DIFFERENTIAL STAINING OF THE SUBUNITS OF DNA-DEPENDENT RNA POLYMERASE

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1. Introduction

Among the bacterial RNA polymerases, the one isolated from *Escherichia coli* has been studied most extensively. The enzyme is composed of 5 subunits: the subunits β' and β, two non-identical peptide chains of high M_r (160 000 and 150 000, respectively) and two copies of the much smaller subunit α (36 500 M_r) are tightly aggregated to form the core enzyme ($\beta'\beta 2\alpha$). The δ-factor (82 000 M_r) is associated with the core enzyme [1].

The RNA polymerases of other bacterial genera show a similar subunit composition, but differences in the molecular weights of the subunits are apparent. Since, on the one hand, the isolated subunits do not catalyze any specific reaction and, on the other hand, enzyme preparations are often contaminated with persistent unidentified proteins it is difficult to decide which protein band represents which polymerase subunit. Therefore it would be highly desirable to find a method which allows the identification of the polymerase subunits quickly without lengthy experimentation. Using RNA polymerase as a size marker in a search for glycoproteins, we found that the subunits of the polymerase were stained differently when they are separated on a polyacrylamide gel and exposed to a carbocyanine dye.

2. Materials and methods

RNA polymerases of *E. coli* and *S. marcescens* were isolated in this laboratory according to [1,2], the *B. stearothermophilus* enzyme was isolated as in [3]. RNA polymerase of *Micrococcus luteus* was a gift of G. Hartmann (Munich). RNase, DNase, nuclease (*St. aureus*) snake venom phosphodiesterase and neuraminidase were purchased from Boehringer (Mannheim), α and β amylase, bovine hyaluronidase from Serva (Heidelberg) and β-1,3-glucanase from Novo Industrias (Copenhagen).

Polyacrylamide electrophoresis was performed according to [4]. The polymerase subunits were separated on 1.5 mm gradient gels (16 × 12 cm). The 5% and 10% acrylamide components were mixed in a gradient mixer in a cold room and poured continuously into the gel form. Electrophoresis was performed in a buffer containing 25 mM Tris-base, 192 mM glycine and 0.1% sodiumdodecyl sulfate (SDS) at 22 mA. To fix the proteins and to remove the SDS after electrophoresis, the gels were kept overnight in 25% isopropanol (SDS interferes with the coloration). The gels were then stained for 2 h in 0.005% 4,5,4',5'-dibenzo-3,3'-diethyl-9-methyl-thiacarbocyanine bromide ('Stains All', Serva) in 50% formamide. They were destained in running tap water. Staining and destaining was carried out in the dark, as intense light destroys the stain entirely; gels can then be restained either with 'Stains All' as described above or with Coomassie blue. The second staining with 'Stains All' often brings more brilliant coloration. The channels were cut out and scanned in a 1 cm cuvette of a Gilford spectrophotometer 2400 at 600 nm and 535 nm to register the blue and the red bands, respectively.

3. Results and discussion

The carbocyanine dye 'Stains All' interacts with a number of macromolecules of biological origin. The binding of the dye molecules to proteins, nucleic acids and other materials allegedly occurs via multiple interactions in orderly arrays, which results in the appearance of new absorption maxima as compared to the

absorption maximum of the dye monomer [5–7]. Thus RNA is stained bluish purple, DNA blue, proteins red, lipoproteins yellow and phosphoproteins as well as glycoproteins blue. Several workers took advantage of this dye to trace specific biological materials in solution [8], in histological tissues [9] and in polyacrylamide gels [10]. Staining polyacrylamide gels with this carbocyanine dye in order to trace the blue bands of gonadotropins which are glycoproteins, we were surprised to find that the δ subunit of RNA polymerase which we used as a size marker, also appeared as a deep blue band. Moreover the β subunit appeared bluish-purple while the subunits β′ and α stained red as expected for proteins. This differential staining was not only observed in a single enzyme preparation: *E. coli* RNA polymerase purchased from Boehringer and RNA polymerase isolated from other sources such as from *S. marcescens, M. luteus* and *B. stearothermophilus* showed an identical coloration of their subunits. This differential staining can be shown when the gels are scanned at 535 nm and at 600 nm (fig.1). At 535 nm the absorption pattern of the subunits is very similar to the pattern which is obtained when the subunits are stained with coomassie blue, i.e., β′ and β absorb approximately equal amounts of light and δ and α as the smaller subunits bind less dye resulting in lower light absorption. At 600 nm, however, the β′ subunit which is stained red, clearly absorbs less light than the bluish-violet β subunit. The blue δ band absorbs relatively more light at 600 nm than at 535. A comparison of the scans obtained from the *B. stearothermophilus* enzymes with those of the other RNA polymerases reveals that in *B. stearothermophilus* the β polypeptide is larger than the β′ subunit. This observation is confirmed by reconstitution experiments where the subunits of the RNA polymerases obtained from *B. stearothermophilus* and from *E. coli* were mutually exchanged [11].

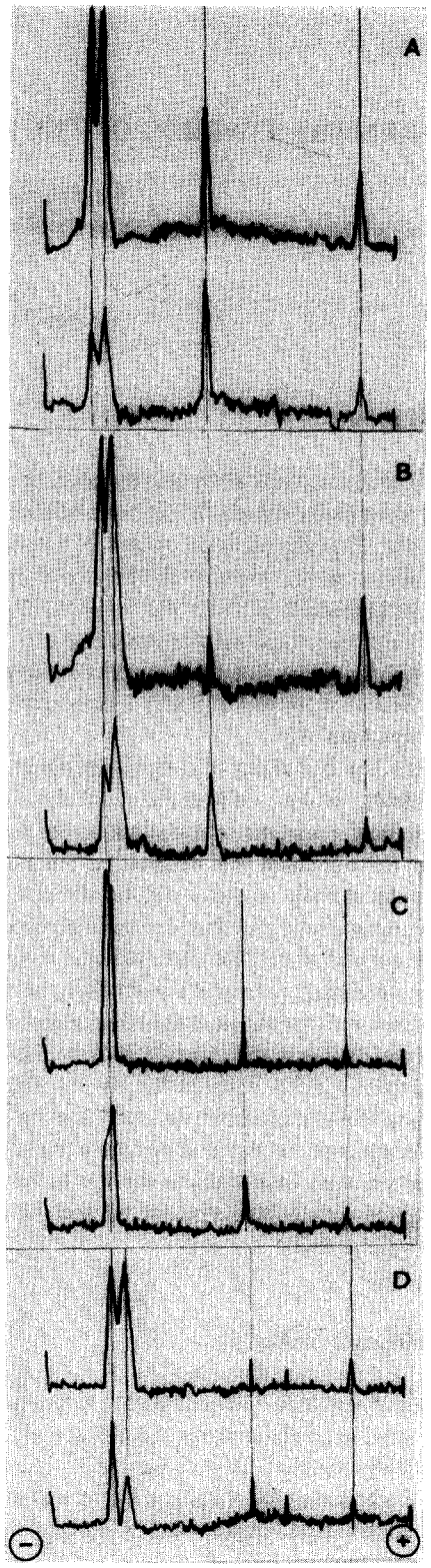

Fig.1. The subunits of ~5 μg of the RNA polymerases isolated from *E. coli* (A), *S. marcescens* (B), *M. luteus* (C) and *B. stearothermophilus* (D) were separated on a polyacrylamide gradient gel. The enzyme subunits were stained with the carbocyanine dye 'Stains All'. Each channel was scanned at 535 nm (top) and at 600 nm (bottom) to register the red, blue violet and the blue bands, The absorption maxima in (A) and (B) represent from left to right the subunits β′β, δ and α. In (C) β′ and β are not entirely separated since their molecular weights are similar to each other. In (D) the sequence of the subunits is β, β′, δ and α. For a more detailed explanation see text.

The question as to why the subunits of RNA polymerase are stained differently can not be answered from our experiments. It is known that phospho- and glycoproteins as well as DNA are stained blue; therefore one could assume that the δ subunit retains residual DNA or that it might carry glyco- or phosphoryl groups. The latter assumption would not be entirely out of place since the phosphorylation of the α-subunit at about 4 min after infection of *E. coli* with phage T4 was reported to be a regulatory event in the transcription of phage DNA [12]. This phosphorylation was identified as an ADP-ribosylation and was confirmed by the sequence analysis of the α subunit [13,14]. We have incubated RNA polymerase with 1 μg each of RNase, DNase, nuclease (*St. aureus*), snake venom phosphodiesterase, α and β amylase, glucanase, hyaluronidase and neuraminidase for 10 min at 37°C prior to gel electrophoresis. No changes in the staining pattern were observed after these treatments (not shown).

However, we have observed that proteins may stain blue when they are precipitated with trichloroacetic acid and neutralized prior to gel electrophoresis, indicating that the reaction with 'Stains All' is sensitive to the conformational state of a polypeptide and/or to its ionic environment. The amino acid sequence analysis seems to be necessary in order to decide whether the RNA polymerase subunit δ is a pure protein or whether it carries additional chemical groups.

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