Journal of Inorganic Biochemistry 176 (2017) 134–139

Contents lists available at ScienceDirect

Journal of Inorganic Biochemistry

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

A small-molecule acts as a 'roadblock' on DNA, hampering its fundamental processes

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ARTICLE INFO

Keywords:
Coordination complex
DNA binding
Fluorescence spectroscopy
DNA polymerase
ESKAPE pathogens

ABSTRACT

DNA replication, RNA and protein synthesis are the most fundamental housekeeping processes involved in an organism's growth. Failure or dysregulation of these pathways are often deleterious to life. Therefore, selective inhibition of such processes can be crucial for the inhibition of the growth of any cell, including cancer cells, pathogenic bacteria or other deadly microbes. In the present study, a Zn^{2+} complex is shown to act as a roadblock of DNA. The Zn^{2+} complex inhibited DNA taq polymerase activity under the *in vitro* conditions of polymerase chain reaction (PCR). Under *in vivo* conditions, it readily crosses the cell wall of gram-negative bacteria (*Escherichia coli*), leading to the reduction of RNA levels as well as protein content. Growth of pathogenic bacteria (*e.g.*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*) was also significantly retarded. The Zn^{2+} complex binds to the grooves of the DNA without inducing conformational changes or exhibiting chemical nuclease activity. To the best current knowledge, this is first coordination complex exhibiting a 'roadblock' property under both *in vitro* and *in vivo* conditions (show at all three levels – DNA, RNA and protein). The label-free approach used in this study may offer an alternative route towards fighting pathogenic bacteria or cancer cells by hampering fundamental cellular processes.

1. Introduction

In cancer cells, most metabolic rates are higher than those found in healthy cells, including fundamental housekeeping processes such as DNA replication, repair and fragmentation [1]. DNA and DNA binding proteins are pivotal to most biochemical processes. For example, RNA polymerase and other factors bind to DNA, resulting in RNA synthesis and eventually protein synthesis. These proteins, in turn, regulate the organisms' growth by participating in biochemical reactions. Therefore, DNA-binding proteins play an essential and critical role in cell growth and development, as they control fundamental biochemical activities including DNA replication and transcription. Therefore, selective inhibition of such processes involving housekeeping proteins can be crucial for the inhibition of growth of any type of cell, including cancer cells, pathogenic bacteria or other deadly microbes [2–4].

There is a continuous effort to develop small molecules, including metal-based coordination complexes, for oncotherapy as well as novel anti-microbial agents [5–8]. Since the discovery of the cisplatin/carboplatin, several coordination complexes without platinum have been synthesized for improved reactivity and activity against broad range of cancer cells [7]. The properties of coordination complexes, such as the metal centre and the organic skeletal of the ligand, can be tuned to suit the desired biological activity. Recently, pyridine-based metal complexes have been developed for DNA binding [9] and thus may act as blockers of DNA-binding/walking proteins [9], inhibiting important biological processes including DNA polymerization [7]. Recently, Yu et al. showed inhibition of Taq polymerase function during PCR using a Ruthenium-based polypyridyl complex [10] and Chandra et al. using Iridium complexes [11]. Many of the currently developed coordination complexes exhibited cytotoxicity under *in vitro* conditions with either an apoptotic mode of action, or more often with an unknown mechanism, without knowing their cellular targets [12]. However, it is of medical interest to find coordination complexes/small molecules which are able to cross the cell wall/membrane barrier and exhibit cell toxicity by targeting the biomolecules responsible for specific housekeeping pathways [5,13].

Redox stability, variability in coordination chemistry and reduced metal toxicity makes Zn(II) one of the most common metal ions found in biological systems [14,15]. Zn-based coordination compounds have been studied for various activities, including toxicity towards infectious organisms [16]. They exhibit more specific functions, such as the inhibition of caspase-3 activity and promotion of ErbB1-ErbB2 heterodimerization by Zinc pyrithione [17], inhibition of cyclin-dependent kinase CDK1 [18]and inhibition of parathyroid hormone activity [13].

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http://dx.doi.org/10.1016/j.jinorgbio.2017.08.023
Received 16 May 2017; Received in revised form 2 August 2017; Accepted 25 August 2017
Available online 06 September 2017

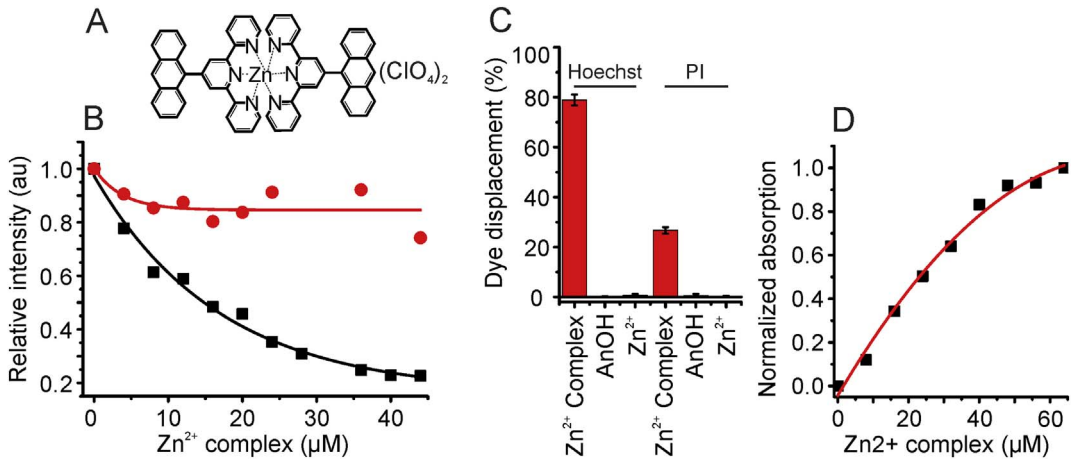

Fig. 1. Interaction of the Zn^{2+} complex with DNA. (A) Chemical structure of the Zn^{2+} complex. (B) Relative fluorescence intensity plot of spectral traces obtained during the dye displacement experiment. Red (—) represents propidium iodide and black (—) represents Hoechst 33,258. (C) Percentage of dye displacement for the Zn^{2+} complex along with the control molecules. (D) Normalized absorption plot of DNA titrated against Zn^{2+} complex. ■ indicate data point and — shows fitting. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Induction of phosphorylation of the Akt downstream effector glycogen synthase kinase 3β and thus proposed to serve as lead structures for developing antidiabetic drugs and useful tools for regulating glucose metabolism to name but a few examples [19]. Zn^{2+} complexes are also known to exhibit antibacterial/antimicrobial, anticancer activities, interacting with DNA and the inducing protein aggregation [20–26].

In the present study, a water-soluble coordination complex of Zn(II) and anthracenyl terpyridine (Figs. 1 and S1) [6,13] has been employed as a roadblock of DNA preventing polymerase activity. The Zn^{2+} complex inhibited DNA taq polymerase activity under the *in vitro* conditions of PCR. Under *in vivo* conditions, it readily crosses the cell wall barrier of gram-negative bacteria (*E. coli*), leading to the reduction in RNA as well as protein content.

2. Results

2.1. Interaction of Zn^{2+} complex to DNA

The fundamental aspect of working as a barrier is a physical interaction with DNA [6,8,27]. A fluorescent dye displacement method can be utilized to evaluate the mode of binding. Propidium iodide and Hoechst 33,258 were chosen as intercalating and groove (minor)-binding dyes, respectively [6]. These dyes have minimal fluorescence when free in aqueous solution. Their fluorescence intensity enhances several-fold once they bind to nucleic acids. Consequently, upon addition of the dye to dsDNA, a significant enhancement in their fluorescence intensity was observed. Gradual addition of the Zn^{2+} complex to a solution containing DNA and propidium iodide leads to a marginal decrease in fluorescence intensity. This shows that the Zn^{2+} complex was not intercalating with the dsDNA. The Zn^{2+} complex was able to displace 20–30% of the propidium iodide intercalated to DNA. On the other hand, addition of the Zn^{2+} complex significantly diminishes Hoechst 33,258 fluorescence intensity. Further analysis suggested that the Zn^{2+} complex is able to displace the Hoechst 33,258 up to 80–90% (Fig. 1B and C). The results clearly indicated that Zn^{2+} complex interactions occur at the groove of the dsDNA. As a control molecule, 9-anthracene methanol or Zn^{2+}-perchlorate were incubated with the DNA, with neither able to displace either dye (Figs. 1C and S2). Absorption studies provide further evidence for the Zn^{2+} complex interaction with DNA. A fit of the data obtained a K_D of 2.7 μM (Figs. 1D and S3). Previous results indicated that replacement of the Zn^{2+} with Cu^{2+} completely changes the binding mode of the coordination complex. The Cu^{2+} complex bearing the same organic skeletal system binds to the dsDNA *via* intercalation [6], in contrast to the groove-binding Zn^{2+} complex herein. The binding mode of the complex is thus directed by the metal ion. The central metal ion might reinforce binding affinity with the negatively charged phosphate groups of DNA *via* electrostatic interactions. Additional molecular interactions are contributed by the organic skeletal system to displace Hoechst 33,258.

2.2. Inhibition of DNA polymerase activity during PCR

In general, the grooves of the DNA expose the most functional groups to biomolecules, hence most biological activity takes place at these sites *via* the recognition of specific sequences to DNA-binding proteins [28]. With the understanding of the groove-binding nature of the Zn^{2+} complex, 'roadblock' activity was investigated during PCR using Taq polymerase. The complete PCR mixture containing plasmid DNA, primers, dNTPs, DNA polymerase, suitable buffer for enzyme activity and an increasing amount of the Zn^{2+} complex, was run for 30 cycles. The PCR products were analysed by 1% agarose gel electrophoresis. The band intensity on the agarose gel could be directly correlated with 'roadblock' activity. A gradual decrease in the PCR product was observed with an increasing amount of the Zn^{2+} complex (Fig. 2A–C). The IC_{50} value (concentration at which the PCR product was equal to 50% of PCR product where no Zn^{2+} complex was added) of the Zn^{2+} complex was found to be 9.23 ± 1.78 μM. Neither 9-anthracene methanol nor Zn^{2+}-perchlorate had any effect on the polymerase activity (Fig. S4A). To confirm that the Zn^{2+} complex is not inhibiting the Taq polymerase directly, but instead the origin of roadblock activity is arising due to DNA binding, a 5-fold excess of Taq polymerase was added, keeping the other parameters constant. At this high concentration, no PCR product was observed (Fig. S4B). This finding confirms that the origin of roadblock activity is due to DNA binding, not by inhibition of enzyme activity.

2.3. Roadblock activity does not alter DNA's conformations

Often, the addition of small molecules bearing hydrophobic moieties to the DNA induces conformational changes in the latter [6]. Therefore, circular dichroism (CD) spectra were recorded in the presence and absence of Zn^{2+} complex. No significant changes in DNA conformation were observed by CD spectroscopy (Fig. 3A) when titrated against the Zn^{2+} complex. Small coordination molecules which intercalate DNA are known to induce such conformational changes [6]. The Zn^{2+} complex, studied here however, binds to the grooves and is thus unable to induce sufficient conformational changes. It is thus impossible that the loss of enzyme activity originates from a ligand-