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MITHRAMYCIN CANNOT BIND TO LEFT-HANDED POLY(dG-m^5dC) IN THE PRESENCE OF Mg^{2+} ION

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SUMMARY: Mithramycin (MTR) is an antitumor compound that inhibits RNA and DNA polymerase action by forming a non covalent complex with double strand DNA, in the presence of divalent cations. We have shown that in the presence of Mg^{2+}, MTR binds to right-handed poly(dG-m^5dC) as a dimer in the right-handed screwness conformation but cannot bind to left-handed poly(dG-m^5dC).

Mithramycin (MTR) (Scheme I) is an antitumor antibiotic closely related to chromomycin A_3. They both belong to the aureolic acid group (1) and are effective against a wide variety of experimental and human tumors (2). The antitumor therapeutic properties of MTR are related to its ability to inhibit RNA and DNA polymerase action by forming a non covalent complex with double stranded DNA (3). Effective binding of MTR to DNA depends on the presence of divalent cations (3-5).

Gao and Patel have demonstrated that chromomycin binds as a dimer at G-C sites in the minor groove of both strands of the self-complementary d[T-T-G-G-C-C-A-A] duplex (6). Using circular dichroism spectroscopy we have shown that MTR binds to DNA as a dimer in the right-handed screwness conformation (7). DNA footprinting studies have shown that MTR recognizes the sequence GpC

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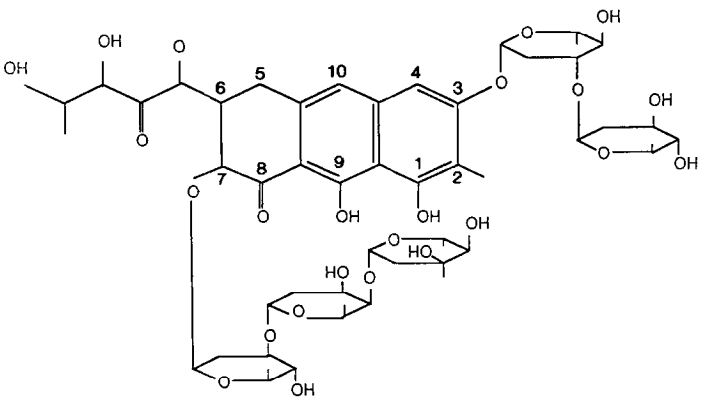

(8,9). Recently Schafer et al. have claimed that chromomycin A_3 can bind simultaneously to both the B and Z conformation of poly (dG-m^5dC) with no effect on the B-Z equilibrium (10).

In this paper we demonstrate that, in the presence of Mg^{2+}, MTR binds to right-handed poly (dG-m^5 dC) as a dimer in the right-handed screwness conformation but cannot bind to left-handed poly(dG- m^5 dC).

Materials and Methods

Poly(dG-m^5dC) and poly(dG-dC) were obtained from Pharmacia-PL Biochemicals. Polynucleotides were dissolved in a buffer consisting of 0.005M HEPES, 0.1M NaCl at pH 7.2. Poly(dG-m^5dC) solutions containing 10mM $CaCl_2$ were heated for 10 min at 50°C in order to complete the B to Z transition (11). An absorption coefficient of 7100 M^{-1} cm^{-1} per nucleotide was used to calculate polynucleotide concentrations from absorbance measurements at 255 nm. Absorption spectra were recorded on a Cary 219 spectrophotometer and circular dichroism (CD) spectra on a Jobin Yvon dichrograph Model Mark V. Results are expressed in terms of ε (molar absorption coefficient) and $\Delta\varepsilon=\varepsilon_L-\varepsilon_R$ (molar CD coefficient). The values of ε and $\Delta\varepsilon$ are expressed in terms of [MTR] the molar concentration of mithramycin.

Results and Discussion

The absorption spectrum of MTR exhibits strong bands at about 400, 330, 280 and 230 nm. According to Harada et al. (12) they can be assigned to $^1A \rightarrow {}^1L_b$,

$^1A \rightarrow {}^1L_a$, $^1A \rightarrow {}^1B_b$ and $^1A \rightarrow {}^1B_a$ respectively. In the region of the $^1A \rightarrow {}^1B_b$ transition, which is polarized along the long axis of the naphtalenoïd moiety, the CD spectrum exhibits two Cotton effects of opposite signs. The summation of the amplitude of the two Cotton effects gives a A-value : $A=\Delta\varepsilon_1-\Delta\varepsilon_2$ where $\Delta\varepsilon_1$ and $\Delta\varepsilon_2$ are the amplitude of the first Cotton effect at longer wavelength and of the second Cotton effect at shorter wavelength respectively (13). The A-value defined the chirality of the species. We have shown that at low concentration ($\leq$ 2µM), in aqueous solution, MTR is always in the dimeric state (7). The conformation of the dimer depends on the pH value and is either right-handed screwness (A>0) when the dimer is neutral $(MitH)_2$, or left-handed screwness (A<0) when the dimer is negatively charged $(Mit^-)_2$.

Interaction of MTR with poly(dG-dC) and poly(dG-m^{5}dC) in the absence of Ca^{2+} and presence of Mg^{2+}. These experiments were performed at 25°C with 2 µM MTR in the presence of either 20 µM poly(dG-dC) or poly(dG-m^{5}dC) in 0.1M NaCl at pH 7.2. In these conditions both polymers are right-handed exhibiting in the 240-300 nm wavelength region a CD signal of the couplet type with a negative band at 255nm ($\Delta\varepsilon$/nucleotide=-10) and a positive one at 285nm ($\Delta\varepsilon$=+2). MTR is a negatively charged dimer with negative chirality (A=-65). The addition of polynucleotides to MTR did not give rise to noticeable modification of the CD spectrum of MTR. We made the hypothesis that the polynucleotide CD spectrum was not or only slightly modified by the addition of MTR and contributions due to polynucleotide in the absence of drug have been subtracted.

It is now well established that the interaction of MTR with DNA or polynucleotide requires the presence of divalent metal ions such as Mg^{2+} or Zn^{2+} (6,14-17) In the present case, the addition of Mg^{2+} to the solution gave rise to strong modification of the CD spectrum indicating a change from a negative

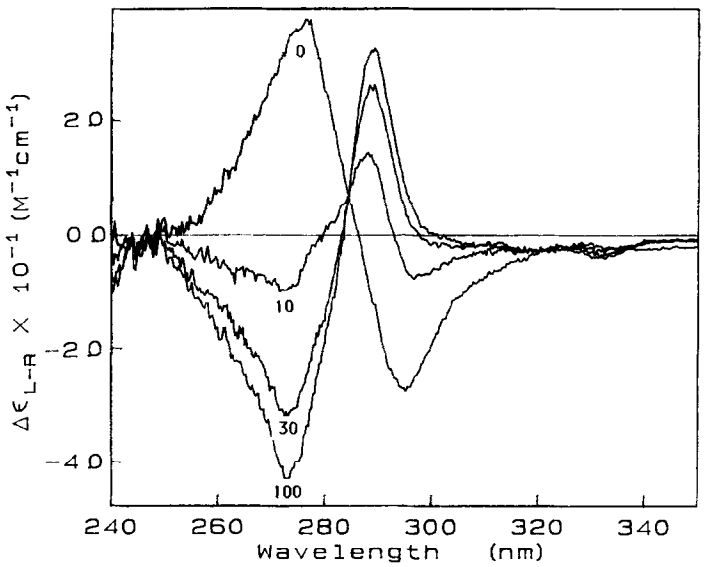

Figure 1. Circular dichroism spectra of mithramycin in aqueous solution in the presence of poly(dG-dC) and various amounts of Mg^{2+}. [MTR]=2μM, [nucleotides]=20μM, [NaCl]=0.1M, HEPES buffer pH 7.2. The molar ratio of Mg^{2+} to MTR was 0 , 10, 30, and 100 as indicated on the figure. The circular dichroism spectrum of poly(dG-dC) has been subtracted.

chirality of the dimer to a positive one (Figure 1). Such a change of chirality can also be induced by the addition of Mg^{2+} in the absence of polynucleotide but in that case this requires a large amount of Mg^{2+} (7).

It is interesting to follow the variation of $\Delta\varepsilon$ at 275 nm for the reasons that at this wavelength i) the variation of the MTR signal is important and strongly sensitive to conformational change ii) the polynucleotide in the B and Z conformation exhibits an isodichroic point. The value of $\Delta\varepsilon$ at 275 nm has been plotted as a function of the molar ratio of Mg^{2+} to MTR (Figure 2). As can be seen 50 % of MTR are in the right-handed screwness conformation and 50 % in the left handed screwness one at a molar ratio of Mg^{2+} to MTR equal to 50:1 in the absence of polynucleotide and to 7:1 and 14:1 in the presence of poly(dG-dC) and poly(dG-m^5dC) respectively. When MTR was 100 % in the right-handed screwness

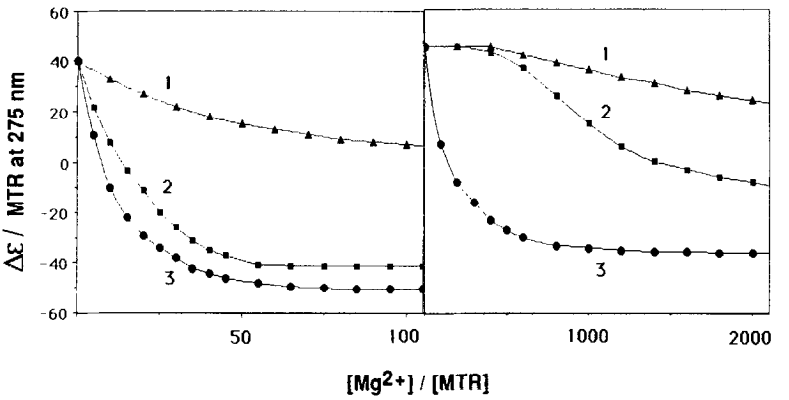

Figure 2. Variation of the circular dichroism spectrum of mithramycin as a function of the molar ratio of Mg^{2+} to mithramycin in the absence (curves 1) or presence of poly(dG-dC) (curves 3) or poly(dG-m^5dC) (curves 2). $\Delta\varepsilon$ at 275 nm has been plotted as a function of the molar ratio of Mg^{2+} to mithramycin. [MTR]=2μM, [NaCl]=0.1M, HEPES buffer pH 7.2. The molar ratio of nucleotide to mithramycin was either 0 (1) or 20.(2 and 3). The experiments were performed in the absence of Ca^{2+} (left) and in the presence of Ca^{2+} 10mM (right).

conformation, A values were -65, +39, +75, +70 at molar ratios MTR:Mg^{2+}:nucleotides equal to 1:0:0, 1:250:0, 1:50:10, 1:50:10 respectively.

Interaction of MTR with poly (dG-dC) and poly (dG-m^5dC) in the presence of Ca^{2+} and Mg^{2+} .In the presence of 10mM Ca^{2+} poly(dG-m^5dC) is in the left conformation. Its CD spectrum exhibits a negative band at 295 nm ($\Delta\varepsilon$/nucl=-9) and a positive one at 260 nm ($\Delta\varepsilon$=+2). In the same conditions poly(dG-dC) is in the right conformation.

We first studied the effect of Ca^{2+} on the MTR conformation in the absence of polynucleotide. We observed that the addition of Ca^{2+} to MTR yield an increase of the amplitude of the CD bands the A-value becoming equal to -76. We have checked that this modification was not due to an increase of the ionic strength: if an ionic strength equal to 0.04 is provided by the addition of NaCl instead of $CaCl_2$ no modification of the CD spectrum of MTR is observed. This

strongly suggests that the left-handed conformation of MTR is stabilized by the presence of Ca^{2+} cation. The addition of Mg^{2+} to MTR in the presence of 10 mM Ca^{2+} yielded a change in the conformation of MTR which became right handed screwness. A molar ratio of Mg^{2+} to MTR equal to about 3500 was required to obtain 50% of MTR in the right-handed screwness conformation. In the presence of poly(dG-dC) a molar ratio of Mg^{2+} to MTR equal to 80:1 brings about 50 % of the transformation. All these data show that the dimer MTR must be in the right-handed screwness conformation to bind polynucleotide (or DNA) in the right conformation.

In the case of poly (dG-m^5dC) in the left conformation, one might have believed that a dimer of MTR in the left-handed screwness conformation would be able to bind to the polynucleotide. However the addition to MTR of poly (dG-m^5dC) in the left conformation did not give rise to modification of the CD spectrum of MTR even after the addition of Mg^{2+} up to a molar ratio Mg^{2+}:MTR equal 500 (Figure 2). The further addition of Mg^{2+} yielded a change in the conformation of both MTR and poly (dG-m^5dC) which adopt the right conformation. As can be seen in Figure 2 the amount of Mg^{2+} requires to induce the conformational change of MTR from left to right is less in the presence of poly(dG-m^5dC) than in its absence despite the fact that the polynucleotide was preliminary in the left conformation.

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