Actinomycin D-binding in vivo: active chromatin preferred

Fu-Li Yu

Department of Biomedical Sciences, University of Illinois, College of Medicine at Rockford, 1601 Parkview Avenue, Rockford, IL 61107, USA

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Escherichia coli RNA polymerase and the endogenous engaged RNA polymerase I were used as specific probes to monitor the physiologically inactive and active nucleolar chromatin template function, respectively. Actinomycin D bound preferentially to the physiologically active regions of rat liver nucleolar chromatin in vivo.

Actinomycin D *Chemical carcinogen* *Nucleolar chromatin* *Nucleolar DNA*
RNA polymerase I *RNA polymerase, of* E. coli

1. INTRODUCTION

Actinomycin D, a widely used chemotherapeutic agent [1] and also a carcinogen [2], is a potent inhibitor of DNA-dependent RNA synthesis [3,4]. The mechanism of actinomycin D-binding to DNA is well studied [5,6], it is clear that with the naturally occurring DNA duplex, deoxyguanosine residues in the DNA are important for the binding. Since the distribution of deoxyguanosine residues in a given cellular DNA is quite general, it is believed that the interaction of actinomycin D with the DNA is non-selective. However, under normal physiological conditions in vivo, DNA is complexed with chromosomal proteins and organized into transcriptionally active and inactive chromatin [7,8]. The question is whether actinomycin D interacts randomly or selectively with chromatin under these physiological conditions in vivo. To answer this question specific probes are needed. RNA polymerase of *Escherichia coli* could transcribe mammalian chromatin, but at random sites [9–11]. This bacterial enzyme can transcribe the physiologically inactive regions of rat liver nucleolar chromatin [12]. The endogenous engaged RNA polymerase I [13,14] transcribes only the physiologically active nucleolar chromatin coding for ribosomal RNA [10,15,16]. Thus, these two enzymes, one of which transcribes chromatin randomly and the other transcribes only the active genes, may be used as specific probes to monitor the binding of chemical carcinogens to chromatin whether it is random or selective. This paper reports that actinomycin D binds preferentially to the physiologically active regions of rat liver nucleolar chromatin in vivo.

2. MATERIALS AND METHODS

2.1. *Actinomycin D administration in vivo*

Male Sprague–Dawley rats (~200 g body wt) were divided into groups of 3 animals. Actinomycin D (0.5 mg/ml H_2O) was injected intraperitoneally at doses of 50, 100, 250, 500 and 1000 μg/100 g body wt, and the animals were killed 30 min later.

2.2. *Isolation of rat liver nucleoli, nucleolar DNA and RNA polymerase I*

The procedure was as in [17.

2.3. *Assay of rat liver nucleolar chromatin template function with endogenous-engaged RNA polymerase I and* E. coli *RNA polymerase after actinomycin D administration in vivo*

The assay conditions for RNA polymerase activities were essentially the same as in [17]. Briefly,

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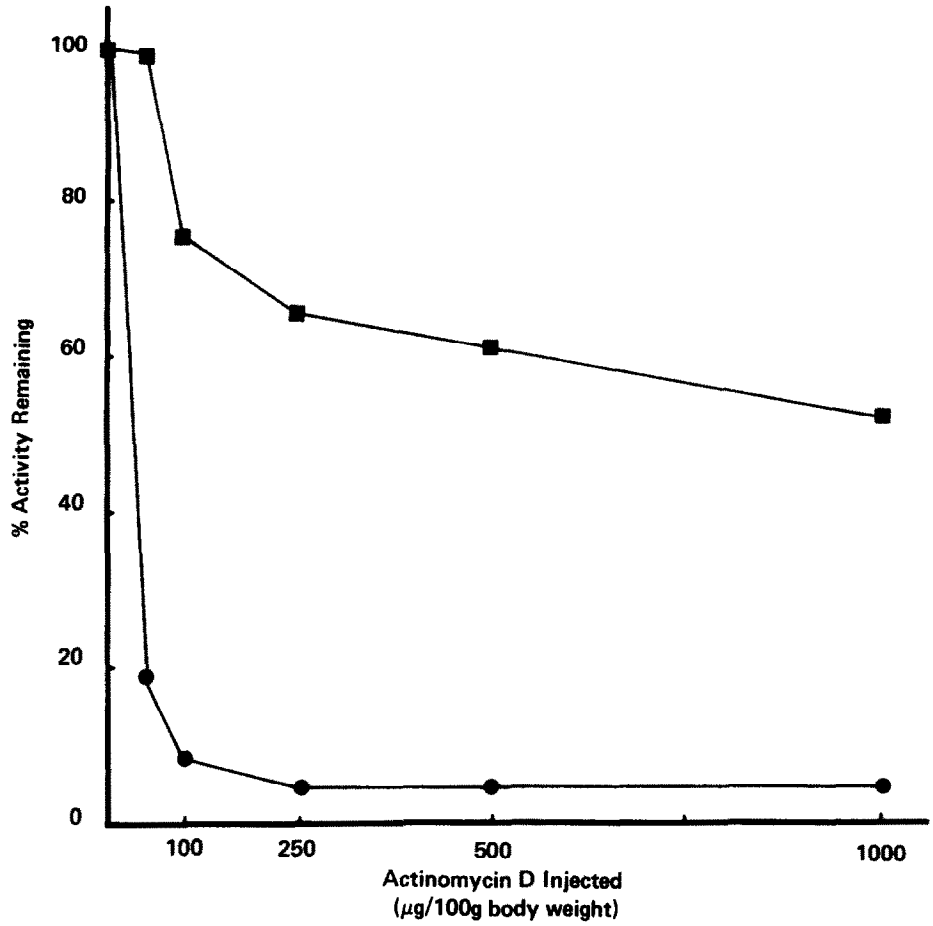

Fig.1. Effect of actinomycin D administered in vivo on rat liver nucleolar chromatin template function measured with the endogenous-engaged RNA polymerase I and RNA polymerase of *E. coli*. Male Sprague–Dawley rats (~200 g body wt) were divided into groups of 3 animals. Actinomycin D at doses of 50, 100, 250, 500 and 1000 μg/100 g body wt was given intraperitoneally 30 min before sacrificing. Liver nucleoli were isolated as in [17]. The template efficiency of nucleolar chromatin (10–15 μg DNA) in supporting RNA synthesis was measured at 37°C for 20 min under standard RNA assay conditions [17] in the absence or presence of 3 units of RNA polymerase of *E. coli* (Worthington). Net *E. coli* RNA polymerase activity (■) is calculated by subtracting the corresponding endogenous-engaged RNA polymerase I activity (●) from each group. Control values (100%) for RNA polymerase I and RNA polymerase of *E. coli* activities were 10345 and 17704 pmol [^{3}H]GMP incorporated/mg DNA, respectively. Data presented are average values from 3 separate expt.

the assay was carried out in vitro with 0.2 ml standard assay medium (medium A) containing: 100 mM of Tris–HCl (pH 7.9 at 23°C), 2 mM $MnCl_2$, 28 mM 2-mercaptoethanol, 70 mM $(NH_4)_2SO_4$, and 0.2 mM each of ATP, GTP, UTP and CTP with 4 μCi [8-^{3}H]GTP (Amersham; spec. act. 10–20 Ci/mmol). The reaction was initiated by the addition of 0.1 ml nucleolar suspension from either control or actinomycin D-treated sources (fig.1) and in the absence or presence of 3 units of RNA polymerase of *E. coli* (holoenzyme, Worthington). The reaction mixture was incubated at 37°C for 20 min with shaking. All the assays were stopped by transfer of the reaction tubes (12 × 75 mm) to chipped ice, followed by immediate addition of 5 ml cold 10% trichloroacetic acid containing 1% sodium pyrophosphate. The acid-insoluble material was collected on Whatman

GF/C filters and washed 7-times with 5 ml 5% cold trichloroacetic acid containing 1% sodium pyrophosphate, and twice with 5 ml 60% ethanol. The filters were air-dried in liquid scintillation vials and the radioactivity was counted in 5 ml Bray's solution [18]. The specific activity of RNA polymerase was expressed as pmol [^{3}H]GMP incorporated/mg DNA. DNA concentrations were determined as in [19].

2.4. *Assay of rat liver nucleolar chromatin template function with endogenous engaged RNA polymerase I and RNA polymerase of* E. coli *after actinomycin D-binding in vitro*

The activities of RNA polymerase were assayed the same way as above except that actinomycin D at various concentrations (fig.2) was added to the 0.2 ml medium A at zero time in the absence or presence of 3 units of RNA polymerase of *E. coli*. RNA synthesis was assayed at 37°C for 20 min. The assays were stopped, radioactive samples were processed and counted as above.

2.5. *Assay of purified rat liver nucleolar DNA template function with solubilized RNA polymerase I and RNA polymerase of* E. coli *in the presence of actinomycin D*

Actinomycin D at the concentrations indicated (fig.3) was added to 0.2 ml medium A containing 3 μg purified nucleolar DNA at zero time, and the DNA template function was measured separately for RNA synthesis at 37°C for 20 min under standard RNA assay conditions as above, with either 3 units of RNA polymerase of *E. coli* or solubilized RNA polymerase I.

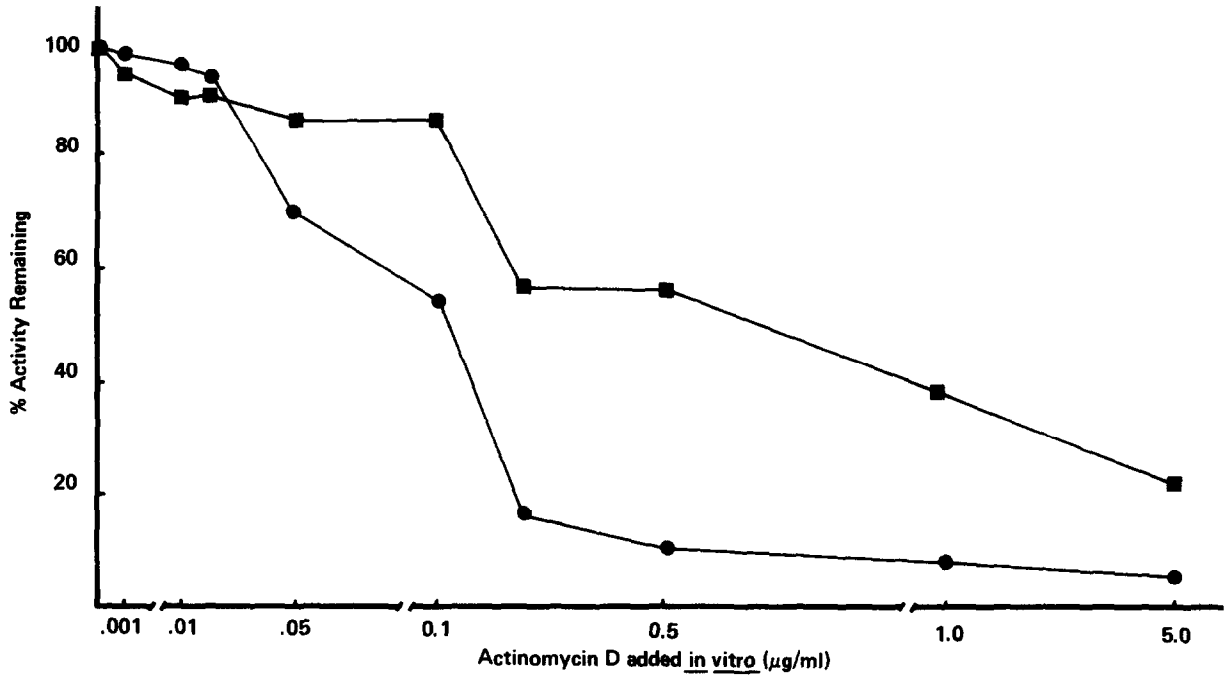

Fig.2. Effect of actinomycin D added in vitro on rat liver nucleolar chromatin template function measured with the endogenous-engaged RNA polymerase I and RNA polymerase of *E. coli*. Rat liver nucleoli were isolated as in [17]. Actinomycin D at the concentrations indicated was added to nucleolar suspensions (10–15 μg DNA) at zero time, and the chromatin template function was measured for RNA synthesis at 37°C for 20 min under standard RNA assay conditions [17] in the absence or presence of 3 units of RNA polymerase of *E. coli* as in fig.1. (■) RNA polymerase activity of *E. coli*; (●) endogenous-engaged RNA polymerase I activity. Control values (100%) for RNA polymerase I and RNA polymerase of *E. coli* activities were 13 360 and 12 778 pmol [^{3}H]GMP incorporated/mg DNA, respectively. Data presented are average values from 2 separate expt.

3. RESULTS AND DISCUSSION

Fig.1 shows that when rats were given intraperitoneal injections of actinomycin D at 50–1000 μg/100 g body wt 30 min prior to sacrifice, the endogenous-engaged RNA polymerase I activity (○—○) is rapidly inhibited. At 50 μg actinomycin D this inhibition is 80%; at ⩾100 μg the inhibition is >90%. On the other hand, transcription of nucleolar chromatin by RNA polymerase of *E. coli* (□—□) is not inhibited at 50 μg actinomycin D and only 20% inhibition at 100 μg. Even at 1000 μg the nucleolar chromatin template still retains close to 50% of its original capacity for RNA polymerase of *E. coli* transcription. Since the inhibition of the engaged RNA polymerase I activity represents the binding of actinomycin D to the active nucleolar chromatin [10,15,16], and the inhibition of RNA polymerase of *E. coli* reflects the non-selective binding of actinomycin D to the general nucleolar chromatin [9–12], these data suggest that at low concentrations actinomycin D binds preferentially to the active regions of the nucleolar chromatin. At higher concentrations, it starts to bind the inactive regions of the nucleolar chromatin as the preferred binding sites in the active regions become saturated. However, these data alone do not rule out the possibility that the binding of actinomycin D still could be random if RNA polymerase of *E. coli* were intrinsically insensitive to actinomycin D inhibition.

We next investigated the binding property of actinomycin D when it was added in vitro to the isolated rat liver nucleoli. Fig.2 shows there is still a differential inhibtion of the endogenous engaged RNA polymerase I activity (○—○) over that of the RNA polymerase activity of *E. coli* (□—□)

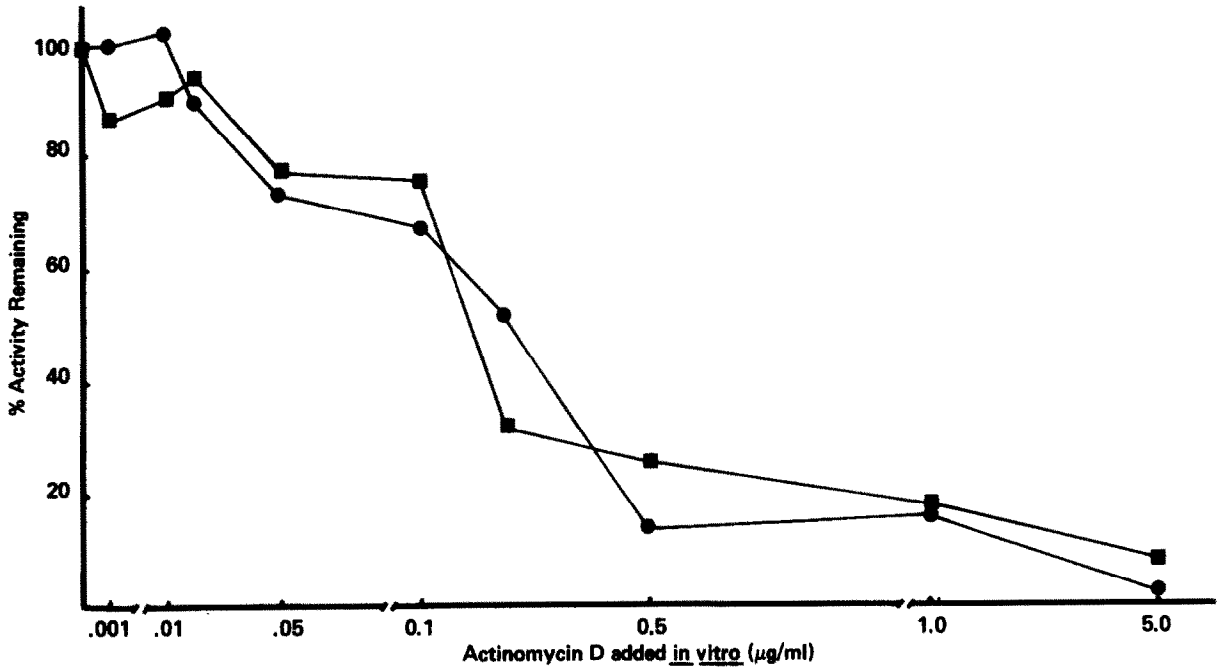

Fig.3. Effect of actinomycin D added in vitro on rat liver nucleolar DNA template function measured with solubilized-engaged RNA polymerase I and RNA polymerase of *E. coli*. Rat liver nucleolar DNA was isolated as in [17]. Actinomycin D at the concentrations indicated was added to nucleolar DNA (3 μg) suspensions at zero time, and the DNA template function was measured separately for RNA synthesis at 37°C for 20 min under standard RNA assay conditions with either 3 units of RNA polymerase of *E. coli* or solubilized RNA polymerase I. (■) RNA polymerase activity of *E. coli*; (●) solubilized RNA polymerase I activity. Control values (100%) for RNA polymerase I and RNA polymerase of *E. coli* activities were 7608 and 453500 pmol [^{3}H]GMP incorporated/mg DNA, respectively. Data presented are average values from 2 separate expt.

over 0.001 μg–5 μg/ml. For example, at 0.1 μg/ml of actinomycin D, RNA polymerase I activity is inhibited 46%, while RNA polymerase activity of *E. coli* is inhibited only 14%. At 0.5 μg/ml, there is 90% inhibition of RNA polymerase I activity, and only 44% inhibition of *E. coli* enzyme activity. These data indicate that although the binding selectivity is not as marked as with in vivo administration of actinomycin D (fig.1), there is still a preferred binding by actinomycin D to the physiologically active regions of the nucleolar chromatin. Again, RNA polymerase of *E. coli* may be intrinsically less sensitive to actinomycin D inhibition.

The evidence that RNA polymerase of *E. coli* is equally sensitive to actinomycin D inhibition as RNA polymerase I is provided by the use of purified nucleolar DNA. Fig.3 shows that when actinomycin D at 0.001–5 μg/ml is added to purified rat liver nucleolar DNA, the activities of both enzymes are now equally inhibited by actinomycin D throughout the concentrations tested. Thus, the data shown in fig.1 are true indications that actinomycin D binds preferentially to the physiologically active regions of nucleolar chromatin in vivo, and that this binding specificity is partially lost when actinomycin D is added to the isolated nucleoli in vitro (fig.2).

This result is similar to preferential DNase I digestion at the active genes of nucleolar chromatin [12,20–23]. It also supports the concept [7] that chromosomal proteins are important regulatory elements conferring additional features that distinguish the active genes from the inactive ones.

This simple method could be used to study the binding specificity of various chromatin- or DNA-reacting agents; e.g., nucleases, hormones and chemical carcinogens. Using this method we have shown [24] that aflatoxin B_1 also preferentially binds to the physiologically active regions of nucleolar chromatin in vivo.

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