DNA-Binding Properties of Heavy-Metal Complexes and Consequences of Structural Changes

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SUMMARY: An overview is presented of the key role for heavy-metal compounds in medicine with a focus on DNA binding, and on Pt compounds used in treatment of cancer. Molecular aspects of the mechanism of action are reviewed in more detail and the consequences of structural distortions will be elucidated.

Introduction

Upon confrontation with the term "heavy metals", many people - including scientists - will primarily have associations with toxicity and dangerous materials. Relatively few will realize that a variety of heavy-metal compounds are extremely useful applied as drugs to cure diseases. A selection of well-known examples includes Silver (to protect the skin after burning wounds), radioactive Technetium compounds (as diagnostics for diseases), Copper (arthritis treatment), Bismuth (treatment of diarrhoea; curing of stomach ulcers), Gold (treatment of arthritis) and Platinum (very efficient tumor curing). Some of these metal compounds are known or suspected to have an interaction with nucleic acids, and the consequences of this binding can be a structural change in the DNA or RNA, with important biological or medicinal follow-up reactions.

According to a long-standing principle all metals are poisons, depending on the dose; however, certain very toxic metals are nevertheless crucial for life, either as a trace element or as a drug. Even though many metal-containing drugs are often quite simple compounds, their mechanism of action is rather complicated, involving multiple and subsequent interactions with e.g. DNA and/or proteins. This manuscript will focus on metal-ion binding to DNA, in relationship to the mechanism of action of Pt antitumor drugs, with preliminary results dealing with ruthenium complexes. Most complexes have the general formula *cis*-$PtX_2(NHR_2)_2$ (R = almost any organic fragment; X = leaving group, such as chloride, sulfate, or bis(carboxylate).

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In particular the multifunctional interactions of nucleic acids and other cellular components with metal compounds, like cisplatin, nowadays often called supramolecular interactions, are an exciting field of study and details of the interaction on the molecular level in the complex cellular mixture remain scarce[1a,b)]. It is also well known that cells of living tissue are complicated compartments, filled with macromolecules, many of which have a strong binding affinity for (certain) metal ions. Some will react very fast, other will react slower, whereas again others might be hardly reactive towards metal species.

A highly relevant question dealing with the application and understanding of the mechanism of metal-containing drugs, is: "How dangerous are heavy metals and their compounds[2)], i.e. the role of curing over the disadvantage of the side effects?" For the chemist the chemical form of heavy metal compounds is crucial, and will determine whether or not such a compound can be used in - for instance - a medicinal application.

A few aspects of current interest in metal-nucleic acid interactions within the cellular environment, but not yet studied in great detail will be addressed. Important topics are listed and from these 1 and 3 will be discussed below in more detail.

1. The role of H bonding in the cellular reactions. We have shown earlier[1)] that the amine N- H group is important for the activity of the Pt compound, probably related to the preferred ionic/dipolar interaction with nucleic acids, such as in discriminating between A and G. Stabilization of the Pt-bound DNA is also crucial, and N-H to phosphate hydrogen bonds may play a key role here.

2. The fact that Pt amine compounds do end up at purine N7 sites is now believed to be related to a migration of Pt from a S donor site to a N7 site. So reactions of S-guanosyl-L-homocysteine (SGH) do yield $[Pt(dien)(SGH\text{-}\underline{S})]^{2+}$ (starting from [PtCl(dien)]Cl at $2<pH<6.5$), but isomerize intramolecularly into [Pt(dien)(SGH-N7) with Pt coordination at N7 of guanosine. Studies with larger nucleopeptide fragments and using $Pt(en)^{2+}$ have confirmed this[3)]. This work clearly suggests that also under *in vivo* conditions Pt species might migrate from S to N donor ligands.

3. The fact that many new (types of) compounds (both unconventional Pt and also Ru species) show antitumor activity and DNA binding, will also be addressed[4)].

Important parameters in these interactions are: The charge and the size of the metal ion; the type of metal ion and its position in the periodic table; the presence and nature of co-ligands. Also the inter-biomacromolecular interactions not dealing with the metal ion (H-bonds, stacking, salt bridges, charge-transfer units) should be mentioned.

Discussion of recent Results

To understand the binding of metal ions to nucleic acids requires to start at the basic coordination sites of the DNA and RNA. In general terms, metal ions (or metal compounds with empty coordination sites) could bind to nucleic acids at:

a) The phosphate back bone with a negative charge on the oxygen atoms;

b) The alcohol groups of the sugar (with or without ionization);

c) The oxygen atoms of the bases (aromatic-linked oxygens still have lone pairs);

d) The nitrogen atoms of the bases (some of them very basic).

It is known from text-book chemistry that heavy-metal compounds do preferentially bind at N-donor sites, when more options are available. This has indeed been found for all heavy-metal ions. A schematic view of metal ion binding sites in the four bases of DNA is redrawn in Figure 1.

Under most conditions the bases will not dehydronate, but in some cases loss of H^+ does occur, and for instance N6 binding at adenine has been found.

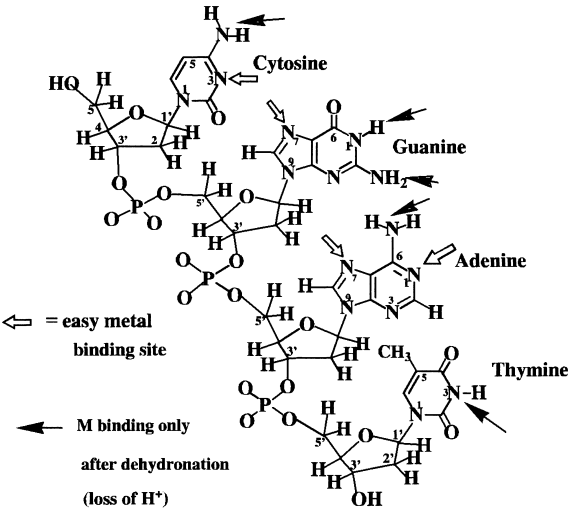

Fig. 1: Schematic binding possibilities of metal ions on the N-donor sites of nucleic acid bases, with an without loss of hydrogen ions.

The strong preference of binding (already at neutral pH) of many metal ions to G-N7, and also of the antitumor drug cisplatin, has been attributed to an intrinsic higher pK_a(base) for the guanine-N7 site, compared to e.g. adenine. However, also a kinetic effect in the approach of the N7 sites, by a hydrogen-bond interaction of an aqua ligand with O6, is likely to play a role; this also is the final stabilization, as has been seen in several crystal structures.

As an example, the binding of the antitumor drug cisplatin to the two neighboring guanine sites in double-stranded DNA will be used below. At first sight two neighboring G-N7 sites are not able to chelate a metal unit with two vacant sites in cis orientation, like e.g. present in *cis*-$[Pt(NH_3)_2]^{2+}$; that this nevertheless happens is a consequence of a structural change resulting from the binding. This binding has been described and reviewed before[1] and will therefore not be repeated in detail here.

Very simple platinum compounds, like *cis*-$PtCl_2(NH_3)_2$ (abbreviated as CDDP, cis-Pt, or cisplatin), have been known since 1845. Remarkably, the biological activity of the parent compound has been reported only 30 years ago[5]. More recently, several derivatives have been reported, initially dealing with variations of the classical species and its antitumor properties . However, increasingly also completely new types have been reported. In Figure 2, a few examples of classical and of these newer types of compounds, showing surprising activity, have been depicted. The trinuclear compound BBR3464 is a variation of a known type of dinuclear compounds[6], but now extended with a 2+ charge in the center, allowing strong interactions within the minor DNA groove, similar to the interactions of spermine-type compounds. The two mononuclear compounds have surprising activity, as they were initially believed not to be so useful. The AMD-473 is a beautiful example of

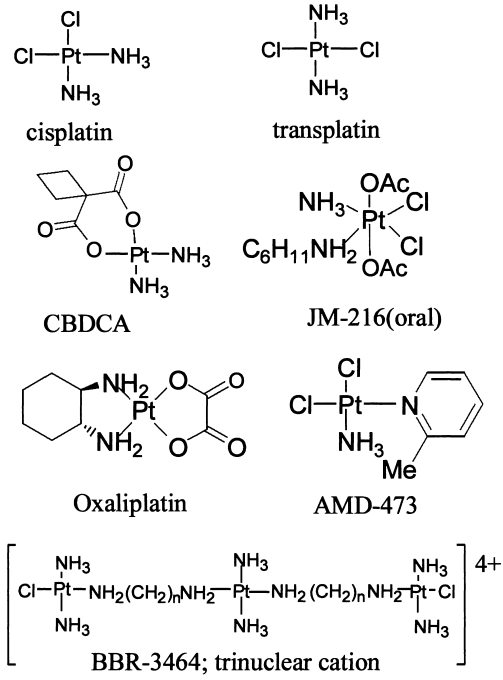

Fig. 2: Six examples of clinically used Platinum compounds, and the inactive transplatin isomer.

kinetic ligand-exchange control by steric effects of the ligand[7,8]. The oxaliplatin[9] belongs to the well-known group of active compounds; it appears promising in the treatment of colorectal tumors when applied in combination therapy with 5-fluorouracil.

Significant improvements of the insight into the mechanism of action of cisplatin (and therefore also in the derivatives), in this case their interaction with biomacromolecules, such as DNA, has recently evolved, and these will be briefly summarized. The crucial elements in its mechanism of action appear to be: (1) a carefully controlled hydrolysis of cisplatin (both kinetically and thermodynamically), transport through membranes and in the cells, eventually followed by binding to DNA; (2) an unusually selective binding at two neighboring guanine bases, resulting in a highly specific distortion of DNA, changing its interactions with proteins. Known other lesions, like the AG adducts and interstrand cross-linked GpG adducts (that have been reported earlier, albeit in much smaller amounts) are likely to be less important[5].

Whether all the most recently reported, active new compounds, like the ones depicted in Figure 2 and other ones, will bind to DNA similarly or in completely different ways, is being studied in many laboratories. At least for the trinuclear compounds (and also for related dinuclear compounds), this type of binding would be predicted to be quite different for geometric reasons[10]. Basically, and when we restrict ourselves to Guanine-N7 sites, one can distinguish 3 major DNA-bindings, namely: (a) intrastrand GG crosslinks; (b) intrastrand $G(X)_nG$ crosslinks; (c) intrastrand G-G crosslinks.

First about the intrastrand crosslink. The DNA structure does alter significantly after monofunctional binding and after chelation of cis-Pt type compounds, albeit it less then originally predicted. A large kink (with variation from 35-80 degrees bending) and unwinding occurs, as proven by NMR and X-ray diffraction studies[11-13]. An example of such a kink is shown in Figure 3. In several other cases very similar structures have been seen, but the degree of distortion and the kink angle very much depend on the actual sequence.

Fig.3: Distorted DNA after chelation of cisplatin at a bis(G-N7) site.

In the case of the $G(X)_nG$ crosslink with cisplatin and transplatin the distortion is again reasonable, as seen from a recent study of n=1 and X=T of Teuben[14], with a clearly visible removed non-basepaired thymidine; this structure is redrawn in Figure 4.

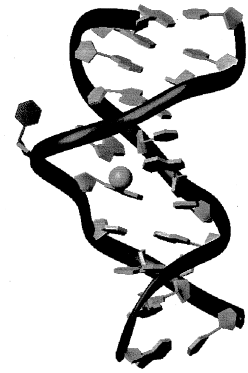

Fig. 4: Structure of ds DNA after Pt chelation at a G-T-G site.

An X-ray structure of the interstrand GN7-GN7 adduct by Malinge et al.[15] has been redrawn in Figure 5. The structure is very regular as a result of the removal of the cytosine from the double-stranded structure.

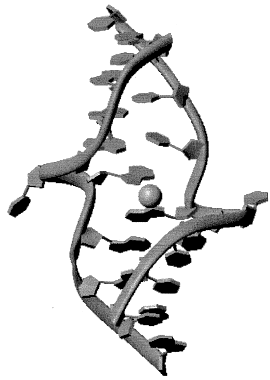

Fig. 5: Interstrand crosslink by cisplatin with bulged-out cytosines.

Although little is known as yet on the newer Pt complexes, at least one different binding form has been found, i.e. hairpin structure formation[16] and also increased interstrand cross-link has been reported. A very exciting question deals with the biological consequences of the altered DNA structure? It appears that certain proteins bind at the platinated DNA, subsequently interfering with gene expression and/or repair and recent work of Lippard[17] has made clear that protein binding is crucial. In fact most recently the crystal structure of a complex of an HMG domain with the major cisplatin d(GG) intrastrand adduct has been determined, as redrawn in Figure 6.

After platinum binding to the DNA, followed by binding of HMG proteins subsequent effects are being studied. These proteins subsequently interact with p53 proteins, which has led to the hypothesis that p53-dependent apoptosis and/or p53-independent cell cycle arrest are crucial[18] and the role of telomere shortening in cisplatin treated cells is under extensive study[19]. Finally, evidence was found that cisplatin forms complexes in the cellular membranes with the phosphatidylserine present[20].

Fig. 6: Ternary cisplatin adduct with ds DNA and a HMG protein.

Certainly the last few years enormous progress was made in the understanding of the mechanism of action of platinum compounds that exhibit anticancer activity and definitely in their DNA binding consequences. Improved antitumor drugs will certainly become available based on the detailed knowledge of the Pt-DNA adducts and on the kinetics of their binding to cellular components, like proteins and DNA.

Finally about other metal ions and DNA binding. The antitumor chemistry of cisplatin has strongly stimulated similar studies on other metals, and it has become clear in recent years that Ru(II) and Ru(III) - each having ligand-exchange kinetics similar to Pt(II) - are very interesting and promising indeed. Some ruthenium ammine complexes showing antitumor activity were reported a long time ago, but most recently other compounds have shown to be more promising and are ready to enter the clinic. The ionic Ru-containing species of formula $[H_2im]$*trans*-$[RuCl_4(dmso)(Him)]$ is close to clinical phase I in Italy[21], and other Ru compounds are closely following[4,22]. Again - just as in the case of Pt - the role of intramolecular hydrogen bonding appears to be very important[4]. Also Ru binding (octahedral species) has quite different steric requirements, influencing the DNA binding, even on the oligonucleotide level, significantly[23].

Concluding remarks

From the results reviewed and discussed in the present paper, it should be clear that metal ions and in particular heavy metal ions play a very important role in every-day life. Their interaction with biological macromolecules is important, and in particular their interactions with nucleic acids are crucial to control, regulate and repair biological processes.

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