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Effect of proflavine and its 2,7-dialkyl substituted derivatives upon initiation of transcription*

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It is generally accepted that inhibition of RNA synthesis *in vitro* by acridines and related fused aromatic heterocycles depends on their intercalation to DNA. However, except of our previous study on total RNA synthesis with bacterial DNA dependent RNA polymerase [1] there have been no experiments reported on such compounds where the ability of the ligand to intercalate DNA had been systematically varied. Proflavine and its 2,7-dialkyl derivatives (methyl to t-butyl, see Fig.1 for the formulae and the abbreviations) form such a series of acridines whose DNA-binding modes are well characterized [2 - 5]. All of them bind to DNA and show biological activity [2 - 5] but the nature of their complexes with DNA differs across the series. The first three members (unsubstituted PF and its methyl and ethyl derivatives, Fig.1) exhibit comparable binding constants and form intercalating complexes [2, 4, 5]. In contrast, DBP interacts with DNA without intercalation, as the bulky t-butyl substituents sterically hinder insertion of the chromophore between the base pairs, leaving the ligand to one of the grooves [2 - 4]. DIP is able to intercalate DNA, but to a very small extent, and has a binding mode more closely related to that of DBP [2, 4, 5]. Our previous experiments have indicated that the inhibitory effects of these drugs depend on the template used [1]. With phage T7 DNA, inhibition of RNA synthesis depends mainly on the mode of ligand binding, with intercalative binding favouring inhibition. With calf thymus DNA inhibition decreases with the increase of the lipophilic character of the compounds, and the mode of binding plays a less important role [1].

The RNA synthesis is a fairly complex process and its inhibition may have resulted from interference of drug at the different steps. The following steps of RNA synthesis may be experimentally distinguished: binding of RNA polymerase to the template, initiation of polynucleotide chains, elongation and termination. The aim of the present experiments is to compare the effect of proflavine and its congeners on early steps of the process.

Phage T7 DNA and a 391 base pairs DNA fragment bearing the *tac* promoter excised from pDR 540 vector serving as templates [6] and *Escherichia coli* DNA-dependent RNA polymerase (EC 2.7.7.6) were used in this study. The data on inhibition of overall RNA synthesis

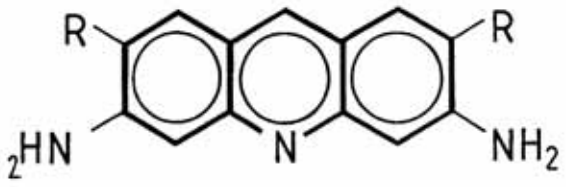

Fig. 1. Structures of proflavine derivatives.
Proflavine (PF), R = H; 2,7-dimethylproflavine (DMP), R = CH_3; 2,7-diethylproflavine (DEP), R = CH_2CH_3; 2,7-diisopropylproflavine (DIP), R = $CH(CH_3)_2$; 2,7-ditertbutylproflavine (DBP), R = $C(CH_3)_3$

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Table 1

Effect of acridines on inhibition of initiation of the transcription.

IC_{50}, micromolar concentration of drug which inhibits to 50% of control value the RNA synthesis on phage T7 DNA (see [1]). IO_{50}, micromolar concentration of drug which inhibits open complex formation on *tac* promoter to 50% of control value. IA_{50}, micromolar concentration of drug which inhibits pppApU synthesis on phage T7 DNA to 50% of control value

Acridine	IC_{50} T7 DNA	IO_{50} *tac* promoter	IA_{50} T7 DNA
PF	19.3	8.5	19.7
DMP	18.5	6.5	16.3
DEP	19.4	13.7	20.3
DIP	93.3	103.5	78.3
DBP	83.6	130.1	87.3

with T7 DNA at 0.1 M KCl [1] have been taken to set drug concentrations in these experiments. Drug concentrations resulting in a 50% decrease in RNA synthesis (IC_{50}) are shown here (Table 1) just for comparison.

Open promoter complexes of RNA polymerase with the ^{32}P-labelled *tac* promoter bearing DNA fragment were formed at different drug concentrations and analysed by electrophoresis in 4% polyacrylamide gel under non-denaturing conditions [7]. The promoter-enzyme complex can be easily separated from the uncomplexed DNA fragment (Fig. 2, lanes 1 and 2). At the temperature of the experiment (37°C) the observed complex corresponds to the open "upper" complex of Straney & Crothers [7]. The amount of the complex formed considerably decreases in the presence of all the acridines studied (Fig. 2, lanes 3 - 7). Note that much higher concentrations of DIP and DBP (lanes 6, 7) are needed to obtain a similar inhibition of the complex formation when compared to the other derivatives tested.

The relative amounts of the promoter-enzyme complexes were estimated by the radioactivity measurements. They were expressed in per-

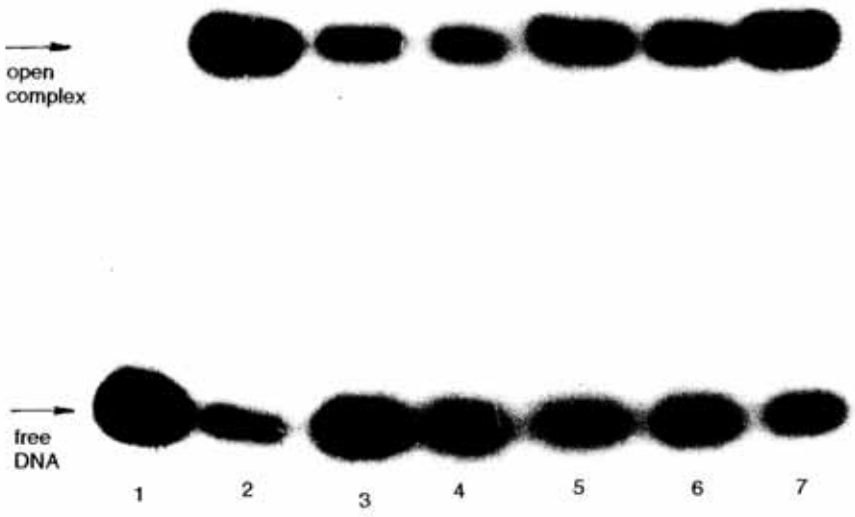

Fig. 2. Acrylamide gel electrophoresis of the open complex of RNA polymerase and a DNA fragment of 391 base pairs carrying the tac promoter, under non-denaturing conditions at the temperature 37°C.
Lane 1, free DNA; lane 2, RNA polymerase/DNA complex alone and the complex in the presence of: lane 3, PF (8.4 μM); lane 4, DMP (7.2 μM); lane 5, DEP (9.0 μM); lane 6, DIP (34.2 μM); lane 7, DBP (37.8 μM)

centage of the corresponding controls, i.e. the assays without any drug added (Fig. 2, lane 2). The binding was assayed at five-six drug concentrations, and drug concentrations decreasing the complex formation to 50% (IO_{50}) were read from the inhibition curves (not shown). The determined IO_{50} values are presented in Table 1. The comparison of these values with the ability of each derivative to intercalate indicates that the inhibitory effect depends strongly on intercalation.

To approach the problem of the drugs effect on the initiation the assay of abortive initiation was used [8]. RNA polymerase in the presence of ATP and UTP repetitively synthesizes pppApU on A1 and A3 promoters of T7 DNA (see [8 - 10]). The amount of the initiating dinucleoside tetraphosphate was quantitated following its separation from the substrates by means of paper chromatography [8]. The estimation of pppApU in the uninhibited controls and at the different acridine concentrations allowed determination IA_{50} values, i.e. drug concentrations decreasing the abortive initiation to 50% (Table 1). The results are very close to those obtained in the assay of the overall RNA synthesis (IC_{50}) and higher than IO_{50} values. The latter is not unexpected as in the experiments reported here the concentration of DNA was comparable in terms of promoters. A considerably higher DNA concentration in terms of mass unit, hence potential acridine binding sites, was used in overall RNA synthesis (IC_{50}) and abortive initiation assays (IA_{50}) than in the binding experiments (IO_{50}).

In separate experiments we have found that the DNA fragment bearing the promoter was able to support the synthesis of short polyribonucleotide chains, with the non-complete substrate set, and that the inhibition by PF and DBP occurred at similar drug concentration as binding (not shown).

The experiments presented here indicate that proflavine derivatives which form short-living complexes with DNA primarily inhibit binding of the enzyme to DNA and that formation of the open promoter complex seems to be crucial for the inhibition. The mechanism seems to be similar to the mechanism of inhibition by ethidium bromide [11]. The inhibitory effect strongly depends on the intercalation of the chromophore. Similar effects on the overall RNA and pppApU syntheses are consistent with the other data indicating that the proflavine effect on the elongation seems to be negligible [12]. Different results were obtained for antracycline antibiotics [9, 10] and furocoumarins adducts [13] which affected the overall RNA synthesis to a greater extent than the synthesis of the initiating dinucleotide. The stability of the ligand-DNA complex seems to be crucial for these differences in the mechanisms of inhibition (see [8] for the discussion of this point).

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