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Further insight into the Zn^{2+}-mediated binding of streptonigrin to DNA☆

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Abstract

The interaction of double- and single-stranded DNA with the antitumor antibiotic streptonigrin (STN) in the presence of zinc ions has been examined by spectrophotometric and circular dichroism techniques. Very little interaction occurs in the absence of the metal ion. When Zn^{2+} is present, the binding process exhibits a bell-shaped dependence upon the ion concentration, consistent with the formation of a ternary complex, splitting into two binary complexes at high content of the metal ion. Evidence is presented for the participation of two STN molecules bridged by Zn^{2+} in the complex with DNA.

Keywords: Streptonigrin; DNA; Zinc; 2:1 Complex

1. Introduction

Streptonigrin (STN, Fig. 1) is a natural antibiotic belonging to the aminoquinoline-dione family. It is characterized by prominent in vivo activity against tumors, including a variety of human cancers [1]. Although clinical trials have been discontinued, due to severe side-effects, STN represents an interesting structure from the mechanistic point of view. In fact, it produces cell toxicity by degradative damage of DNA. Two different mechanisms of DNA-strand scission have been shown to occur: one, mediated by transition metal ions, rests on the production of active radical species [2,3], the other, protein mediated, is related to the stabilization of the topoisomerase II–DNA cleavable complex [4,5]. The important features of the interaction of STN with metal ions and DNA have been thoroughly discussed in a recent review [6]. A direct interaction between STN and DNA is evident in the presence of transition metal ions (e.g. Zn^{2+}) only. NMR studies with double-stranded synthetic oligonucleotides are suggestive of the formation of a complex preferentially oriented towards the major groove of the duplex and dominated by electrostatic contributions from the phosphate backbone [7]. To further address the interesting issue of STN binding to DNA we have performed a spectrophotometric and circular dichroism (CD) investigation at various STN:Zn^{2+}:DNA ratios. We show evidence that the complex species acting on DNA probably comprises two drug molecules coordinated to the metal ion.

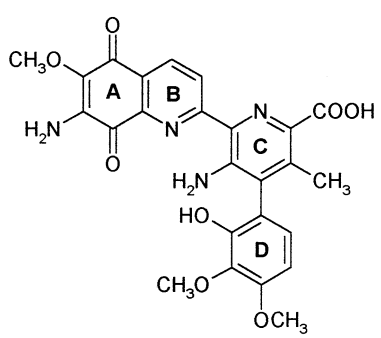

Fig. 1. Chemical structure of STN.

☆ Dedicated to Professor Antonio Maccioni.
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2. Experimental

2.1. Materials

STN was obtained from the Drug Synthesis and Chemistry Branch, National Cancer Institute (Bethesda, MD). Stock solutions of the antibiotic were prepared dissolving a weighed amount of STN (about 1 mg) in 1 ml acetonitrile and diluting to 50 ml with the desired Tris–NaCl buffer. The STN concentration was confirmed spectrophotometrically using a molar absorptivity of 15 500 M^{-1}/cm. Double-stranded calf thymus DNA (highly polymerized sodium salt) was purchased from Sigma (St. Louis, MO). It was deproteinized by phenol extraction. Its concentrations were determined using a molar absorptivity (per residue) of 6600 M^{-1}/cm. Single-stranded DNA (molar absorptivity 8350 M^{-1}/cm) was provided by Crinos S.p.A. (Villaguardia, Italy). Analytical grade zinc nitrate was obtained from Fluka as the hexahydrate salt. A stock solution was prepared dissolving about 2 g of this salt in 25 ml of Tris–NaCl buffer. The zinc concentration was confirmed by atomic absorption measurements at 213.9 nm.

2.2. Methods

All spectroscopic investigations were carried out at 23°C, 10 mM in Tris, 20 mM NaCl, pH 7.0.

Experiments were usually carried out at STN concentrations of the order of 10^{-5} M. Under these conditions the solutions were stable and no drug precipitation was observed.

Ternary complex titrations were performed either at a fixed nucleic acid concentration (normally in the millimolar range) and variable metal ion or at fixed Zn^{2+} and variable DNA concentration.

Spectrophotometric measurements were performed with a Perkin–Elmer Lambda 5 apparatus equipped with an Haake F3-C thermostat. Quartz cells with 1 cm optical path length were employed.

CD studies were performed using a Jasco J-500 spectropolarimeter interfaced to a J-500 N computing station. Four to eight scans were accumulated for each measurement. An Haake F3-C thermostat was used to control temperature.

Atomic absorption measurements were made using a Perkin–Elmer 360 apparatus. A specific hollow cathode lamp was used for the quantitative determination of zinc.

3. Results and discussion

3.1. STN binding to zinc(II) ions

It has been proposed by other authors that STN binds to Zn^{2+} in aqueous solution as a 1:1 complex. In this stoichiometry the metal ion coordinates either the bipyridyl nitrogens or the pyridyl nitrogen in ring B and the amino nitrogen of ring C [6,8]. In organic media, however, a 2:1 stoichiometry is also possible [8,9].

Before investigating the interaction with DNA, we performed spectrophotometric measurements to characterize the drug–metal ion complex in our experimental conditions (10 mM Tris, 20 mM NaCl, pH 7.0). The spectral modifications of STN upon increasing Zn^{2+} concentration were not characterized by an isosbestic point. This could be due either to a different distribution of the possible 1:1 species, or to the simultaneous presence of complexes with different stoichiometries. To obtain further information, we took advantage of the drug's optical activity by performing CD measurements. The CD pattern of STN in the presence of Zn^{2+} is shown in Fig. 2(a) and (b). The behavior is clearly

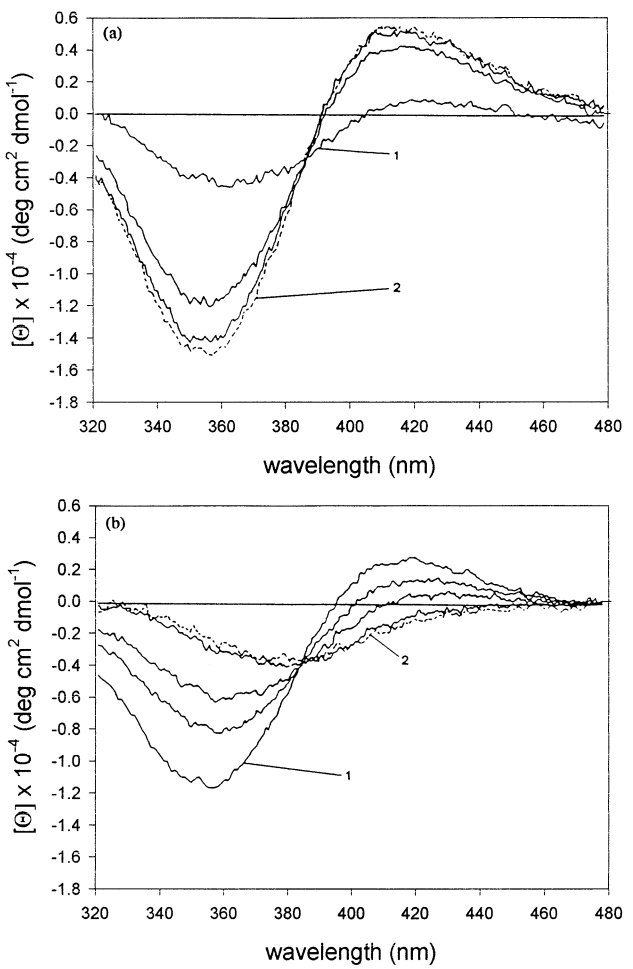

Fig. 2. (a) CD spectra of STN (3.2×10^{-5} M) in the presence of increasing amounts of Zn^{2+}. Curve 1 refers to free STN, curve 2 to a Zn/STN ratio of 1.92. Measurements were made in 10 mM Tris, 20 mM NaCl, pH 7.0, 23°C. (b) CD spectra of STN (3.2×10^{-5} M) in the presence of increasing amounts of Zn^{2+}. Curves 1 and 2 refer to Zn/STN ratios of 3.15 and 200, respectively. Measurements were made in 10 mM Tris, 20 mM NaCl, pH 7.0, 23°C.

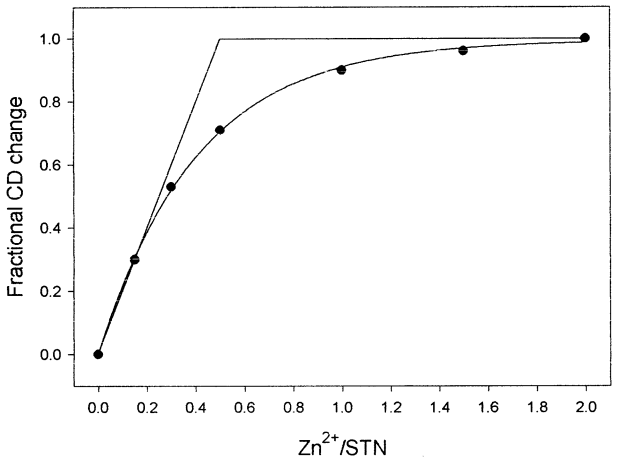

Fig. 3. Plot of the fractional CD change (change in molar ellipticity of STN in the presence of a given Zn^{2+} concentration over the maximum change observed, see Fig. 2(a)) at 360 nm as a function of the Zn/STN ratio. Drug concentration and buffer conditions as in Fig. 2(a).

biphasic as an increased intensity of the bands is observed until Zn^{2+}/STN ratios of the order of 2, followed by a progressive decrease and by more dramatic spectral changes at zinc concentrations in the millimolar range. The form of the CD spectrum in the presence of the metal ion is consistent with an exciton splitting occurring in the system. In fact, the cross-over in the CD spectrum of STN in the presence of Zn^{2+} is close to the wavelength corresponding to the absorption maximum of the complex. A similar behavior has been reported simply increasing STN concentration in the absence of metal ions, and has been interpreted in terms of STN–STN interactions due to the production of aggregated species [10]. Hence, the data suggest the formation of (at least in part) a 2:1 STN–Zn^{2+} complex at low concentration of the metal ion, evolving into a 1:1 system as more Zn^{2+} is added. This is further supported by the plot of the fractional change in molar ellipticity at 360 nm versus the Zn^{2+}/STN molar ratio, which exhibits an initial slope of 2 (Fig. 3).

3.2. STN binding to double-stranded (ds) DNA

The absence of a measurable interaction between STN as such and DNA is confirmed by our results. As expected, the presence of a metal ion like Zn^{2+} is required for effective DNA-binding of the drug [6]. Spectrophotometric titrations of a 1:100 STN–DNA mixture show a new band approximately located at 405 nm, red shifted of more than 40 nm with reference to STN, which can be safely attributed to the ternary STN:Zn^{2+}:DNA complex. At metal ion concentrations of the order of 1 mM or higher (Zn^{2+}:STN > 100) a progressive blue shift of the spectrum occurs (to about 390 nm), the new band closely resembling that of the STN–Zn^{2+} complex in the absence of DNA.

CD spectra were also recorded as a function of Zn^{2+} concentration at DNA:STN fixed ratios of the order of 100. The results are summarized in Fig. 4. The complex is characterized by two bands, a negative one centered at about 370 nm and a positive one, having practically the same rotational strength, located at 430 nm. The cross-over corresponds to the position of the absorption band of the above-mentioned ternary complex. These data can be clearly interpreted in terms of the stabilization of a 2:1 STN–Zn^{2+} stoichiometry bound to the DNA template. It can be also appreciated that the rotational strength of the ternary complex is remarkably increased when the drug is bound to DNA. This could be the result of a reduced rotational freedom of the δ bond of STN when bound to the nucleic acid and the metal.

The intensity of the CD band exhibits a maximum as a function of the metal ion:STN ratio (Fig. 5).

A reasonable explanation for these and the spectrophotometric results can be proposed recalling that, besides STN, also DNA is able to bind zinc ions. In fact, two kinds of sites are available for the complexation of the transition metal to the nucleic acid: the sugar–phosphate backbone and (especially for Zn^{2+}) the purine N-7 [11].

The relevant equilibria will be:

$$\mathrm{STN} + \mathrm{Zn} \rightleftarrows \mathrm{STN{-}Zn} \quad (1)$$

$$\mathrm{DNA} + \mathrm{Zn} \rightleftarrows \mathrm{DNA{-}Zn} \quad (2)$$

$$\mathrm{STN} + \mathrm{DNA} + \mathrm{Zn} \rightleftarrows \mathrm{STN{-}DNA{-}Zn}$$

(ternary complex) (3)

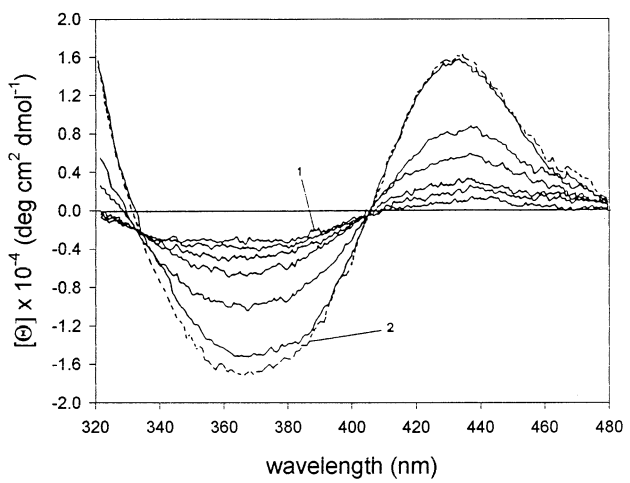

Fig. 4. CD titration of a STN/ds-DNA mixture ([STN] = 2.0×10^{-5} M, DNA/STN = 50) with increasing amounts of Zn^{2+}. Curve 1 is recorded in the absence of Zn^{2+}, curve 2 at a Zn/STN ratio of 109. Measurements are made in 10 mM Tris, 20 mM NaCl, pH 7.0, 23°C.

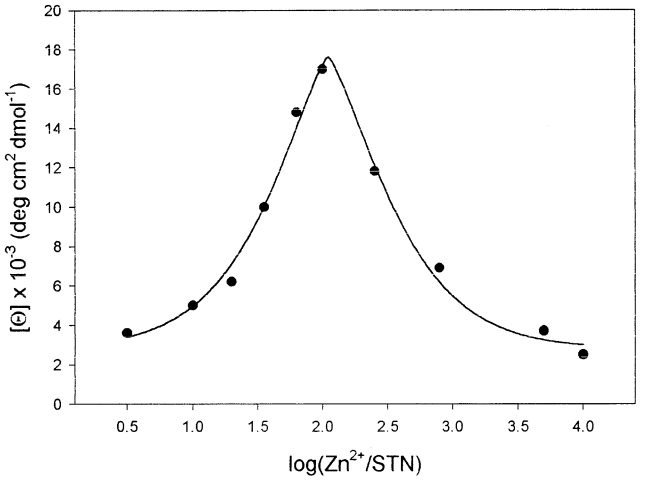

Fig. 5. Effects of increasing amounts of Zn^{2+} on the molar ellipticity at 370 nm of a STN/ds-DNA mixture ([STN] = 2.0×10^{-5} M, DNA/STN = 50). Buffer conditions as in Fig. 4.

$$STN-DNA-Zn + Zn \rightleftarrows STN-Zn + DNA-Zn \quad (4)$$

here, net charges, counterions and stoichiometries are omitted for simplicity.

A consequence of the above equilibria is that the relative amount of ternary and binary complexes will be modulated by the concentration of zinc ions. In particular, there will be an optimal Zn^{2+} concentration for ternary complex formation, above which dissociation to binary complexes (equilibrium 4) occurs. The Zn^{2+} dependence of the CD bands attributed to the ternary complex (Fig. 5) fully supports this hypothesis.

3.3. STN binding to single-stranded (ss) DNA

Spectrophotometric and chiroptical measurements similar to those carried out with ds-DNA were performed using a single-stranded nucleic acid sample. The main problem connected with these experiments has to do with the tendency to precipitation of the DNA–zinc complex. Hence, the useful Zn^{2+} concentration range is remarkably restricted. A dichroic titration of a mixture of STN and ss-DNA as a function of Zn^{2+} is shown in Fig. 6. The spectral changes recorded are very similar to those observed in the presence of ds-DNA at corresponding experimental conditions, which suggests a model for the ternary complex close to the one previously discussed. Spectrophotometric measurements are in total agreement with the CD data. Hence, in addition to double stranded structures, it appears that also single stranded regions of the DNA are efficiently recognized by STN. This is consistent with the reported affinity of reduced STN for ss-DNA in the presence of Zn^{2+} [12].

In conclusion, a species containing two STN molecules coordinated to one zinc appears to be involved in interactions with DNA both in a single and in a double stranded conformation. This species could be pharmacologically relevant as it brings two reactive drug molecules close to the nucleic acid chain, using only one metal ion carrier. Considering the mechanism of ion-mediated DNA-cleavage, the 2:1 complex could produce more efficient binding to the nucleic acid, along with a higher local production of radical species. It is worth recalling that another antibiotic like chromomycin is known to bind DNA as a dimer stabilized by a metal ion bridge [13]. As far as the topoisomerase II-mediated mechanism is concerned, a double preference of STN for positions +2 (T) and +3 (A) from the cleavage site has been demonstrated [5]. Interestingly, the χ^2 values of biased drug distribution are almost identical at the two positions. This might suggest the simultaneous presence of two drug molecules located symmetrically with reference to the cut operated by the enzyme at each strand. The in vitro assays are carried out in the absence of Zn^{2+}. Hence, we cannot infer a metal ion mediated mechanism, unless we consider Mg^{2+}, present at mM concentrations, as a suitable bivalent positively charged center. However, the physiological presence of zinc, would facilitate dimerization at the staggered bases in the cleavable complex in vivo. Moreover, the affinity exhibited by the STN–Zn^{2+} complex for single-stranded DNA might suggest a possible interference with the DNA-resealing step operated by the enzyme on the sticky ends of the cleaved nucleic acid. Further studies on the effects of zinc ions on the topoisomerase-mediated STN-stimulated cleavage of DNA are warranted to clarify the relevance of the proposed mechanism.

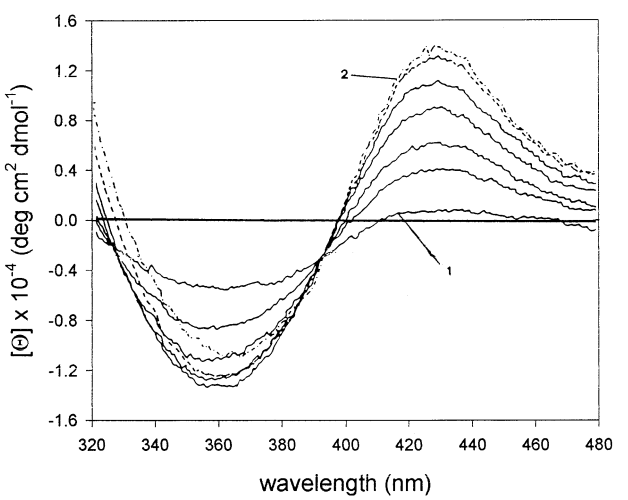

Fig. 6. CD titration of a STN/ss-DNA mixture ([STN] = 3.8×10^{-5} M, DNA/STN = 50) with increasing amounts of Zn^{2+}. Curve 1 is recorded in the absence of Zn^{2+}, curve 2 at a Zn/STN ratio of 19.2. Measurements are made in 10 mM Tris, 20 mM NaCl, pH 7.0, 23°C.

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