure cell and the resulting extract centrifuged at 25,000 g for 30 min. The supernatant was retained and aliquots assayed for GAR and protein content.

GAR accumulation was assayed by the procedure of Nierlich and Magasanik (1965), the conditions being as

described by Gross and Woods (1971). In the presence of IMP and a 15-30% ethanol fraction of chicken liver GAR is transformylated to FGAR with the co-formation of aminoimidazolecarboxamide ribotide (AICAR):

$$GAR + IMP + H_2O \longrightarrow FGAR + AICAR$$

The AICAR is assayed by the Bratton Marshall reaction (Flaks and Lukens, 1963). Results are expressed as nmoles GAR/gm protein in the cell-free extract. Controls lacking chicken liver enzyme and extract were included in each experiment.

FGAR in cell-free extracts was hydrolysed to GAR by incubating 0.9 ml extract with 0.1 ml 1.0 N HCl at 100°C for 15 min. The tubes were chilled in ice and contents neutralised with 0.1 N NaOH.

The 15-30% ethanol fraction of chicken liver was prepared by Dr. T.S. Gross according to the method of Flaks and Lukens (1963). Protein contents were estimated by the method of Lowry et al. (1951).

RESULTS AND DISCUSSION

The results of testing for GAR accumulation in mutants of the genes epistatic to ade2 are listed in Table 1. GAR is present in extracts of ade3, and ade8; it is also detectable in extracts of ade6 and ade7 after acid hydrolysis. It can be seen that the GAR is labile, 50-60% is lost on acid hydrolysis of the ade3 extract.

To check that these accumulations were due to blocks in purine biosynthesis we assayed accumulation in cultures

Table 1. GAR accumulation in cell-free extracts of ade mutants

Strain	Culture yield (mg/ml)	GAR (nmoles/gm protein) untreated	hydrolysed
ade3	1.20	17,600	8,020
ade4	0.87	0	0
ade5	0.91	0	0
ade6	0.98	0	8,000
ade7	0.91	0	3,890
ade8	0.86	8,350	-

Table 2. Effects of adenine concentration on GAR accumulation

Strain	Adenine (μM)	Culture yield (mg/ml)	GAR (nmoles/gm protein) untreated	hydrolysed
ade3	40	0.87	16,100	-
	80	1.20	9,050	-
	160	1.28	2,240	-
	320	1.27	1,030	-
ade6	40	1.16	0	7,300
	80	1.52	0	8,250
	160	1.73	0	3,600
	320	1.75	0	1,550
ade7	40	1.17	0	6,300
	80	1.82	0	3,710
	160	1.74	0	2,230
	320	1.89	0	0
ade8	40	0.86	8,350	-
	80	1.36	7,710	-
	160	1.42	2,660	-
	320	1.54	1,550	-

Table 3. GAR accumulation in double mutant strains

Genotype	Culture yield (mg/ml)	GAR (nmoles/gm protein) untreated	hydrolysed
ade3/ade4	0.98	0	-
ade3/ade5	0.93	0	-
ade3/ade6	0.90	10,000	-
ade3/ade8	0.85	9,000	-
ade4/ade6	0.78	0	0
ade4/ade8	1.06	0	-
ade5/ade6	1.02	0	0
ade5/ade8	0.91	0	-
ade6/ade8	0.89	5,200	-

grown in a range of adenine concentrations. The results, in Table 2 show that accumulation is strongly repressed by high adenine in all four mutant strains.

As a further test we assayed GAR accumulation in a series of double mutants involving ade3, ade4, ade5, ade6 and ade8. The results in Table 3 clearly show that ade4 and ade5 are epistatic to ade3, ade6 and ade8, which is in accord with the established biochemical lesions in these two (Gross and Woods, 1971). It is also clear that ade3 and ade8 are epistatic to ade6 as the double mutants ade3 ade6 and ade6 ade8 accumulate GAR.

Our results show that two of the mutants which are epistatic to ade2 accumulate GAR. The effects of adenine on the levels of accumulation indicate that the GAR detected is an intermediate in purine biosynthesis; the epistasis of ade4 and ade5 is consistent with this conclusion. Since ade3

is defective in the synthesis of a co-factor for GAR transformylase it is reasonable to conclude either that ade8 is the structural gene for this enzyme or that the ade8 product is required for its activity in vivo.

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REFERENCES

Flaks, J.G. and Lukens, L.N. Methods in Enzymology, Vol. 6. Academic Press, New York, 54 (1963), eds S.P. Colowick and N.O. Kaplan.
Gross, T.S. and Woods, R.A. Biochim. Biophys. Acta 247, 13 (1971).
Jones, E.W. and Magasanik, B. Biochem. Biophys. Res. Commun. 29, 600 (1967).
Lazowska, J. and Luzzati, M. Biochem. Biophys. Res. Commun. 39, 34 (1970).
Lomax, C.A., Gross, T.S. and Woods, R.A. J. Bacteriol. 107, 1 (1971).
Lowry, O.H., Rosebrough, N.J., Farr, A.C. and Randall, R.J. J. Biol. Chem. 193, 265 (1951).
Nierlich, D.P. and Magasanik, B. J. Biol. Chem. 240, 366 (1965).
Roman, H. C.R. Trav. Lab. Carlsberg, Ser. Physiol. 26, 299 (1956).
Silver, J.M. Ph.D. Thesis, City University of New York (1969).
Silver, J.M. and Eaton, N.R. Biochem. Biophys. Res. Commun. 34, 301 (1969).