Stimulation by silybin, a eukaryotic feature of archaebacterial RNA polymerases

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Transcription of native DNA by DNA-dependent RNA polymerases of members of the thermoacidophilic branch of the archaebacteria like that of eukaryotic RNA polymerase A of rat liver, is stimulated by the flavonolignane derivative silybin. Eubacterial RNA polymerases are not affected. For both RNA polymerase A of rat liver and RNA polymerase of *Thermoplasma acidophilum* the stimulatory effect is confined to the enhancement of the elongation reaction suggesting a similar mechanism for both enzymes. These results further support a specific phylogenetic relationship between the archaebacteria and the eukaryotic cytoplasm.

Transcription *Silybin* *Archaebacteria* *Eubacteria* *Eukaryotis* *Evolution*

1. INTRODUCTION

The prokaryotic domain has been divided into the urkingdoms of the eubacteria and the archaebacteria on the basis of the comparison of 16 S rRNA sequences [1–3]. Further evidence for this division is provided by differences in cell envelopes [4], membrane lipids [5], the translation apparatus [6–8] and the transcription machinery [9,10]. Archaebacteria resemble eukaryotes rather than eubacteria in several features, like the ADP-ribosylation of their EF-2s by *Diphtheria* toxin [11], the sequence of ribosomal A proteins [6,7] and of initiator tRNAs [12], the rifampicin and the streptolydigin insensitivity and the component patterns of their DNA-dependent RNA polymerases [9,10]. This paper presents further evidence that archaebacterial RNA polymerases are phylogenetically related to those of eukaryotes. The flavonolignane derivative silybin which was isolated from the thistle *Marianum silybium* is known to stimulate rat liver RNA polymerase A [13]. The influence of silybin on archaebacterial transcription has been investigated.

2. MATERIALS AND METHODS

Silybin was a gift of Madaus (Köln). It was dissolved in water by titrating the suspension to pH 10.5 with 1 N NaOH. After clearance of the solution the pH was readjusted to 9.5 with 1 N HCl. Due to the hydrophobic nature of the flavonolignane it is difficult to maintain definite silybin concentrations. Also, the protein concentration (even BSA) influences the assay, presumably because of an unspecific but competitive binding of silybin. The compound was applied in 0.1–100 μg/ml. The observed activation varied between the different enzyme preparations.

DNA-dependent RNA polymerases were isolated and assayed as in [13–18]. Phage T7 DNA (10 μg) was used as a template for all prokaryotic RNA polymerases, calf-thymus DNA (8 μg) for eukaryotic RNA polymerase A. The following amounts of RNA polymerase were used: *Escherichia coli*, 250 μU; *Lactobacillus curvatus*, 150 μU; *Sulfolobus acidocaldarius*, 175 μU; *Thermoproteus tenax*, 340 μU; *Desulfurococcus mucosus*, 375 μU; *Thermoplasma acidophilum*,

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9 μU; polymerase A, 1.5 μU; polymerase B, 1.3 μU. [^{3}H]UTP with a spec. act. 80 Ci/mol was used for polymerase A and B from rat liver, [^{14}C]ATP with a spec. act. 10 Ci/mol for the RNA polymerase from *T. acidophilum*, and 1 Ci/mol for all others.

The ternary complex was prepared by preincubating the complete assay mixture for 4 min. After cooling to 0°C, 200 μg/ml heparin and the indicated amount of silybin were added. After 5 min on ice incubation was continued and aliquots were taken after various times.

3. RESULTS AND DISCUSSION

The DNA-dependent RNA polymerase A(I) of rat liver which transcribes the ribosomal genes is stimulated by the flavonolignane derivative silybin up to a factor of 1.5 at 20 μg/ml [13] (fig. 1). RNA polymerase B and the RNA polymerases of the eubacteria *L. curvatus* and *E. coli* are not affected at lower concentrations and are inhibited at higher concentrations. The enzymes of the archaebacteria *T. acidophilum*, *S. acidocaldarius*, *T. tenax* and *D. mucosus*, all members of the thermoacidophilic branch, are activated to the same extent as RNA polymerase A(I) (fig. 1).

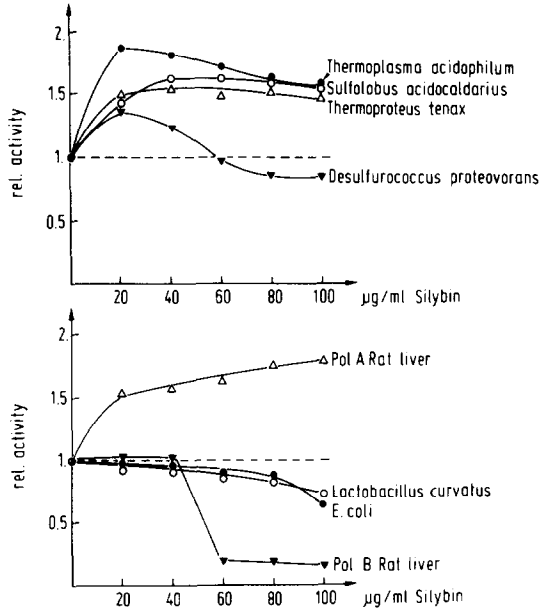

Fig. 1. Influence of silybin on the transcription by eubacterial, archaebacterial and eukaryotic RNA polymerases. The relative activity of each enzyme in the absence of silybin was taken as 1.

To determine the point of attack of the compound in the multi-step transcription process, the elongation rate was measured employing ternary complexes prepared by adding heparin to preincubated assay mixtures, thus inhibiting all free enzyme molecules and blocking any further initiation. The elongation was then followed with and without addition of silybin. Fig. 2 shows that it proceeeds ~2-times faster with *T. acidophilum* RNA polymerase and 1.4-times faster with rat liver RNA polymerase when silybin is added. This accounts completely for the net activation in the overall assay which was 1.8- and 1.4-fold, respectively. Though the mechanism of action of silybin is unknown, the fact that archaebacterial RNA polymerases and eukaryotic RNA polymerase A are both affected in the elongation reaction indicates a similar mode of elongation for both enzymes.

As shown in [13] the K_M-values for the 4 nucleotides are not changed by the addition of silybin but V_{max} is increased, as expected for an allosteric effector binding to a site different from the active center. The possible existence of cellular effectors for the elongation phase of transcription gains some probability by the occurrence of an 'artificial' effector.

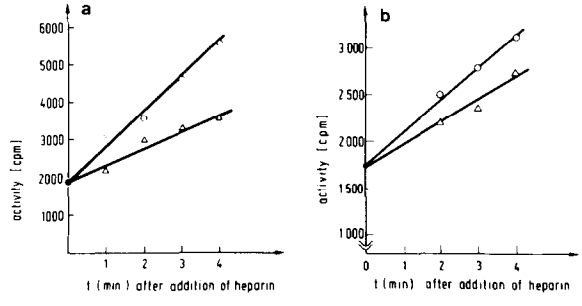

Fig. 2. Influence of silybin on the elongation of transcription by: (a) RNA polymerase of *Thermoplasma acidophilum*; (b) RNA polymerase A of rat liver. (△) without addition of silybin; (○) with silybin, 20 μg/ml for *Thermoplasma acidophilum* RNA polymerase, 100 μg/ml for polymerase A. Controls show that heparin and silybin do not interfere with each other.

These results show that archaebacterial RNA polymerases are specifically related to the eukaryotic RNA polymerase A as already suggested by their component patterns and their insensitivity to rifampicin and streptolydigin which are strong inhibitors of eubacterial RNA polymerases. Further support for this notion is provided by the cross-reaction of antibodies against several components of RNA polymerase A of yeast with components of archaebacterial RNA polymerases (J. Huet, R. Schnabel, A. Sentenac and W. Zillig, unpublished). In the reverse experiment, antibodies raised against single components of RNA polymerase of *S. acidocaldarius* cross-react with components of polymerase A of yeast (R. Schnabel, unpublished).

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