Journal of Inorganic Biochemistry 149 (2015) 59–67

Contents lists available at ScienceDirect

Journal of Inorganic Biochemistry

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

A molecular level mechanism for uranium (VI) toxicity through Ca^{2+} displacement in pyrroloquinoline quinone-dependent bacterial dehydrogenase

Katherine A. Burbank [a], Robert A. Walker [a], Brent M. Peyton [b,*]

[a] Department of Chemistry and Biochemistry, Montana State University, Bozeman, MT, USA
[b] Department of Chemical and Biological Engineering, Montana State University, Bozeman, MT, USA

ARTICLE INFO

Article history:
Received 17 June 2014
Received in revised form 24 November 2014
Accepted 25 November 2014
Available online 3 December 2014

Keywords:
Uranyl
Dipicolinic acid
UV–VIS spectroscopy
ESI-Mass spectrometry
DFT

ABSTRACT

Dipicolinic acid (DPA), a small molecule analogue for the pyrroloquinoline quinone (PQQ) bacterial dehydrogenase cofactor, was used to model displacement of the complexing ion, Ca^{2+}, by a uranium (VI) dioxo-cation, UO_2^{2+}. Complexation of UO_2^{2+} with DPA through the displacement of Ca^{2+} was examined with UV/visible spectroscopy, ESI (electrospray ionization)-Mass spectrometry, and density functional theory based-modeling. The UO_2^{2+} displacement of other biologically important metal cations (Zn^{2+}, Cu^{2+}, Ni^{2+}, and Fe^{3+}) from DPA was also examined. Results show that UO_2^{2+} has a distinctly higher binding affinity ($\log\beta = 10.2 \pm 0.1$) for DPA compared to that of Ca^{2+} ($\log\beta = 4.6 \pm 0.1$), and provide molecular level insight into the mechanism of uranium toxicity associated with the {ONO} site. These results support those of VanEngelen et al. (2011) where a key interaction between PQQ and UO_2^{2+} produced significant uranium toxicity in bacteria. The observed toxicity mechanism was determined to be the displacement of a Ca^{2+} cation bound to the {ONO} site on PQQ and was observed even at submicromolar UO_2^{2+} concentrations. Here we couple experimental findings with density functional theory (DFT) calculations to investigate the electronic and structural properties that make the {ONO} site so distinctively favorable for UO_2^{2+} binding. This novel approach using integrated experimental and fundamental atomic based models opens the path to identify a library of potential uranium interactions with critical biological molecules.

1. Introduction

Uranium is an environmental contaminant that produces the highly mobile, dioxouranium (VI) cation, UO_2^{2+} or uranyl, in aqueous environments. In this form, UO_2^{2+} can deliver both radiological and chemical toxicity to living organisms [1,2]. Though naturally present in varying concentrations throughout environmental ecosystems, the wide use of uranium for industrial and military applications has increased the risk for UO_2^{2+} exposure through mining activities, uranium processing, and leaching of radioactive waste [3–6].

Uranium presence in ground water is monitored and allowed by the United States Environmental Protection Agency at concentrations less than 126 nM (30 μg/L) [7]. Increased distribution of uranium into the environment, more specifically through groundwater, should be of concern considering there is known chemical toxicity associated with ingestion [1,2], although toxicity mechanisms existing in the literature have yet to present a molecular basis for UO_2^{2+} presence in living organisms. Published data concerning the mechanism of uranium interaction with proteins and metabolites at a molecular level is limited [8–15], and few quantitative studies have investigated the binding properties of UO_2^{2+} with small, biologically relevant molecules [14,16–23].

Consequently, a recent combined *in vivo*, *in vitro*, and *in silico* study by Van Engelen et al. proposed a key interaction of pyrroloquinoline quinone (PQQ) with UO_2^{2+} that was responsible for a specific toxicity mechanism in bacteria [24]. The measurements showed that the presence of UO_2^{2+} nearly completely inhibited bacterial growth at ~0.5 μM concentrations [24]. Additional mass spectrometry and computational modeling determined that the inhibition was due to a UO_2^{2+} interaction with the {ONO} site on the PQQ cofactor (Fig. 1), a non-covalently bound *ortho*-quinone cofactor used by a number of bacterial dehydrogenases [25]. A crystal structure of the quinone-dependent methanol dehydrogenase (QMDH) [26] shows this site to be coordinated to a Ca^{2+} that serves both catalytic [27] and structural [28] roles by anchoring PQQ within the holoenzyme.

The mechanism of UO_2^{2+} toxicity inferred from the PQQ-dependent growth conditions [24] was explained using the biotic ligand model (BLM) of acute metal toxicity. The basic assumption of the BLM is that acute metal toxicity results from the complexation of a metal (uranium) with physiologically active binding sites ({ONO} site), to the exclusion of competing ions (calcium) [29,30]. This competitive UO_2^{2+} binding relative to Ca^{2+} on active sites has been reported subsequently in recent publications investigating albumin [15], C-reactive protein [21], and calmodulin [16].

While the role of PQQ in non-bacterial organisms is under debate [31,32], the molecular basis of the interaction between the {ONO} active

* Corresponding author. Tel.: +1 406 994 7419.
E-mail address: bpeyton@coe.montana.edu (B.M. Peyton).

http://dx.doi.org/10.1016/j.jinorgbio.2014.11.007
0162-0134/

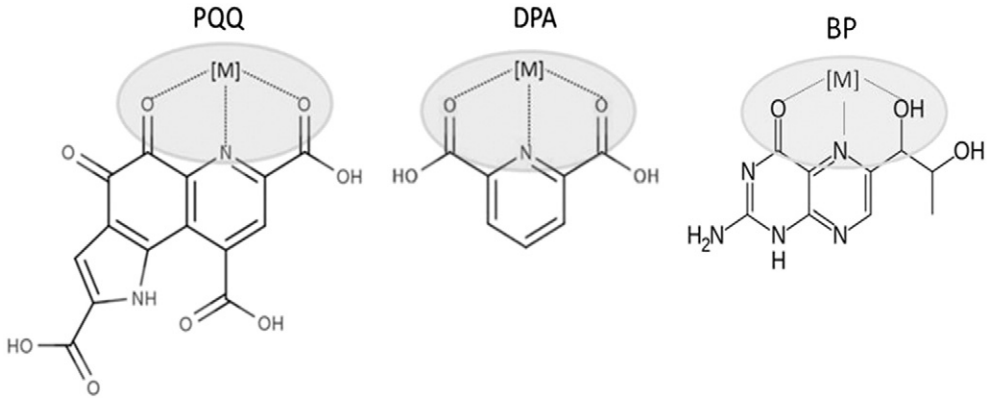

Fig. 1. {ONO} binding site on PQQ, DPA, and BP. [M] indicates the complexing metal cation and can be UO_2^{2+}, Ca^{2+}, Zn^{2+}, Cu^{2+}, Ni^{2+} and Fe^{3+}.

site of PQQ and UO_2^{2+} has implications for understanding uranium toxicity in other organisms, including humans. By examining the structural and electronic properties of the {ONO} site, additional active sites with the potential for uranium coordination to the exclusion of competing ions can be identified. As a supporting example to this hypothesis, the {ONO}-site of PQQ shows structural similarity to the tricyclic alloxazine catalytic center of flavoprotein monoamine oxidase that may allow UO_2^{2+} coordination and an earlier study has indicated that UO_2^{2+} inhibits flavoprotein monoamine oxidase function in rat kidney [33]. Tetrahydrobiopterin additionally contains an {ONO}-site and is involved in numerous biological processes that are relevant to human health. The {ONO} site binding properties of a derivative of tetrahydrobiopterin (biopterin, BP Fig. 1.) were therefore investigated in this work as a means to extend the significance of the {ONO}-site to biomolecules that are important to human life.

In the work presented here, UV/Visible spectroscopic analysis provided an experimental probe to follow the competitive UO_2^{2+} binding with Ca^{2+} for the {ONO} site through changes in absorbance spectra. Changes in absorption intensities provided a means for evaluating the electronic structure of the metal–ligand complexes formed by each cation. Electrospray ionization mass spectrometry (ESI-MS) and computational modeling resolved remaining uncertainties about these metal–ligand complex structures. Since UO_2^{2+} is a di-cation, it can compete electrostatically with Ca^{2+} for binding sites. Thus, UO_2^{2+} binding to the {ONO} site was compared with other biologically relevant divalent cations including Zn^{2+}, Ni^{2+}, and Cu^{2+}. By considering the high valence of the uranium central ion with empty 5*d* and 4*f* shells, the coordination properties of UO_2^{2+} can also be considered electronically similar to Fe^{3+} ions. This comparison was also investigated given the importance of Fe^{3+} in biological systems.

Competitive binding between the above mentioned metal cations and UO_2^{2+} for the {ONO} binding motif was thus investigated. In doing so, possible interactions of uranium with similar sites found in biological organisms were identified from the insight gained by the evaluation of a specific binding motif ({ONO} site) that is known to have inhibitive effects from uranium at submicromolar concentrations. Characterizing UO_2^{2+} interactions at a molecular mechanism level has consequences for understanding of electronic and structural factors that govern UO_2^{2+} binding to small molecules relevant to human health. Research in this area of study may additionally assist in developing new molecules that could be used for uranium biodetection and bioremediation purposes [34–39].

2. Experimental and theoretical methods

2.1. Chemicals

All chemicals were purchased from Sigma Aldrich except for PQQ (Ark Pharmaceuticals). Calcium, zinc (II), copper (II), nickel (II), and iron (III) were provided as hydrated perchlorate salts. UO_2^{2+} was added as a pentahydrate di-nitrate salt. Milli-Q water (18 mΩ) was used in the preparation of all the solutions. All experiments were conducted at (23 ± 2) °C. The concentration of U (VI), Zn (II), Cu (II), Ni (II), and Fe (III) in the stock solution was determined by absorption spectrophotometry. Dipicolinic acid (pyridine-2,6-dicarboxylic acid, DPA, 98%) solutions were prepared as a pH = 7.0 solution by neutralizing weighed amounts of DPA with a standard NaOH solution (0.10 M) and diluted to appropriate concentrations with Milli-Q water (18 mΩ).

2.2. Spectrophotometry

Competitive spectrophotometric titrations were measured on a Cary 6000i spectrophotometer (Varian, Inc.) to determine the extent of formation of [UO_2DPA] complexes with the competing cations. Absorption spectra were collected in the wavelength region of 200–400 nm (0.1 nm interval), where the species of DPA and its complexes with UO_2^{2+} and Ca^{2+} (as well as Fe^{3+}, Ni^{2+}, Zn^{2+} and Cu^{2+}) showed unique spectral features at a pH of 7.0. After an initial titration with NaOH stock solution, the pKa for DPA was determined to be 5.6. Therefore, the solutions used for the spectrophotometric titrations were buffered to a pH of 7.0 with NaOH stock solution to ensure that the carboxylate groups were deprotonated.

For a typical titration, 2.00 mL of metal cation:DPA complex or DPA solution was placed in a quartz cuvette (1.0 cm optical path) into which appropriate aliquots of UO_2^{2+} or Ca^{2+}solutions were added and mixed thoroughly for 1–2 min before the spectra were collected. Preliminary kinetic experiments showed that the complexation reaction was fast and the absorbance became stable within 30 s of mixing. For a typical sample, 10–25 additions were made, generating a set of 11–26 spectra in each titration. Multiple titrations with different concentrations of [CaDPA] and UO_2^{2+} were performed.

2.3. Stability constants

The stability constants (K) for [CaPQQ] and [CaDPA] complexes were calculated by the modified Rose–Drago expression [40], where for example, in the case of the [CaDPA] complex, the expression for the stability constant is as follows:

$$K_{\mathrm{CaDPA}} = \frac{[\mathrm{CaDPA}]}{[\mathrm{Ca}^{2+}][\mathrm{DPA}^{2-}]} \tag{1}$$

The corresponding modified Rose–Drago expression was:

$$\frac{\left([\mathrm{DPA}^{2-}] + [\mathrm{Ca}^{2+}]\right)}{\Delta\varepsilon + (K * \Delta\varepsilon)} = \frac{[\mathrm{DPA}^{2-}][\mathrm{Ca}^{2+}]}{\Delta A} + \frac{\Delta A}{\Delta\varepsilon^2} \tag{2}$$