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POLYAMINE EFFECTS ON THE STABILITY OF DNA-ACTINOMYCIN D COMPLEX

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SUMMARY: Simultaneous interactions of spermidine and actinomycin D on the DNA molecule were measured in vitro by means of the variations in the absorption spectrum of the actinomycin D- DNA complex after addition of polyamines. Putrescine did not show detectable effects, spermidine produced a small hyperchromism and spermine a marked hyperchromism. The separation of the actinomycin D-DNA complex labelled with [^{3}H] actinomycin by filtration trough Sephadex columns, shows a great decrease of relative radioactivity bound to DNA when spermine is added to the medium.

INTRODUCTION

Polyamines take part in many important metabolic processes in prokaryotes and eukaryotes (1,2). In particular they are involved in the mechanisms of nucleic acid and protein synthesis (3,4,5). Their action mainly depends on their ability to form complexes with nucleic acids, stabilizing them in their natural conformation (6,7,8,9,10). Polyamines may be substituted by bivalent cations such as Mg^{2+} (for example in binding the two ribosomal subunits), but bivalent cations do not have the same efficiency of polyamines in binding to DNA and RNA. In various growing tissues polyamine synthesis accompanies or precedes the nucleic acid synthesis (11, 12). The increased synthesis of RNA also depends on the stimulating effect of polyamines on RNA polymerases. On the contrary actinomycin D (AMD) is a very powerful antibiotic that forms a complex with p-dG-dC sequences of DNA, according to Jain and

Abbreviation: AMD, actinomycin D.

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Sobell model (13,14,15), not permitting the elongation of the growing RNA chains (16). A previous work (17) showed that the inhibition of DNA and RNA synthesis due to 1.5 μM AMD in activated slices of tubers of Helianthus tuberosus was removed by 100 μM spermidine administered either at the same time or after five hours. In order to verify if this effect depends on interactions of AMD and polyamines on the DNA molecule, we have studied the formation of the DNA-AMD complex in vitro in the presence of various polyamines.

MATERIALS AND METHODS

Actinomycin D was a gift of Merck, Sharp and Dohme; [^{3}H] actinomycin D (7.9 Ci/mmol) was obtained from The Radiochemical Centre, Amersham; high molecular weight DNA of Micrococcus lysodeikticus (73% G+C) was purchased from Sigma; putrescine-dihydrochloride, spermidine-trihydrochloride and spermine-tetrahydrochloride from Fluka. All solutions were prepared with a 0.01 M Na-phosphate buffer (pH 7). Spectrophotometric measurements were carried out with a Varian Techtron and a Cary 15 spectrophotometer by adding into 1 ml of 14.4 μM or 17.6 μM actinomycin D, 40 μl of 1 g/l DNA then 10 μl of 10 mM polyamine and by measuring the absorption after each addition; dilution effects have been taken into account. Reported data refer to 17.6 μM actinomycin.

Prepacked columns PD-10 containing 9.1 ml Sephadex G-25 medium were used for gel filtration. The solutions had the same concentrations of those prepared for spectrophotometric analyses, but only spermine was used. The presence of 5 μCi [^{3}H] actinomycin D (approximately 630 pmol) did not practically change the concentration of actinomycin D. The volume of each fraction was 0.5 ml

RESULTS

The spectrum of DNA-ADM complex changes significantly when 10.0 μl of 10 mM spermine are added to 1.0 ml of a solution containing AMD and DNA of M. lysodeikticus obtaining a 100 uM polyamine final concentration (Fig. 1). The spectrum presents an increase in absorption in the blue region of the spectrum (13 per cent at 425 nm) and a small decrease in the red region. Spermidine produces the same effects, but to a lesser extent

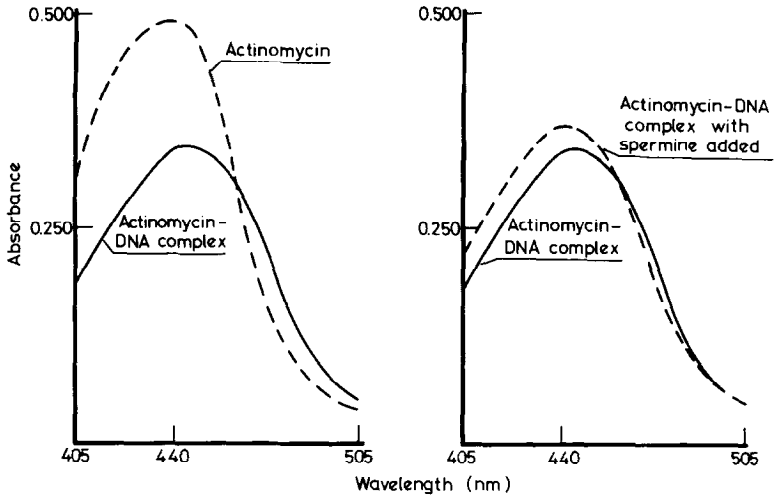

Figure 1. Left: comparison between the absorption spectra of 1 ml of 17.6 μM AMD before and after the addition of 40 μg DNA.
Right: Comparison between the absorption spectra of the above AMD-DNA solution after the addition of 100 nmol of spermine.

(7 per cent at 425 nm); putrescine evidences no effects, in the concentrations used. The isolation of the complex labelled with [^{3}H] AMD through Sephadex, shows a marked decrease (about 18%) of the relative amount of AMD bound to DNA, in the presence of spermine (Fig. 2). M. lysodeikticus DNA is eluted in exclusion volume in the fractions from 1 to 12, but the relative amount of DNA in the fractions 11 and 12 is less than 0.5 per cent of the total DNA; so we can calculate the percentage of free and bound AMD with the equations:

$$(1) \quad AMD_f = \frac{1 - \sum_{1}^{10} i \, R_i^{DNA}}{1 - \sum_{1}^{10} i \, R_i} \quad ; \quad (2) \quad AMD_b = 1 - AMD_f$$

where AMD_f and AMD_b are respectively the percentages of free and bound AMD, R_i^{DNA} and R_i are the relative radioactivity in

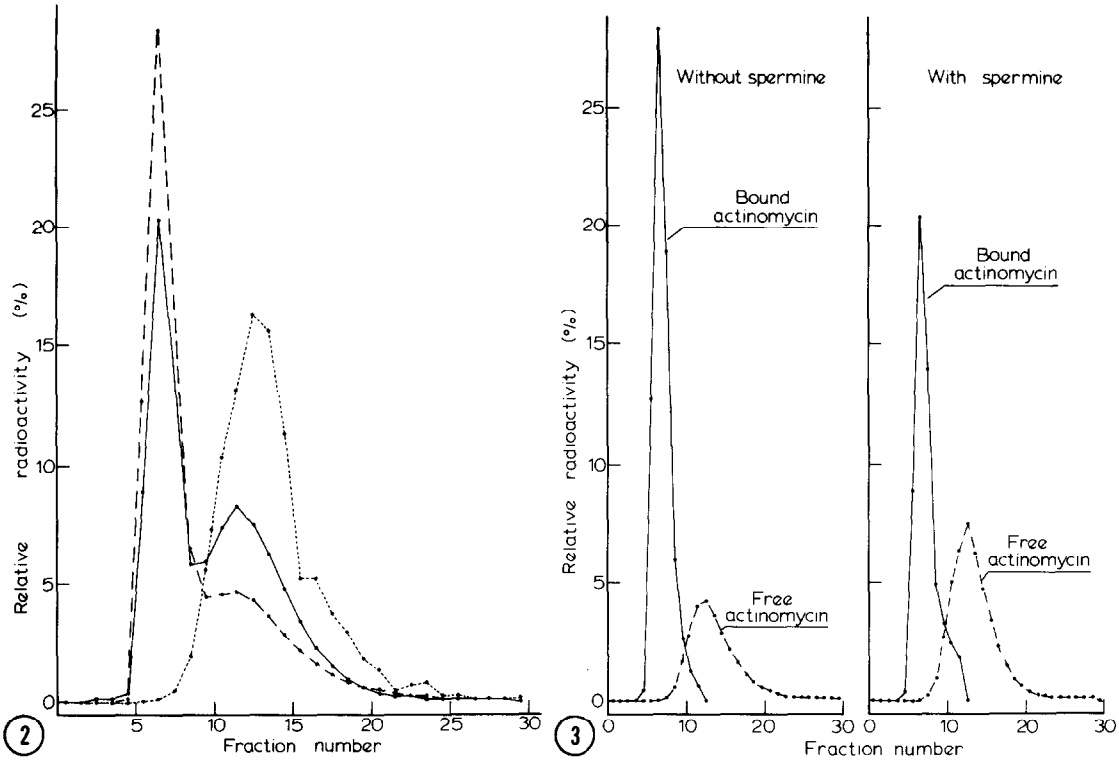

Figure 2. Radioactivity distribution through Sephadex G 25. Dotted line: 1 ml of 14.4 μM AMD; interrupted line: 1 ml of 14.4 μM AMD with 40 μg DNA; solid line: AMD with DNA (at the same concentrations) with 100 nmol spermine.

Figure 3. Distribution of free and bound AMD according to equations (1) and (2) (1 ml of 14.4 μM AMD with 40 μg DNA, with or without 100 nmol of spermine).

each fraction in the presence and in the absence of DNA and 10 is the number of the fractions used in the integration.

From the data of figure 2 we obtain $AMD_f = 0.31$ and $AMD_b = 0.69$ in the absence of spermine and $AMD_f = 0.49$, $AMD_b = 0.51$ in its presence.

The apparent binding constant is approximately evaluated with the equation:

$$(3) \quad K_{app} = \frac{AMD_b}{[SITES_T] \cdot AMD_f - [AMD_T] \cdot AMD_b \cdot AMD_f}$$

where $SITES_T$ and AMD_T are respectively the total concentration of DNA binding sites and the total concentration of AMD.

The values of K_{app} are $1.8 \cdot 10^6$ in the absence of spermine and $2.8 \cdot 10^5$ in its presence. Figure 3 shows the distribution of bound and free AMD.

DISCUSSION

The concentration of polyamines used in this work (100 μM) is the same as the one used in the previous *in vivo* work (17).

In spite of the fact that changes in the DNA-AMD complex spectrum induced by spermine or spermidine are quantitatively small, they are qualitatively significant, as polyamines induce contrary effects on the spectrum of AMD in comparison with those induced by DNA. This feature may be interpreted as a reduction of the binding affinity of AMD to DNA when polyamines are involved. The Sephadex separation confirms this hypothesis. The K_{app} is of the same magnitude as that found in previous works (18,19) and it is lowered 6.4 times by 100 uM spermine. The addition of polyamines in our conditions should not significantly affect the ionic strength, the increase of which could reduce the binding affinity of AMD to DNA (18,19).

These effects may be tentatively interpreted as follows:

1) spermine (and spermidine), having a binding constant to DNA of 10^4, can interfere with AMD binding, causing a steric impediment.
2) polyamines induce a DNA conformation that binds AMD less efficiently.

The present *in vitro* data is the first support to the ones obtained *in vivo* (17) on the reversal of the inhibition of RNA synthesis due to AMD caused by spermidine.

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