Inhibition of isolated rat liver RNA polymerases I and II by aminoacridines

A. Zoncheddu, R. Accomando, E. Calendi[1] and M. Orunesu

Istituto di Fisiologia Generale, Università di Genova, Corso Europa, I-16132 Genova (Italy), 2 January 1980

Summary. 9-Aminoacridine and 2 derivatives which contain hydroxyalkylic or aminoalkylic side chains in the 9-position totally inhibit the transcription of calf thymus DNA by rat liver RNA polymerases I and II. This inhibitory action does not always appear to be completely related to the ability of aminoacridines to intercalate into the DNA template.

Among the widespread biological effects of acridines the inhibition of RNA synthesis is certainly one of the most evident[2]. The well-known ability of these planar heterocyclic compounds to bind strongly to DNA[3] by a process which is believed to involve intercalation between adjacent base pairs of the DNA double helix[4] provides one of the conceivable mechanisms by which RNA synthesis may be impaired. Actually, several acridine derivatives, especially aminoacridines, have been shown to prevent the transcription of a number of natural or synthetic templates by bacterial[5,6] and viral[7] RNA polymerases. As far as we know, however, this evidence is lacking for the eukaryotic RNA-synthesizing enzymes, though studies on the inhibition of RNA synthesis in vivo by acridine derivatives support the conclusion that DNA is also a probable target for these compounds in higher organisms[8,9].

The present paper deals with the effect of some aminoacridines on the transcription of calf thymus DNA by isolated rat liver RNA polymerases I and II, the 2 major nuclear RNA-synthesizing enzymes responsible in vivo for the transcription of ribosomal and messenger RNA coding sequences, respectively[10]. The compounds tested were 9-aminoacridine and 2 derivatives which contain hydroxyalkylic or aminoalkylic side chains in the 9-position[11].

Materials and methods. 9-Aminoacridine hydrochloride was the product of ICN Pharmaceuticals. 9-(3′-hydroxypropylamino)Acridine hydrochloride (derivative 1) and 9-(3′-diethylaminopropylamino)Acridine dihydrochloride (derivative 2) were synthesized at the Institute of Pharmaceutical Chemistry, University of Sassari[11]. Highly polymerized calf thymus DNA (Sigma) was used throughout the experiments. RNA polymerases I and II were isolated from rat liver nuclei[12] by the high-salt sonication procedure of Roeder and Rutter[13] as modified by Schwartz et al.[14]. The 2 enzymes were separated by DEAE-Sephadex A-25 chromatography[13], concentrated by ammonium sulfate precipitation and stored at −80 °C.

Results and discussion. Figure 1 shows that increasing amounts of all 3 aminoacridines tested gradually and completely inhibit the transcription of calf thymus DNA by isolated rat liver RNA polymerases I (panel A) and II (panel B). On a molar basis the inhibitory activities were in the order derivative 2 > derivative 1 > 9-aminoacridine. Still on a molar basis, all 3 aminoacridines appear to inhibit RNA polymerase I more markedly than RNA polymerase II.

Figure 2 shows the binding curves of the 3 aminoacridines to the calf thymus DNA used as a template for RNA polymerases I and II from rat liver. Such curves, i.e. a plot of the amount (r) of aminoacridine bound per mole of DNA nucleotides against the concentration (c) of free unbound aminoacridine, show the characteristic plateau region occurring at a value of r which represents the maximum extent of strong binding[3], i.e. intercalation. This value was approximately 0.24 for derivative 2, 0.23 for 9-aminoacridine and 0.20 for derivative 1 when determined under the ionic conditions used to assay RNA polymerase I activity (panel A). Under the ionic conditions used to assay RNA polymerase II activity maximum values of r decreased to approximately 0.20 for derivative 2, 0.19 for 9-aminoacridine and 0.17 for derivative 1 (panel B).

At this point attempts were made to interpret the inhibitory action of the 3 aminoacridines in terms of the amount of drug actually bound to the DNA template. To achieve this, the values of r for the particular total aminoacridine concentrations used in the enzyme inhibition experiments were calculated using the relation: $T_L = T_A \cdot r + c$, where T_L and T_A are the total concentration of aminoacridine and DNA, respectively. By substituting corresponding pairs of r and c values from figure 2 into the above relation and with T_A equal to that in the enzyme assay, a series of T_L values was obtained and plotted against r. This graph enabled r to be evaluated for every total aminoacridine concentration[5]. The variation of enzyme inhibition with r, evaluated by using the data of figure 1 as well as other data from similar experiments performed with different DNA and aminoacridine concentrations, is shown in figure 3. With regard to the activity of RNA polymerase I (panel A), it has been found

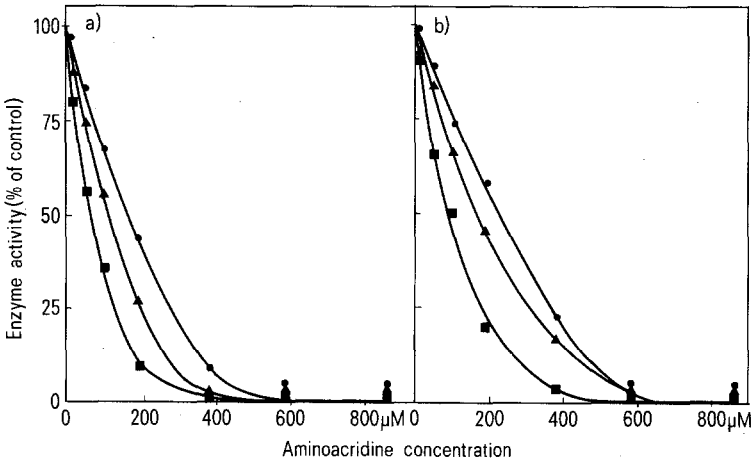

Fig. 1. Inhibition of rat liver RNA polymerase I (panel A) and II (panel B) by 9-aminoacridine (●—●), derivative 1 (▲—▲) and derivative 2 (■—■). The standard assay mixture contained: 50 mM Tris-HCl pH 7.9; 6 mM NaF; 7 mM dithiothreitol; 0.9 mM ATP, GTP, CTP and 0.045 mM UTP; 5–10 μCi ^{3}H-UTP (10 Ci/mmole, The Radiochemical Centre); 25 μg calf thymus DNA; 40 μg enzyme preparation. Assay for RNA polymerase I contained in addition 0.04 M $(NH_4)_2SO_4$ and 6 mM $MgSO_4$; RNA polymerase II assays contained 0.14 M $(NH_4)_2SO_4$ and 2 mM $MnCl_2$. Aminoacridines (20 μl) were present at the final concentration indicated in the legend to the figures. After incubation for 20 min at 30 °C the assay mixtures (0.1 ml) were spotted onto Whatman GF/C glass fibre filters which were then processed and counted for radioactivity as described elsewhere[15].

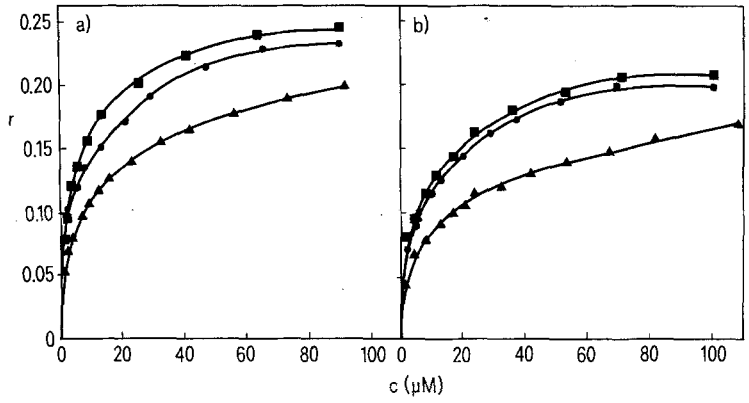

Fig. 2. Binding curves of 9-aminoacridine (●—●), derivative 1 (▲—▲) and derivative 2 (■—■) on calf thymus DNA determined under the ionic conditions used in the assay of RNA polymerase I (panel A) and II (panel B). Assays were performed according to the spectrophotometric method of Peacocke and Skerett[3] based on the change of spectrum of the acridines which occurs when they are strongly bound to DNA. The wavelength chosen for measurements was the one at which the largest change in optical density occurred on binding, namely 397 nm for 9-aminoacridine, 408 nm for derivative 1 and 410 nm for derivative 2.

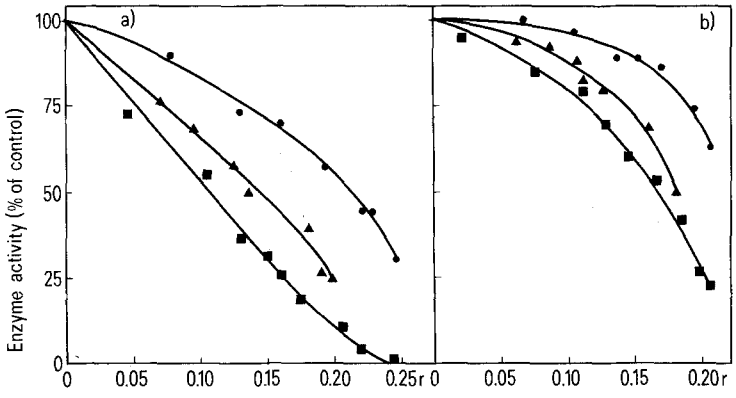

Fig. 3. Inhibition of rat liver RNA polymerase I (panel A) and II (panel B) as a function of the amount of 9-aminoacridine (●—●), derivative 1 (▲—▲) and derivative 2 (■—■) bound to the DNA template. Aminoacridines were present at the different concentrations giving the indicated values of r.

that at the values of r corresponding to the completion of binding of each aminoacridine to DNA inhibition of 100%, 75% and 70% was attained with derivative 2, derivative 1 and 9-aminoacridine, respectively. With RNA polymerase II (panel B) the completion of binding of the 3 aminoacridines was associated with a lower degree of enzyme inhibition which was about 75%, 50% and 35% in the case of derivative 2, derivative 1 and 9-aminoacridine, respectively. From these figures it is clear that the contribution to enzyme inhibition given by the binding to DNA of all 3 drugs is more significant for RNA polymerase I than for RNA polymerase II. This fact may be consistent with the greater extent of binding attainable under the ionic conditions used to assay RNA polymerase I. Similarly, the different contributions given to the inhibition of both enzymes by the interaction with DNA of derivative 2 and 1 agree reasonably well with their relative extent of binding. The different behaviour of 9-aminoacridine is, however, worth mentioning as its relatively great extent of binding to DNA gives the smallest contribution to enzyme inhibition. This may indicate that the functional alteration of the DNA template is not simply related to the mere amount of the bound acridines but also to the chemical nature of the intercalated molecules.

A critical point which clearly emerges from the above data is that the completion of strong binding of the 3 aminoacridines to the DNA template is not always associated with the total enzyme inhibition attainable at higher concentrations of the drugs (figure 1). This indicates that the inhibitory action of aminoacridines may involve additional mechanisms other than their intercalation into DNA. Among them, the weaker, external binding of the drugs to DNA predominating at higher r values[16] and/or the interaction with the enzymes themselves or the substrate nucleoside triphosphates[5] are conceivable possibilities.

In conclusion, evidence is presented for the first time that aminoacridines interfere with the activity of isolated eukaryotic DNA-dependent RNA polymerases. Clearly, further studies are required to answer the many questions raised by this observation.

1 Istituto di Genetica, Università di Sassari, Viale Mancini 5, 07100 Sassari, Italy.

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