# BIOCHEMICAL DEFICIENCY ASSOCIATED WITH ad3 MUTATIONS IN SACCHAROMYCES CEREVISIAE

## I. LEVELS OF THREE ENZYMES OF TETRAHYDROFOLATE METABOLISM

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Received February 3, 1970

SUMMARY : In ad3 mutants, the specific activities for formyltetrahydrofolate synthetase and methenyltetrahydrofolate cyclohydrolase, represent ca. 10 % of wild type activity and the activity for methylenetetrahydrofolate dehydrogenase can represent 50 % of wild type activity under appropriate assay conditions.

Mutations at the ad3 locus in S. cerevisiae cause a simultaneous requirement for adenine and histidine (11). Jones and Magasanik (5) reported that ad3 mutations result in "a total absence of formyltetrahydrofolate synthetase (EC 6.3.4.3.), an absence of methenyltetrahydrofolate cyclohydrolase (EC 3.5.4.9.), and 10 to 15 % of the wild type activity for methylenetetrahydrofolate dehydrogenase (EC 1.5.1.5.)". These enzymes being related to the interconversion of folic acid derivatives, their absence explains in a satisfactory way the adenine requirement in ad3 mutants. Similar results have been obtained in Schizosaccharomyces pombe (8). Some indirect experiments (7) suggested that the simultaneous histidine requirement resulted from an inhibition of one early step in histidine biosynthesis by AICAR accumulated in ad3 strains. However the precise step is still unknown.

In this report we will present data which shows that formyltetrahydrofolate synthetase and methenyltetrahydrofolate cyclohydrolase activities are not completely missing in ad3 mutants. Furthermore, the activity of methylenetetrahydrofolate dehydrogenase is a function of the conditions of the assay.

## MATERIAL AND METHODS

The ad3 strains used were : ad3-10, a deletion of 2/3 of the gene (4), ad3-39 and ad3-3, two point mutations at ad3 locus. The first two strains were kindly furnished by E.W. Jones. The wild type strain is a hi7 mutant.

The cultures were grown at 28°C up to exponential phase in a synthetic minimal medium (1) supplemented with either adenine (20 mg/l) and histidine (10 mg/l) (ad3 strains) or histidine (10 mg/l) (wild type). The cells (0.3-0.6 g wet weight) were harvested, washed twice with 0.1 M potassium phosphate buffer, pH 7.5, resuspended in 8 ml of the same buffer and disrupted in a Braun desintegrator with 8 g of glass beads. After centrifugation at 23,000 g for 30 min the supernatant was either dialysed overnight against 0,01 M phosphate buffer, pH 7.5, or passed through a Sephadex G-25 column.

The enzymes were assayed by slight modifications of methods already described. Formyltetrahydrofolate synthetase (12) (13) : 50 µmoles Tris, pH 7.5, 50 µmoles 2-mercaptoethanol, 150 µmoles sodium formate, 145 µmoles $NH_4Cl$, 4 µmoles $MgCl_2$, 1.25 µmoles ATP, 1 µmole phosphoenolpyruvate, 2 µmoles tetrahydrofolic acid, protein (up to 0.3 mg for wild type and up to 1.0 mg for ad3) from the dialysed extract, and water to a final volume of 1 ml is incubated at 37°C. Immediately after the addition of protein, and thereafter at various times, 0.1 ml samples are removed and added to 1.9 ml perchloric acid (3.5 %). After 10 minutes the precipitated protein is removed and the absorbance of the supernatant at 355 nm is measured against a blank, prepared by adding 0.1 ml of a mixture identical to the one above, but lacking protein, to 1.9 ml of perchloric acid.

Methenyltetrahydrofolate cyclohydrolase (9) : 1 ml of incubation mixture contained 200 µmoles potassium maleate buffer, pH 7.0, 280 µmoles 2-mercaptoethanol, protein (from the wild type strain extract up to 0.3 mg and from ad3 strain extract up to 4 mg). The reaction is started by the addition of 0.075 µmoles of $N^5$-$N^{10}$ methenyl tetrahydrofolate and the decrease of absorbance at 355 nm is recorded on a Guilford spectrophotometer. A blank is made without enzyme and the difference between the two initial velocity is calculated.

Methylenetetrahydrofolate dehydrogenase (10) : 1.2 ml of mixture containing 50 mM potassium phosphate buffer pH 7.5 ; 6.6 mM 2-mercaptoethanol ; 0.2 mM tetrahydrofolic acid ; 0.6 mM NADP ; 0.59 mM formaldehyde 0.2-1.0 mg protein is incubated at 37°C. The reference cell contained all the reactants except formaldehyde and enzyme. NADP is added to start the

reaction and the absorbance increase at 340 nm is recorded over 1 min intervals on a Beckmann DU spectrophotometer.

Chemicals were obtained from the following sources : d,l-L-tetrahydrofolic acid (THF) from Sigma Chemical Co., or prepared as described by Hafeti et al. (2) ; $N^5$-$N^{10}$ methenyl tetrahydrofolic acid was prepared as described by Huennekens et al. (3). All the chemicals were checked by their spectra before use. Biuret (6) was used for protein determination.

## RESULTS AND DISCUSSION

Formyltetrahydrofolate synthetase : Fig. 1A shows a typical experiment : enzymic activity is proportional to the amount of protein in both strains, and the specific activity in the ad3 extract is about 10 % of the one in the wild type extract. The main improvement in the assay consisted in increasing the tetrahydrofolate concentration from 1 mM to 2 mM and the incubation temperature from 30°C to 37°C. For assays of ad3 extracts, the

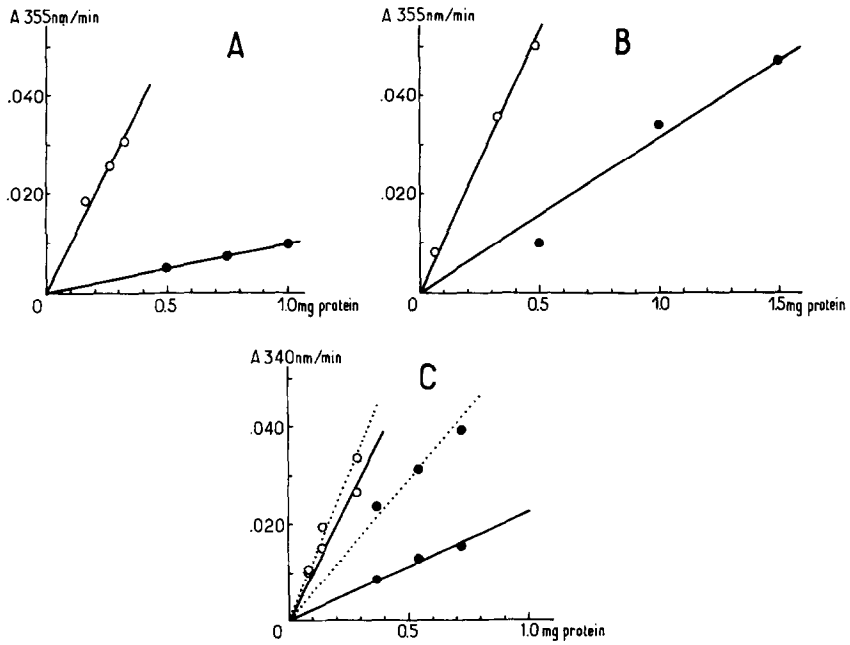


Fig. 1 : Enzymic activity as a function of protein in crude extracts of wild type (open circles) and ad3 (closed circles) strains.

A. Formyltetrahydrofolate synthetase
B. Methenyltetrahydrofolate cyclohydrolase
C. Methylenetetrahydrofolate dehydrogenase
full line : assay at a concentration of NADP 0.6 mM and phosphate buffer 50 mM
dotted line : assay at a concentration of NADP 3 mM and phosphate buffer 217 mM.

amount of protein and the length of incubation time has to be increased : if not, the enzymic activity is negligible.

Methenyltetrahydrofolate cyclohydrolase : Fig. 1B shows an experiment, similar to the one shown in Fig. 1A : the specific activity in ad3 extracts is about 30 % of the specific activity in wild type extracts. The pH of the incubation mixture has been increased from 5.5 to 7.0, the optimum pH of the assay. Spontaneous transformation of $N^5$-$N^{10}$ methenyl tetrahydrofolate into $N^{10}$- formyltetrahydrofolate, has to be deducted from the experimental value obtained in the presence of the extract.

Methylenetetrahydrofolate dehydrogenase : As in the preceding figures, Fig. 1C shows the dehydrogenase activity in wild type and in ad3 extracts. It can be seen that the activity in ad3 extracts is dependent of the conditions of the assay : a 2-fold increase is obtained by changing the ionic strength and NADP concentration for ad3 extracts, while very little difference is observed in wild type extracts. The rationale of the assay conditions are the following : dehydrogenase activity is more sensitive to changes in phosphate molarity in ad3 extracts than in wild type extracts, as can be seen in Fig. 2. With regard to NADP concentration, ad3 extracts show a different $K_m$ for NADP than wild type extract, as will be discussed in the accompanying paper.

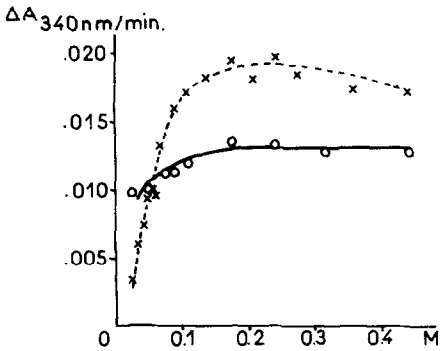


Fig. 2 : Methylenetetrahydrofolate dehydrogenase
Effect of phosphate molarity on the enzymic activity in crude extracts of wild strain (0,28 mg protein) and ad3 strain (0.84 mg protein)
Full line : wild type strain
dashed line : ad3 strain

## CONCLUSIONS

The specific activities for the three enzymes in extracts of three different ad3 strains, two point mutations and one deletion, as

TABLE I

Specific activities of different enzymes in crude extracts of wild type strains and of some ad3 strains.

| | Wt | ad3-3 | ad3-39 | ad-3-10 |
|---|---|---|---|---|
| FORMYLTETRAHYDROFOLATE SYNTHETASE<br>nmoles $N^{10}$-formyl THF/min/mg protein | 70.0 | 4.5 | 10.0 | 8.0 |
| METHENYLTETRAHYDROFOLATE CYCLOHYDROLASE<br>nmoles $N^{5}$-$N^{10}$ methenyl THF/min/mg prot. | 40.8 | 6.3 | 5.0 | 5.2 |
| METHYLENETETRAHYDROFOLATE DEHYDROGENASE<br>nmoles NADPH/min/mg protein | | | | |
| standard assay conditions | 8.2 | 2.5 | 2.7 | 2.9 |
| high NADP (3mM) and high phosphate molarity (217 mM) assay conditions | 9.2 | 3.9 | 5.3 | 6.0 |

compared to wild type extract, are summarized in table 1. It can be seen that the synthetase and cyclohydrolase activities in all three ad3 mutants are far from being negligible, and the dehydrogenase activity can represent ca. 50 % of the activity in the wild type under appropriate assay conditions.

Assuming that ad3 is a structural gene, these experiments do not allow to say which is the primary alteration caused by ad3 mutation. When a deletion mutation which covers 2/3 of ad3 locus still has the activities for the three enzymes studied, it is difficult to imagine that any of these proteins correspond to the product of the $ad3^{+}$ gene, unless the residual enzymic activities are due to different enzyme forms. This point has been investigated in the accompanying paper.

ACKNOWLEDGEMENTS

We are grateful to Pr. P. Slonimski for discussion and suggestions. This work was supported by a grant from D.G.R.S.T. Biol. Molec. N°66.00.168

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