Journal of Inorganic Biochemistry 182 (2018) 177–183

Contents lists available at ScienceDirect

Journal of Inorganic Biochemistry

journal homepage: www.elsevier.com/locate/jinorgbio

Ruthenium anticancer agent KP1019 binds more tightly than NAMI-A to tRNA^{Phe}

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ARTICLE INFO

Keywords:
NAMI-A
KP1019
RNA
tRNA
Anticancer drug
Metallodrug
Ruthenium

ABSTRACT

The ruthenium-based anticancer agent NAMI-A (ImH[*trans*-$RuCl_4$(dmso)(Im)], where Im = imidazole) has been shown to interact with RNA in vivo and in vitro. We hypothesized that the similarly structured drug KP1019 (IndH[*trans*-$RuCl_4(Ind)_2$], where Ind = indazole) binds to RNA as well. Fluorescence spectroscopy was employed to assay the interactions between either NAMI-A or KP1019 and tRNA^{Phe} through an intrinsic fluorophore wybutosine (Y) base and by extrinsic displacement of the intercalating agent ethidium bromide. In both the intrinsic Y-base and extrinsic ethidium bromide studies, KP1019 exhibited tighter binding to phenylalanine-specific tRNA (tRNA^{Phe}) than NAMI-A. In the ethidium bromide study, reducing both drugs from Ru^{III} to Ru^{II} resulted in a significant decrease in binding. Our findings suggest that the relatively large heteroaromatic indazole ligands of KP1019 intercalate in the π-stacks of tRNA^{Phe} within structurally complex binding pockets. In addition, NAMI-A appears to be sensitive to destabilizing electrostatic interactions with the negative phosphate backbone of tRNA^{Phe}. Interactions with additional tRNA molecules and other types of RNA require further evaluation to determine the role of RNA in the mechanisms of action for KP1019 and to better understand how Ru drugs fundamentally interact with biomolecules that are more structurally sophisticated than short DNA oligonucleotides. To the best of our knowledge, this is the first study to report KP1019 binding interactions with RNA.

1. Introduction

When NAMI-A (ImH[*trans*-$RuCl_4$(dmso)(Im)], where Im = imidazole) became the first Ru anticancer agent to enter clinical trials, it was thought to cause apoptosis by binding to DNA, similar to the cytotoxic chemotherapeutic cisplatin (Fig. 1) [1]. NAMI-A was found to bind not only to DNA [2,3] but also to RNA [4] and proteins [1]. Although NAMI-A showed tremendous promise as an antimetastatic agent [5,6] due to multiple interactions inside and outside of the cell, it was deemed only moderately tolerable in a phase I/II clinical trial [6]. While the clinical focus has shifted to KP1019 (IndH[*trans*-$RuCl_4(Ind)_2$], where Ind = indazole) and NKP-1339 (Na[*trans*-$RuCl_4(Ind)_2$]) (Fig. 1), as well as ruthenium(II)-arene based drugs [7], NAMI-A continues to serve an important role in understanding the biochemical and pharmacological properties of Ru-based drugs.

KP1019 and NKP-1339, the sodium salt analogue of KP1019, have undergone clinical evaluations [8,9] and because of their identical Ru coordination spheres they are expected to have similar modes of action [10,11]. Although NKP-1339 was found to accumulate preferentially in the cell nucleus (approximately 90%) and KP1019 mainly localized in the cytoplasm (approximately 75%), both Ru drugs were found to induce apoptosis via similar pathways regardless of different intracellular distribution patterns [10]. The cytotoxic effect of NKP-1339 and KP1019 appears to result from endoplasmic reticulum (ER) stress due to generation of reactive oxygen species (ROS) [12]; however, the molecular target(s) for inducing ER and ROS stress remain(s) unknown. Although it has been speculated that proteins could be the drug target [12–14], interactions with single nucleotides, oligonucleotides, and RNA molecules in the cytoplasm may play a role in the mechanism of action even if they are not the primary target [15].

Previous studies examining the interactions of NAMI-A and KP1019 with nucleic acids have been limited to short single or double-stranded oligonucleotides [15,16]. Interactions with short oligonucleotides are largely characterized by interactions with specific bases, whereas more sophisticated nucleic acid structures such as tRNA provide varied binding pockets due to their rich tertiary structures. Mass spectrometry and two-dimensional NMR studies found that both Ru drugs exhibited significant preferential binding to guanine in short DNA

Abbreviations: KP1019, IndH[*trans*-$RuCl_4(Ind)_2$]; NAMI-A, ImH[*trans*-$RuCl_4$(dmso)(Im)]; Ind, Indazole; Im, Imidazole; DMSO, Dimethylsulfoxide; tRNA, Transfer RNA; tRNA^{Phe}, Phenylalanine-specific tRNA; FTIR, Fourier transform infrared; PAGE, Polyacrylamide electrophoresis

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https://doi.org/10.1016/j.jinorgbio.2018.02.019
Received 16 January 2018; Received in revised form 22 February 2018; Accepted 23 February 2018
Available online 24 February 2018
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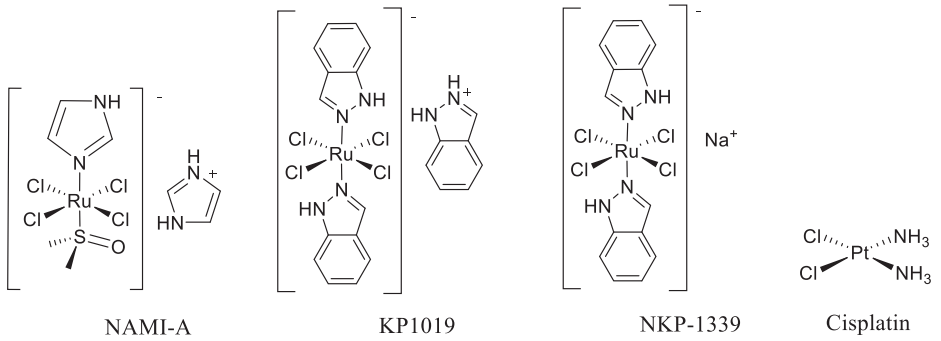

Fig. 1. Structures of NAMI-A, KP1019, NKP-1339, and cisplatin.

oligonucleotides through direct Ru-guanine coordination as well as intercalation and stacking interactions [15,17]. In this work, we investigated how the structural differences between two Ru-based drugs affect their binding affinities to a biologically ubiquitous and more structurally complex nucleic acid model molecule, tRNA.

Phenylalanine-specific tRNA ($tRNA^{Phe}$) serves as an excellent RNA model due to its rich structural features such as loops, bulges, and stems. Because all forms of tRNA have a conserved tertiary structure (Fig. 2), molecules that bind $tRNA^{Phe}$ are likely to bind to other tRNA molecules. Furthermore, tRNA is the most abundant, stable, soluble form of RNA in the cytosol [18]. Since tRNA plays a key role in senescence [19], which is being increasingly invoked as a relevant issue in creating new modalities of cancer treatment [20,21], the finding that several Ru- [22,23] and Fe-based [24] compounds induce senescence further motivates using tRNA as a molecular model.

Many metals and small molecules, including drugs, have been shown to bind to $tRNA^{Phe}$ and affect its tertiary structure [25–27]. NAMI-A was found to bind to RNA in vitro and in vivo in *Saccharomyces cerevisiae* following incubation in a 450 μM NAMI-A solution [4]. Due to the structural similarities of NAMI-A and KP1019 (Fig. 1), we hypothesized that KP1019 binds RNA as well. This is the first study to the best of our knowledge to report that KP1019 interacts with RNA. Because ascorbate can readily reduce NAMI-A and KP1019 from Ru^{III} to Ru^{II} [28–31], the effect of reducing these drugs on their binding to tRNA was also explored.

Fluorescence spectroscopy has been used to monitor structural changes via the Y-base fluorescence and effectiveness of a small molecule to displace an intercalator. The fluorophore wybutosine (Y) base at position 37 in $tRNA^{Phe}$ (Fig. 2) equilibrates between two conformations: sandwiched within internal hydrophobic π-stacks which enhances fluorescence, and "flipped out" which exposes it to aqueous solvent and quenches its fluorescence [25,32]. Accordingly, the Y-base serves as an intrinsic probe of $tRNA^{Phe}$ conformational change; any agent that binds to $tRNA^{Phe}$ and preferentially stabilizes one of the two Y-base conformations alters its fluorescence intensity.

In the extrinsic displacement study, the well-known intercalator ethidium bromide may be ejected from the π-stacks of $tRNA^{Phe}$ following binding of a drug complex. Thus, the binding interactions of KP1019, NAMI-A, and their reduced species were further explored by determining whether the drugs could displace ethidium bromide from $tRNA^{Phe}$, thereby quenching ethidium bromide fluorescence. Our goals were to specifically ascertain whether these ruthenium-based compounds affect the structure of $tRNA^{Phe}$ and more generally to contribute to the foundational knowledge of Ru drug-nucleic acid interactions in a structurally rich and biologically active model.

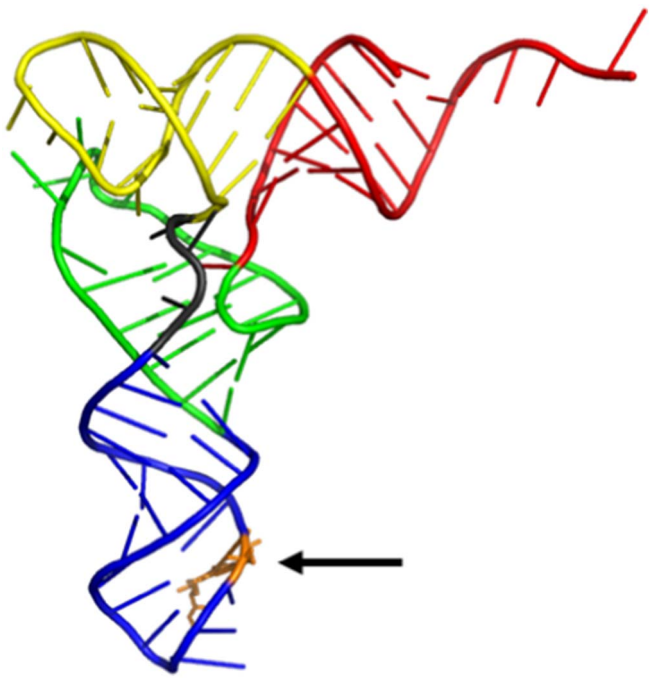

Fig. 2. The tertiary structure of $tRNA^{Phe}$. The arrow points at the Y-base in orange.

2. Materials and methods

2.1. Instrumentation

A Bruker Avance III 500 MHz High Performance Digital NMR Spectrometer was employed for ^1H NMR spectroscopy. A Nicolet iS10 FT-IR spectrometer with an attenuated total reflectance assembly was used for measurements in the mid-infrared region, while IR absorbance in the far-infrared region was measured with a Bruker Tensor 27. A Cary Eclipse Fluorimeter was used for fluorescence spectroscopy studies.

2.2. Synthesis and characterization of Ru drugs

All reagents, including $tRNA^{Phe}$, were purchased from Sigma-Aldrich unless stated otherwise. Details of the synthetic procedures and characterization are in the Supplementary Information. Briefly, NAMI-A was synthesized in a two-step procedure according to Alessio et al. [33]. UV–vis [34], FT-IR [34], and ^1H NMR [35] spectroscopy were employed to characterize NAMI-A (Supplementary Information, Tables S1-S2). KP1019 was synthesized in a two-step procedure according to Lipponer et al. [36] and was characterized with UV–vis [37], FT-IR [36], and ^1H NMR spectroscopy [36] (Supplementary Information, Tables S3–S4). Reduction of KP1019 and NAMI-A occurred immediately upon addition and mixing with equimolar quantities of aqueous ascorbic acid.