'Red pigment' from ADE-2 mutants of *S. cerevisiae* prevents DNA cleavage by restriction endonucleases

Arturas Meškauskas*, Vladimir Ksenzenko, Mikhail Shlyapnikov, Valentin Kryukov and Donaldas Čitavičius*

*Institute of Botany, Lithuanian SSR Academy of Sciences, Vilnius, 232027 Lithuanian SSR, and Institute of Biochemistry and Physiology of Microorganisms, USSR Academy of Sciences, Pustchino on the Oka, 142292 Moscow region, USSR

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Protection of DNA from cleavage by restriction endonucleases *Eco*RI, *Hin*dIII, *Bam*HI, and *Bgl*II with red pigment, produced by ADE-2 mutants of *Saccharomyces cerevisiae* is demonstrated. Purification of yeast DNA from pigment can be achieved by chromatography on hydroxyapatite columns.

ADE-2 locus *Red pigment* *Restriction endonuclease* (S. cerevisiae)

1. INTRODUCTION

The adenine biosynthesis pathway in the yeast *Saccharomyces cerevisiae* is controlled by at least 13 genes [1]. Mutations in the ade-2 locus prevent the conversion of (5-phosphoribosyl)amino-imidazole into its carboxylated (in the 4th position) derivative [2]. It is considered that the so-called 'red pigment', accumulated by ade-1 and ade-2 mutants, represents a polymeric form of this intermediate [3]. On investigating the ade-2 locus we encountered a problem of incomplete restriction of DNA, isolated from these mutants. Here we attempted to elucidate the possible reasons for this phenomenon.

2. MATERIALS AND METHODS

Wild-type *S. cerevisiae* strains and ade-2 mutants, isolated in our laboratory, have been used. Yeasts were grown on YEPD medium to the late log phase. DNA was isolated from 10 g wet wt as in [3]. Further purification was performed by chromatography on hydroxyapatite columns (Bio-Gel HT, Bio-Rad), equilibrated with K-P buffer. After DNA sorption the columns were washed with 5 vols K-P buffer (0.05 M K_2HPO_4-KH_2PO_4, pH 6.8), followed by 5 vols of 0.16 M KPO_4, pH 6.8. DNA was eluted with 0.4 M KPO_4, pH 6.8. Red pigment, not adsorbed by hydroxyapatite, was extensively dialyzed against 10 mM Tris-HCl, pH 7.5, precipitated with 3 vols ethanol, and dissolved in 300 μl of 10 mM Tris-HCl, pH 7.5.

3. RESULTS AND DISCUSSION

In the analysis of yeast DNA isolated from ade-2 mutants of *S. cerevisiae* we encountered some difficulties in achieving complete cleavage of DNA by restriction endonucleases. However, under identical conditions DNA from ade strains was cleaved to completion. The distinguishing feature of ade-2 mutants is intracellular accumulation of the so-called red pigment. We suppose that the inhibitory activity is associated with the pigment copurifying with DNA during isolation. We have tried a number of methods to purify DNA from the pigment. Satisfactory purification was achieved by chromatography on hydroxyapatite columns. The pigment essentially was not adsorbed by carrier. The remaining amounts of the dye were washed with 0.16 M K-P buffer. Among other methods tried, satisfactory results were obtained by chromatography on glass fiber filter paper (Whatman GF/C) [5]. In the following experiments we investigated the effect of red pigment on the cleavage of phage λ DNA by a number of restriction endonucleases. The fraction of the pigment,

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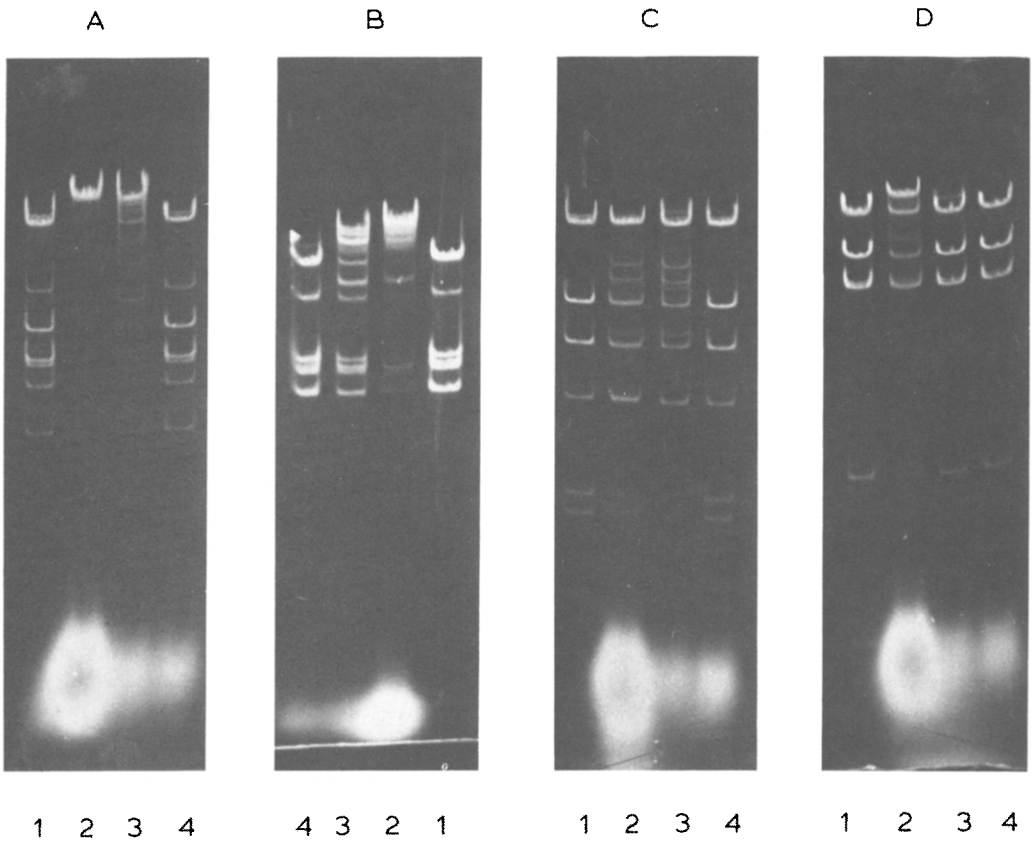

Fig.1. Influence of red pigment from ade-2 mutants of *S. cerevisiae* on the restriction of phage λ c1857 DNA by *Eco*RI (A), *Bam*HI (B), *Hin*dIII (C) and *Bgl*II (D) restriction endonucleases. The enzyme/DNA ratio in the reaction mixture was 1 unit per μg (lanes 1-3) and 10 units per μg (lane 4). 5 μl (lane 2) and 0.5 μl (lanes 3, 4) of red pigment (see section 2) were added to the reaction mixture, containing 1 μg DNA. Lane 1, restriction in the absence of pigment. Electrophoresis was carried out in 0.7% agarose gel.

not adsorbed by hydroxyapatite, was dialysed, precipitated with ethanol and dissolved in 10 mM Tris-HCl, pH 7.5. Addition of red pigment aliquots to the reaction mixture resulted in partial or complete inhibition of λ DNA cleavage by *Eco*RI, *Hin*dIII, *Bam*HI and *Bgl*II restriction endonucleases. As shown in fig.1 the level of inhibition was different for each enzyme tested. The highest level of inhibition (at an identical ratio of enzyme/DNA/pigment) was achieved for *Eco*RI cleavage, the lowest for *Bgl*II. Increasing the enzyme/DNA ratio under unchanged pigment concentration resulted in a decrease in the inhibitory effect. Unfortunately, the absence of reliable spectral characteristics of the red pigment, its polymerization heterogeneity, as well as the absence of precise data on its chemical structure do not allow more definite quantitative analysis of the inhibitory effect of the red pigment on DNA restriction. For the same reasons it is difficult to elucidate the mechanism of its action.

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