FUNCTIONAL BLOCKS OF THE AD_1 AND AD_2 MUTANTS OF SACCHAROMYCES CEREVISIAE

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SUMMARY

Spectrophotometric analyses of Bratton-Marshall chromophores in deproteinized extracts of ad_1 and ad_2 mutants of S. cerevisiae indicated that ad_2 was blocked at the conversion of AIR to CAIR and ad_1 at the conversion of CAIR to SAICAR. The demonstration, that the introduction of an ad_2 marker into an ad_1 strain results in the elimination of a C^{14}-glycine-labelled, Pauly-positive compound characteristic of the ad_1 strain, further supports the conclusion that the block of ad_2 precedes that of ad_1.

RESULTS AND DISCUSSION

The biochemical blocks in the adenine-requiring mutants, ad_1 and ad_2, of Saccharomyces cerevisiae (Roman, 1956) have not been precisely established. Assignment of the functions controlled by these loci have depended upon the identification of AIR (aminoimidazole ribotide) and CAIR (5-amino-4-imidazole-carboxylic acid ribotide), which yield Bratton-Marshall chromophores with absorption maxima of 500 and 520mμ respectively (Lukens and Buchanan, 1959), as the intermediates accumulated by these mutants.

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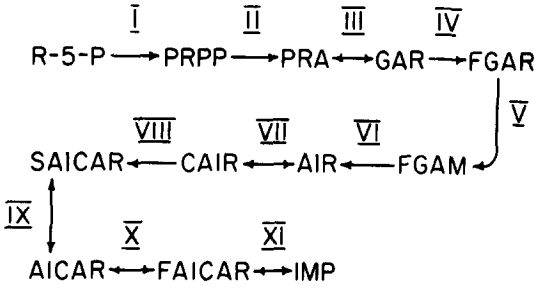

Fig. 1. Reaction steps and intermediates of purine biosynthesis. Abbreviations: R-5-P, ribose-5-phosphate; PRPP, 5-phosphoribosylpyrophosphate; PRA, 5-phosphoribosylamine; GAR, glycinamide ribotide; FGAR, formylglycinamide ribotide; FGAM, formylglycinamidine ribotide; AIR, aminoimidazole ribotide; CAIR, 5-amino-4-imidazole carboxylic acid ribotide; SAICAR, 5-amino-4-imidazole-N-succino-carboxamide ribotide; AICAR, 5-amino-4-imidazole-carboxamide ribotide; FAICAR, 5-formamido-4-imidazole-carboxamide ribotide; IMP, inosinic acid.

Considerable difficulty is encountered however, since both mutants may accumulate AIR as a result of a block either at step VIII or at step VII (Fig. 1), and AIR may also be formed by the breakdown of CAIR, which is labile (Lukens and Buchanan, 1959). In addition, in the presence of oxygen AIR will polymerize to form a dark red pigment, which can interfere both with the spectrophotometric analyses and with the chromatographic resolution of components of cell extracts. Further difficulty may result from a lack of strict control of the pH at which the Bratton-Marshall test is carried out (Lukens and Buchanan, 1959).

It was considered desirable to reinvestigate the nature of the ad_1 and ad_2 mutational blocks, using conditions which avoid some of the above difficulties. Extracts of ad_1 and ad_2, which were to be analyzed by absorption spectra of the Bratton-Marshall reaction products, were therefore made from cells grown anaerobically, since less interfering pigment is

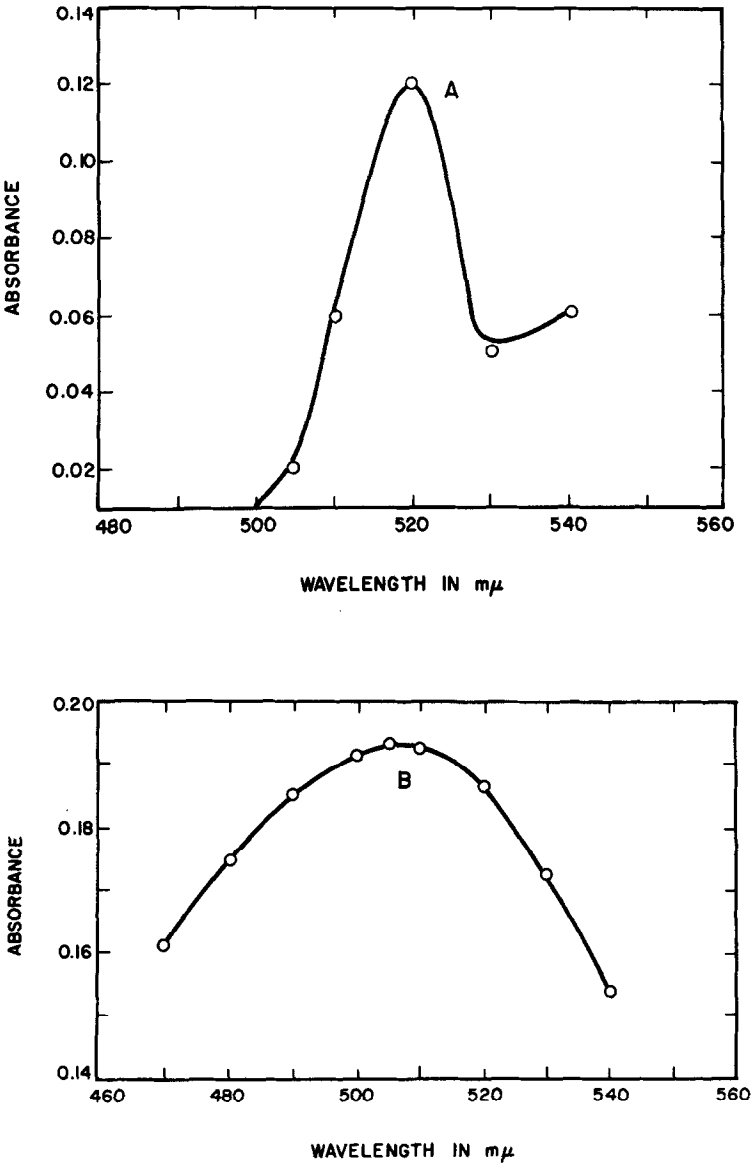

Fig. 2. Absorption spectra of Bratton-Marshall chromophores of ad_1 and ad_2. (A) absorption spectrum of the Bratton-Marshall chromophore in ad_1. (B) absorption spectrum of the Bratton-Marshall chromophore in ad_2. The Bratton-Marshall test for aryl-amines, as modified by Flaks and Lukens (1963) was applied to deproteinized extracts after treatment of the preparations with 10% acetic anhydride for 20 minutes. Solutions were acidified prior to assay with 1.33M potassium phosphate in 20% trichloroacetic acid adjusted to pH 1.4 with KOH (Flaks and Lukens, 1963).

formed under these conditions, and all Bratton-Marshall reactions were carried out at a pH of 1.4. The results are shown in Fig. 2. The absorption maximum of the Bratton-Marshall chromophore in ad_2 extracts is close to the range of 500-502 mμ reported for AIR (Bernstein, 1961), whereas that of the ad_1 extract is identical to that reported for CAIR, (520 mμ; Lukens and Buchanan, 1959). These data indicate that ad_2 is blocked at the conversion of AIR to CAIR and that ad_1 is blocked at the conversion of CAIR to SAICAR.

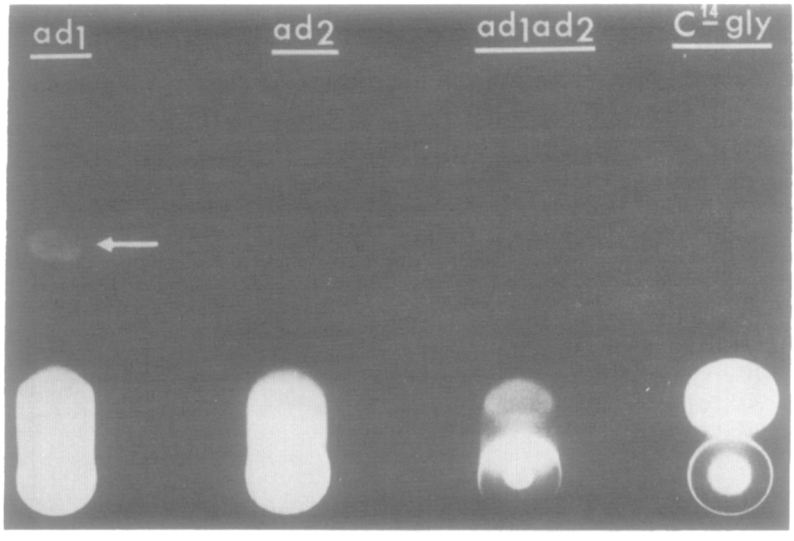

Fig. 3. Radioautogram chromatographed extracts of C^{14}-glycine-labelled ad_1, ad_2 and ad_1ad_2.
Cells were grown without shaking for 48 hours at 30° in a synthetic complete medium containing 0.025μC/ml, C^{14}-U. L-glycine Ethanol deproteinized extracts were chromatographed on a thin layer cellulose plate in n-butanol-glacial acetic acid-water (40:20:20). Radioactive compounds were located by autoradiography. Imidazole compounds were localized with Pauly reagent (Ames and Mitchell, 1962), countersprayed with 5% Na_2CO_3. The arrow indicates the location of a C^{14}-glycine-labelled imidazole characteristic of ad_1.

A similar conclusion was tentatively reached by Dorfman (1964), who found that acidification of an ad_1 extract caused the absorption maximum of the Bratton-Marshall derivative of this mutant to approach the absorption maximum characteristic of AIR, while that of the ad_2 derivative remained the same. This behaviour of the material in the ad_1 extract is consistent with that expected of CAIR which is decarboxylated by acid to AIR (Lukens and Buchanan, 1959).

The disappearance of a characteristic accumulation of a mutant will occur if the introduction of an additional adenine marker into the strain corresponds to the introduction of an earlier enzymatic block. The introduction of an ad_2 marker into an ad_1 strain results in the elimination of a C^{14}-glycine-labelled, Pauly-positive compound which is characteristic of the ad_1 strain, further supporting the conclusion that the block of ad_2 precedes that of ad_1 (Fig. 3). This order of the functional blocks is supported also by Fisher (1969) who found that extracts of the ad_2 strain could convert SAICAR to CAIR whereas extracts of the ad_1 strain lack this activity.

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