NATURE OF INTERACTION OF ANTITUMOR ANTIBIOTIC MITHRAMYCIN WITH DNA

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1. Introduction

Mithramycin is an antitumor antibiotic effective against a variety of experimental and human tumors [1–4]. In particular it has been found useful in treating human testicular tumors [2,4]. The mechanism of action of this antibiotic has been investigated and it has been shown that the antibiotic binds to DNA and inhibits DNA dependent RNA synthesis [5–7]. For this drug to bind to DNA, a divalent cation like Mg^{2+} is necessary [7], and mithramycin and other structurally related antibiotics like chromomycin A_3 and olivomycin are known to be anionic in nature [8]. Nayak et al. [9] have shown that the divalent cation is required to counterbalance the electrostatic repulsion between the anionic drug and the negatively charged phosphate groups of DNA. However, it is not clear whether Mg^{2+} is actually a part of the drug–DNA complex or is independent of it. The earlier studies indicated that mithramycin does not affect the hydrodynamic behaviour of DNA and therefore does not bind by intercalation [7–10]. On the other hand, Behr et al. [11] have suggested the possibility, that chromomycin A_3, which has a chromophore similar to mithramycin, binds by partial intercalation.

Since the molecular details of binding of mithramycin to DNA and in particular the role of Mg^{2+} remain unclear, we have examined this question by the use of the chelating agent EDTA which chelates Mg^{2+}.

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2. Materials and methods

DNA was isolated from Yoshida ascites sarcoma by the method of Kay et al [12]. The tumor was grown in Wistar A/Iisc rats by serial i.p. transplantation every fifth day. Mithramycin was a generous gift from Pfizer, Maywood, N. J. Spectrophotometric titrations were carried out at room temperature (29°C) in 0.01 M Tris–HCl buffer, pH 8.0, and spectra were recorded by either an Unicam SP 700 or a Carl Zeiss VSU-2 spectrophotometer.

3. Results and discussion

Addition of Mg^{2+} to mithramycin produces spectral changes similar to those produced by DNA in the presence of small amounts of Mg^{2+}. It was therefore necessary to investigate Mg^{2+}–mithramycin interaction and then evaluate its effect on DNA–mithramycin complex formation. It was found that high concentrations of Mg^{2+} (in the order of 10^{-2}M) are necessary to produce spectral changes similar in magnitude to those obtained in DNA–mithramycin interaction (fig.1). The $pK_{Mg^{2+}}$ of the Mg^{2+}–mithramycin complex was calculated from the concentration-dependent spectral change and was found to be 3.5 (fig.2). It is clear therefore, that at the concentration of Mg^{2+} employed in DNA–mithramycin interactions (Mg^{2+} concentration 15×10^{-5}M) the contribution by the Mg^{2+} towards the observed spectral change is negligible. Further, A_{440} of free mithramycin is always measured in the presence of magnesium.

The DNA–mithramycin interaction was studied by adding various amounts of DNA to mithramycin

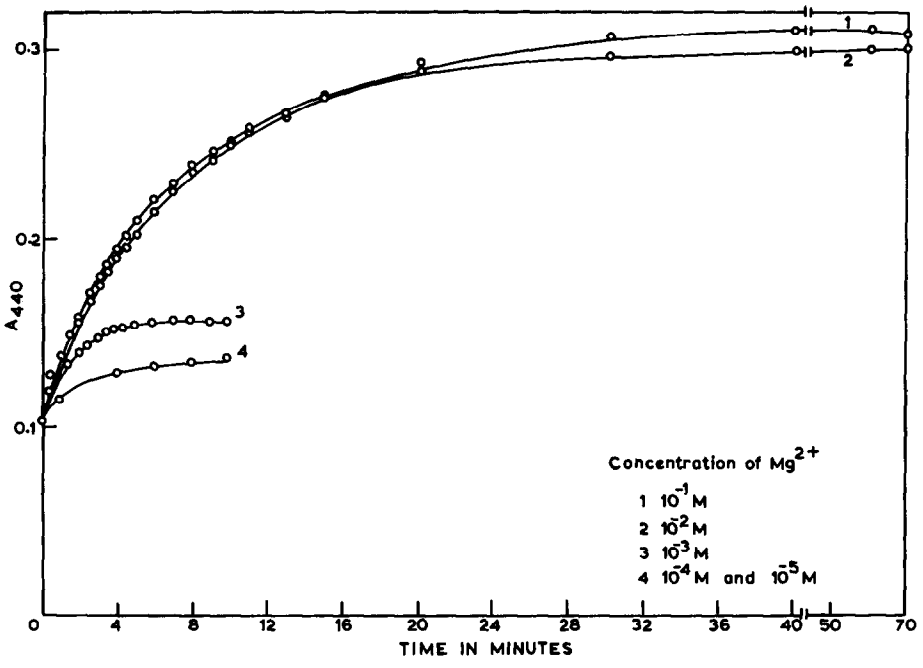

Fig.1. Time course of interaction of mithramycin (6.4×10^{-5} M) with Mg^{2+} in 0.01 M Tris–HCl buffer, pH 8.0.

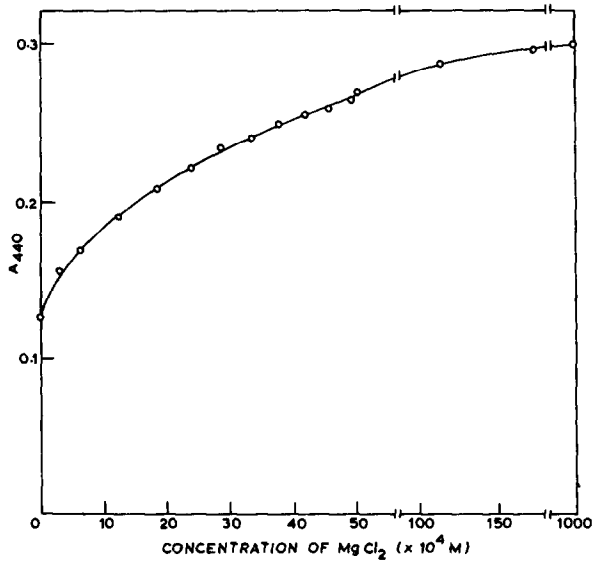

Fig.2. Change in absorbance of mithramycin (6.4×10^{-5} M) at 440 nm as a function of Mg^{2+} concentration. Tris–HCl buffer, 0.01 M, pH 8.0 was used.

(4.75×10^{-5} M) and Mg^{2+} (15×10^{-5} M) in Tris–HCl buffer. The mixture was equilibrated for 30 min at room temperature and spectra were recorded. The spectral titration curves of a typical experiment are shown in fig.3. The spectral changes are similar to those observed for chromomycin A_3 [9,13], the absorption maximum shifts from 405–440 nm with an isobestic point at 411 nm. Similar spectral changes also occur with DNA– proflavin, DNA–ethidium bromide and DNA–actinomycin D interactions for all of which intercalation is the preferred mode of binding [14–16]. The association constant of DNA–mithramycin binding, calculated from the Scatchard plots of the data, is 2×10^5 M^{-1}.

The time course of interaction of DNA with mithramycin is given in fig.4. To check if Mg^{2+} is actually present in the complex, EDTA was added to the equilibrated DNA–mithramycin complex and spectral changes at 440 nm were recorded. From the time-dependent change in absorbance produced by EDTA it is clear that rapid dissociation of the complex is induced. The half-life of dissociation is 0.7 min as against 4.5 min needed for formation of the DNA–

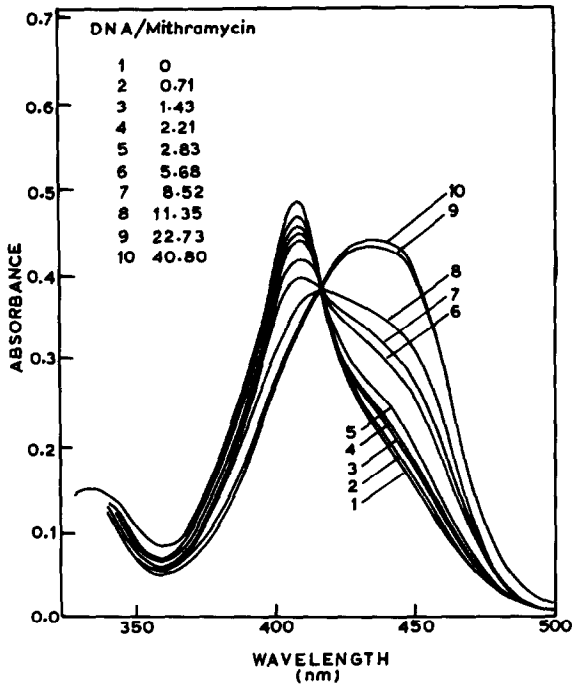

Fig.3. Spectrophotometric titration of mithramycin (4.75×10^{-5} M) with Yoshida ascites sarcoma DNA in 0.01 M Tris–HCl buffer, pH 8.0 and 15×10^{-5} M $MgCl_2$. The numbers indicate molar ratio of DNA phosphate to mithramycin.

mithramycin complex. However, addition of EDTA to the complex does not produce the spectrum characteristic of free mithramycin indicating that a fraction of the drug probably still remains in the complexed form. Higher concentrations of EDTA did not decrease the absorbance any further and, much more significantly, EDTA by itself did not induce any changes in the spectrum of mithramycin. In contrast, addition of EDTA to mithramycin–Mg^{2+} results in a spectrum characteristic of the free drug. It is thus reasonable to assume that at the concentrations of EDTA employed no Mg^{2+} will be available for DNA–mithramycin complex formation. The extent of spectral change produced by EDTA indicates that 78% of the drug present is dependent on Mg^{2+} in order to bind with DNA. This Mg^{2+}–dependent binding of the drug is presumably due to external binding where a divalent cation is always needed to shield the negative charges of anionic mithramycin ($pK_a = 5$) from that of the phosphate groups of DNA.

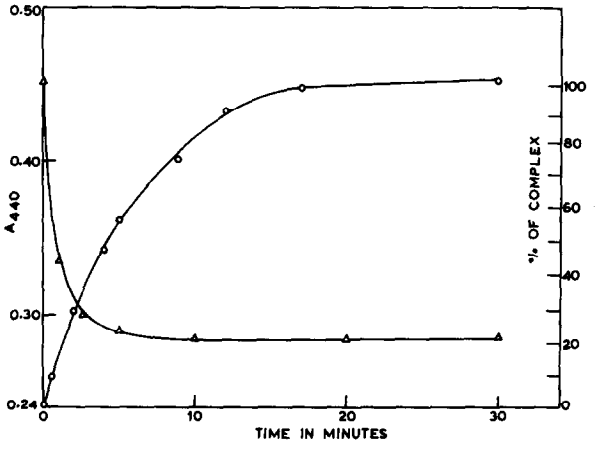

Fig.4. Time course of DNA (2.7×10^{-4} M)–mithramycin (5.77×10^{-5} M) complex formation in presence of $MgCl_2$ (15×10^{-5} M) (●—●); and dissociation of the above complex by EDTA (2×10^{-2} M) (△—△). The titration was carried out at 29°C in 0.01 M Tris–HCl buffer, pH 8.0.

The fraction of the drug that remains complexed to DNA even after chelation of Mg^{2+} is probably due to the intercalated form of the drug since no divalent cations would be needed for such intercalation. It is observed that when ethidium bromide, a typical intercalating dye, is added to mithramycin–DNA, a fraction of the bound drug is displaced. This result suggests that a fraction of mithramycin is in fact intercalated and it is this intercalated complex that is displaced by ethidium bromide. However, it is not clear whether Mg^{2+} is necessary to facilitate the entry of the drug to the site of intercalation although physico-chemical considerations would require such cations in order to overcome the electrostatic repulsion the reactants would intially encounter.

The complex between mithramycin and DNA, which can exist even in the absence of Mg^{2+}, can be considered as highly stable, having a relatively slow dissociation rate (fig.4). Muller and Crothers have suggested that in the case of such complexes dissociation rates are more important than association rates in determining the biological activity of the drug [16]; that is, a slower dissociation of the complex implies a higher biological activity. It follows then that it is the Mg^{2+}–independent DNA–mithramycin complex (which presumably has mithramycin in an intercalated form) which is largely responsible for the observed inhibition of transcription.

Acknowledgments

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