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Amino-acid-linked platinum(II) analogues have altered specificity for RNA compared to cisplatin

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Cisplatin can be modified with various ligands to alter the size and charge distribution of the complex. Several amino-acid-linked platinum(II) complexes were synthesized, and a reactivity study with 16S ribosomal RNA was carried out. The amino-acid-linked analogues show altered specificity compared to the parental compound cisplatin.

Cis-diamminedichloridoplatinum(II), or cisplatin, is a clinically used antitumor drug that has long been believed to react preferentially with DNA over RNA.1, 2 The cross-links at d(GpG) sequences are considered to be crucial for its biological activity.3

The main coordination site of cisplatin on DNA is the N7 of guanine, and the complex forms adducts mainly at runs of consecutive Gs, with less reactivity at d(GpA) and d(ApG) sequences.4, 5 In order to improve the effectiveness of the drug, many cisplatin analogues have been generated.2 Some analogues display altered sequence preferences. For example, a tethered 9-aminoacridine-4-carboxamide platinum(II) complex, which contains an additional amino group and positive charge compared to cisplatin, shows increased reactivity at d(GpA) sequences.6

Similarly, promising new Pt(II) complexes with antitumor activity have been developed, which coordinate to single nucleobases and participate in different mechanisms of DNA damage and downstream events.7, 8 Other modifications include charged, neutral, or hydrophobic amino acids and peptides as ligands to replace the amines.9, 10

In addition to its use as an antitumor agent, cisplatin has also been used to probe accessible guanines in RNA such as the 16S ribosomal RNA either isolated or in the context of complete ribosomes, both in vitro and in cellulo.11, 12 Recently, in vitro and in vivo RNA labeling by a novel compound picazoplatin was reported.13 However, the potential of cisplatin analogues to react with RNA compared to DNA has not been fully explored. As discovered through the development of novel platinum antitumor drugs, one of the key advantages of metal-based probes such as cisplatin is the ease with which their properties such as size and charge can be altered. In an attempt to vary the coordination sites from G-rich sequences to other sequences, and gain further information about accessibility of RNA secondary and tertiary structures towards various ligands, the platinum(II) complexes were modified to contain different functionalities. Specifically, amino-acid-linked platinum(II) complexes (Figure 1), employing lysine (Kplatin), ornithine (Oplatin), arginine (Rplatin), and aspartate (Dplatin) as ligands, were used to probe the 16S rRNA at a region known as helix 24 (h24 or 790 loop). This region has been shown previously to be a strong target site of cisplatin.11

The amino-acid-linked platinum(II) complexes were converted to the moaunoaquated species and used to probe 16S rRNA. The moaunoaquated species (Figure 1) have a different distribution of positive charge compared to the parental compound cisplatin, as well as different sizes and hydrogen-bonding capabilities. Therefore, a change in reactivity with the rRNA was anticipated. The reactive sites of the moaunoaquated complexes were mapped on 16S rRNA by using primer extension analysis.11 The 16S rRNA was isolated from E. coli MRE 600 and renatured as described previously.12 The reactions were carried out with a 1:20 molar ratio of 16S rRNA: metal complex in 20 mM HEPES, pH 6.5, at 37 °C in the dark for 5 hours. The reaction was quenched with 0.2 M NaCl, followed by immediate freezing and ethanol precipitation. To compare the platinum reactive sites directly with cisplatin, the functionally important rRNA region of h24 was selected for analysis. The adduct sites were mapped with a 32P-5'-end-labeled DNA primer (5'-CCAAGTCGACATCGTTT-3'), which anneals at residues 831–814 of h24 (E. coli numbering), reverse transcription (RT), and comparison to deoxy sequencing reactions on 8% denaturing polyacrylamide gels.

The magnitudes of platinum reactivity were classified by intensities of the bands on autoradiograms. Coordination sites of the amino-acid-linked complexes were determined by comparing with a control sample treated in a similar manner, but lacking metal complex. The RT stops occur one nucleotide prior to the coordination site. The mapping results show clear differences in the reactive sites of amino-acid-linked complexes and cisplatin (Figures 2 and S1, Supplementary Information). As shown previously, cisplatin reacts with consecutive Gs, as well as Gs in mismatched or loop regions.13, 14 In contrast, Kplatin, Oplatin, Rplatin, and Dplatin show quite different patterns of reactivity than cisplatin. For example, consecutive Gs in h24 that show
strong reactivity with cisplatin (e.g., G799-800) are much less reactive towards the amino-acid-linked complexes. Instead, Kplatin and Oplatin are observed to react with A residues in the loop region of h24.

In h24, the strong cisplatin coordination site that was observed at G803 displays only moderate reactivity with monoaquated Oplatin. In contrast, several strong reactive sites are indicated in the h24 loop region for these complexes (Figure 2). For example, strong RT stops occur at C795, G791, and U788, which are all located on the 3′ side of A residues (A, ApG, and GpA sites, respectively). A moderate RT stop is also observed at U804 for Kplatin, which corresponds to a reactive ApG site. When the coordinating ligand is changed to arginine, the resulting complex (Rplatin) shows a different strong RT stop than Oplatin or Kplatin at G778. This reaction occurs at the three-nucleotide asymmetric bulge region of h24, specifically at an A residue (A777). Only minor reactivity by Rplatin is observed at the sites that show strong reactivity with Kplatin and Oplatin. In contrast, there are no strong RT stops observed with Dplatin.

Together, the probing results indicate that electrostatics and structure are likely to be important factors for the platinum complexes to bind and coordinate to RNA at specific sites. The strong cisplatin RT stop at A787 neighbors consecutive Gs in the stem region and occurs at an A-C mismatch. In contrast, the monoaquated complexes of Oplatin and Kplatin appear to react at G786 and A787 (GpA), thus shifting the preferred site by one nucleotide compared to cisplatin (G785 and G786, or GpG). Various other RT stop sites are observed in all lanes of Figure 2, including the control lane, due to secondary structures in the RNA template that block reverse transcriptase.

Probing studies on h24 of 16S rRNA reveal that the coordination site preferences for Oplatin and Kplatin are quite similar, comprising A, ApG, or GpA sequences in loop or bulge regions. This similarity in reactivity by Oplatin and Kplatin is not surprising, because these two complexes differ only by a CH₂ group and contain the same positively charged side chain. Rplatin shows differences in reactivity compared to the other amino-acid analogues tested, such as a strong preference to a nucleotide in a bulge region. Compared to cisplatin, the reactivity profiles of the amino-acid complexes are altered considerably. The amino acids increase the bulkiness of the ammine or cis ligand and have altered charge distribution, which in turn alters interactions with the negatively charged nucleic acid. In contrast, the negatively charged complex Dplatin does not show any significant reactivity with h24 compared to the other complexes at the same reaction ratios, suggesting that electrostatic repulsion prevents interactions with the RNA target.

Previously, the coordination sites of Kplatin were mapped on an 82-bp DNA fragment by exonuclease. This mapping study revealed that Kplatin reacted at both d(ApG) and d(GpG) sites in DNA with no apparent preference for either sequence. In the present study with RNA, several ApG and GpA sites show strong reactivity with Oplatin and Kplatin, whereas most of the GpG sequences within h24 do not show any reactivity. The Rplatin reactivity is quite different than the other complexes. Rather than a sequence preference, this complex shows a structural preference, with strongest reactivity at the three-nucleotide bulge (A777) and minor reactivity at the loop-closing base pair (A794) of h24. This difference in reactivity compared to Oplatin and Kplatin likely arises from the altered ligand structure. Interestingly, the coordination of Rplatin at the internal bulge on h24 resembles the TAR-Tat interaction in which the arginine-rich Tat protein binds at the three-nucleotide bulge region of TAR RNA. Arginine is an ideal RNA-binding molecule because of its positive charge, planar hydrogen-bonding pattern, and potential for stacking interactions with the RNA bases. It has been noted previously that binding to RNA occurs at such bulge sites because the major groove is more accessible.

In order to examine more closely the nucleotide preferences of the amino-acid-linked analogues, monoaquated Oplatin was reacted with adenosine and guanosine at different ratios and lengths of time. The reactivity was monitored by reverse-phase (C18) HPLC, in which unreacted nucleoside was quantified at different time points, and the results were compared to the control reactions without Oplatin (the peak area of unreacted nucleoside was divided by the corresponding nucleoside peak area of the control reaction). With both 10-fold and 50-fold excess monoaquated Oplatin, the level of reactivity with adenosine is significantly higher than guanosine (Figure S2, Supplementary Information). For example, after 2 hours (1:50 ratio), more than 70% adenosine has reacted compared to 30% of guanosine under identical conditions.

![Image](55x383 to 286x565)

Figure 2. Autoradiogram of probing results with amino-acid-linked complexes on helix 24 (h24) of 16S rRNA. A) The secondary structure map of 16S rRNA at h24 is shown. Strong reactive sites with cisplatin (▲), Oplatin and Kplatin (★), and Rplatin (○), and moderate reactive sites with cisplatin (▲), Oplatin and Kplatin (★), and Rplatin (○) are indicated. B) The autoradiogram shows the RT stops resulting from platination. The sequencing lanes are indicated by C, U, A, and G. The control contained no metal complex in the reaction mixture. The remaining lanes are 16S rRNA treated with various amino-acid-linked platinum(II) complexes at a complex: nucleotide ratio of 1:75. Strong and moderate RT stop sites are indicated with symbols and corresponding nucleotide numbers.
A competition reaction with both purine nucleosides and excess monoaquated Oplatin was also carried out (Figure 3). In both experiments (10 or 50-fold excess platinum) with a time course of 0 to 10 h, the reaction of adenosine was greater than guanosine (at a 1:1:50 ratio, after 2 hours, 70% of adenosine has reacted compared to 20% of guanosine, panel B). This result reveals the preference of Oplatin for adenosine over guanosine, and is in contrast to prior studies that showed a preference of cisplatin for guanosine residues in DNA. The Oplatin has a different ligand size and charge distribution compared to cisplatin, which clearly leads to an altered nucleoside or nucleotide preference in RNA.

Together, these results on 16S rRNA and single nucleosides show that positively charged amino-acid-linked complexes coordinate to RNA with different sequence selectivity than cisplatin, with an apparent preference for A, ApG, and GpA sites in the rRNA h24. The substitution of one ammine ligand and placement of an amino or guanidinium group on the side chain alter both the structure and charge distribution of the complexes, leading to alternate target sites compared to the parental compound cisplatin. These results demonstrate that the accessibility of adenosines within the 16S rRNA or other large, biologically relevant RNAs can be monitored. Furthermore, the higher reactivity of Rplatin to the bulge or loop provides additional information about accessibility to RNA motifs for ligand binding. The fact that these metal complexes are small (MW ~ 400), charged, and also coordinate to RNA, will allow them to be used to monitor the kinetics and illustrate how other charged molecules recognize their target sites in complex systems.

Considering these results on rRNA h24, newly designed Pt(II) analogues might also have different reactivity profiles in DNA versus RNA, which needs to be further explored. Overall, the future applications of these complexes are two fold. First, they can be exploited as chemical probes to map the accessible sites and electrostatic environments in complex systems such as the ribosome, which could serve as valuable targets for future drug design. Second, the identification of in vivo RNA targets could have broader applications to explore Pt(II)-based drugs with reduced dose-dependent side effects.

Notes and references

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‡ Electronic Supplementary Information (ESI) available: detailed experimental procedures, synthesis, platination reaction, and HPLC. See DOI: 10.1039/b000000x/.

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