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Structural features of uranium-protein complexes

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ABSTRACT

Uranium toxicity depends on its chemical properties rather than on its radioactivity and involves its interaction with macromolecules. Here, a systematic survey of the structural features of the uranyl sites observed in protein crystal structures deposited in the Protein Data Bank is reported. Beside the two uranyl oxygens, which occupy the axial positions, uranium tends to be coordinated by five other oxygen atoms, which occupy the equatorial vertices of a pentagonal bipyramid. Even if one or more of these equatorial positions are sometime empty, they can be occupied only by oxygen atoms that belong to the carboxylate groups of Glu and Asp side-chains, usually acting as monodentate ligands, to water molecules, or to acetate anions. Although several uranium sites appear undefined or unrefined, with a single uranium atom that lacks the two uranyl oxygen atoms, this problem seems to become less frequent in recent years. However, it is clear that the crystallographic refinements of the uranyl sites are not always well restrained and a better parametrization of these restraints seems to be necessary.

1. Introduction

The bioinorganic chemistry of uranium received little attention, since there are no biological processes that require this heavy and radioactive metal [1]. Increasing attention is however devoted to uranium toxicity and to the molecular mechanisms behind it.

Uranium is a chemo-toxic, radiotoxic and a carcinogenic element [2]. The chemical toxicity of uranium is the major environmental health hazard, with its radioactivity a secondary problem [3,4]. This implies that uranium can interact with macromolecules and tissues and alter their functionality. In particular, it is considered to be a classic nephrotoxin [5,6] and its deleterious effects on the nephrons in the kidney have been described for the first time about one century ago [7], though other deleterious effects on human health, including various types of cancer, have been observed [8].

Natural exposure to uranium is highly unlikely since the natural concentration of uranium is extremely low. In fact, despite uranium is more abundant than silver or mercury on the heart crust [9], it is ubiquitously distributed and rare – and strategically important – are its mines. Nevertheless, accidental uranium contamination is possible, for example amongst workers employed in uranium industry and nuclear power stations. A recently discovered risk is the exposure of soldiers to depleted uranium, which is copiously used in military applications, including armor plating and armor-piercing projectiles [10]. Moreover, non-occupational exposure is possible, the most common being due to naturally occurring uranium sources contamination of drinking water [11], sometime close to former mining sites [12].

Uranyl cation, UO_2^{2+}, is the largely predominant species of water-soluble uranium in the environment. In the bloodstream it forms carbonates, $[UO_2(CO_3)_2(H_2O)_n]^{2-}$ and $[UO_2(CO_3)_3(H_2O)_n]^{4-}$, which may diffuse into tissues (liver, bones, kidneys, etc.), and it forms protein complexes, which are eliminated from the kidney [13] and here uranyl manifests its nephrotoxicity by damaging the peritubular cell membrane or disrupting the para-aminohippurate transporter system [14–16]. Other mechanisms of uranyl toxicity have been discovered, like hydrolysis of the DNA backbone [17] or displacement of magnesium(II) in hexokinase and disruption of the first step in glycolysis [18]. Uranium exposure cause also several other effects, including reprotoxicity [19], perturbation of mitochondrial metabolism [20], oxidative stress [21] and imbalance of the redox regulation [22].

Early studies showed that uranyl is known to interact with several proteins, like transferrin [23], albumin [24,25], ceruloplasmin [26], hemopexin [26]. More recently, a proteomic analysis of human kidney-2 soluble cell extracts based on immobilized metal-affinity chromatography identified 64 proteins that interact with uranium and that display varied functions [27,28]. In another, very recent proteomic study, Eb-Levadoux and colleagues observed protein-uranyl interaction in 20 proteins of zebrafish ovaries, including proteins that have completely different functions like actin and copper-zinc superoxide dismutase [29].

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Uranyl-protein interactions have been studied with several techniques and in different experimental conditions. Reitz and colleagues analyzed the interaction between uranyl and the proteinaceous surface layer of the archeal *Sulfolobus acidocaldarius* by means of extended X-ray absorption fine structure (EXAFS by using the uranium L_{III} band) and time-resolved laser-induced fluorescence spectroscopy (TRLFS) [30]. Similarly, uranyl interactions with the proteinaceous surface layer of *Bacillus sphaericus* was studied with EXAFS by Merroun and colleagues [31]. A wide assortment of experimental and computational techniques, including attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR), TRLFS, isothermal titration calorimetry (ITC), EXAFS, density functional theory calculations (DFT), was used to characterize the uranyl binding sites in several macromolecules, including phosvitin from egg yolk [32], a Osteopontin model [33], cell wall lipopolysaccharides from *Pseudomona aeruginosa* [34], and it was observed that uranyl tends to interact with water molecules and with carboxylate and phosphate groups, with some preference for the latter. Particular attention has been devoted to uranyl interaction with *Arabidopsis thaliana* calmodulin, which is about thousand times more affine for uranyl than for calcium. Based on TRLFS, FTIR, EXAFS, DFT and molecular dynamics data and on protein engineering, it has been concluded that uranyl strongly interacts with mono- and bidentate carboxylates and that phosphorylation plays a determining role in uranyl binding [35–37].

Several studies have been devoted to the control of uranium release into groundwater. One possible way is the reduction of soluble uranium (VI) species to less soluble uranium(IV) species. This can be catalyzed by cytochrome *c*7 of *Geobacter sufurreducens* [38,39]. Another way is the uranium(VI) kidnapping by chelators. High affinity ligands are necessary to bind uranyl in aqueous solutions, where it strongly interacts with carbonates, which are in general present at high concentration. Two guidelines are followed to maximize both affinity and selectivity towards uranyl [40]. The presence of five or six equatorial ligands with the formation of pentagonal or hexagonal bipyramids, where the two uranyl oxygen atoms occupy the axial positions, allows the separation of uranyl from most of the transition, alkali, and alkaline metal ions. The presence of the axial uranyl oxygens, which can be hydrogen bonded, distinguishes uranyl complexes from the complexes of other metal ions.

On these bases, Zhou and co-workers were able, by using a sophisticated bioinformatics screening of the Protein Data Bank and by empirical sequence mutations, to engineer a small, thermostable protein of unknown function from *Mathanobacterium thermautotriphicom* and to get a femtomolar affinity towards uranyl ($K_d = 7.4 \times 10^{-15}$ M) at pH 8.9 [40]. Smaller, nanomolar affinities were observed by using an engineered NikR, a nickel-dependent transcriptional repressor protein from *Escherichia coli* [41]. Micromolar uranyl affinities were observed by using an engineered calmodulin peptide from *Paramecium tetraurelia* [42]. Cyclic peptides have also been designed with affinities towards uranyl in the nano-picomolar range [43–46].

Another computational approach for predicting uranyl binding sites in proteins has been developed, based on the statistical analyses of experimental data [47], and it has been successfully applied to the identification of the uranyl binding site in C-reactive protein [48]. This is the physiological calcium binding site and the apparent binding affinity of uranyl for the native protein is ca. 100-fold higher than that of calcium [48].

This manuscript describes the structural features of the uranium uranyl site in protein crystal structures. Uranium atoms are always bound to the two uranyl oxygens in axial positions and up to five donor atoms in equatorial position, with the formation of pentagonal bipyramid, where some of the five equatorial positions can be empty. These five donor atoms are always oxygen atoms, either of water molecules, small anions (actetate) or Glu and Asp side-chains. Carboxylates have a strong tendency to be monodentate. Curiously, often the uranyl cation is not directly bound to protein atoms but only two water molecules or small anions that are hydrogen bonded to protein atoms.

Many uranium sites appear undefined or unrefined, with a single uranium atom that lacks the two uranyl oxygen atoms. In these cases, it is impossible to analyze the structural features of the protein-uranium interactions. However, this problem seems to become less frequent in recent years. However, it is clear that the crystallographic refinements of the uranyl sites are not always well restrained and a better parametrization of these restraints seems to be necessary.

Table 1
Identification codes of the Protein Data Bank files that contain uranium. In bold those that contain at least one complete uranyl cation.[a]

1anv 1b05 1b0h 1b1h 1b2h 1b32 1b3f 1b3g 1b3h 1b3l 1b40 1b46 1b4h 1b4z 1b51 1b52 1b58 1b5h 1b5i 1b5j 1b6h 1b7h 1b9j 1bzo 1ct9 1efq 1fe4 1jet 1jeu 1jev 1nci 1ncj 1ola 1olc 1qka 1qkb 1t9h 1uyj 2gic 2olb 2rkm 2veo 3dgc 3l0o 3mko 3pto 3ptx 3pu0 3pu1 3pu4 4fzp 4ild

[a] Each PDB file is identified by a four letter code; further details are given in Table S3 of the Supplementary Material.

2. Material and methods

Protein crystal structures containing uranium were searched in the Protein Data Bank [49,50] by means of the "Chemical component search" service available at www.rcsb.org/pdb/ligand/chemAdvSearch.do and by searching entries containing uranium (in any form) or uranyl. 52 protein structures were identified (Table 1). No filters based on the amino acid sequence similarity were used to reduce sequence redundancy since the presence of non-native, exogenous inorganic ions is substantially independent of sequence.

Atoms were considered to coordinate uranium if closer than 3 Å to the metal atom. The stereochemistry of the first coordination sphere was analyzed and visually inspected by means of the "Metal Geometry" tools of the Chimera package (version 1.11) [51]. Hydrogen bonds were identified with the program HBplus program (version 3.2) [52] by using default parameters.

3. Results and discussion

Uranium compounds are seldom used in macromolecular crystallography to solve the phase problem [53] and this is the main reason why some of the PDB entries contain it. Despite its presence is often subsidiary, its interaction with protein atoms may bring useful information in understanding uranium-protein interactions. It is however mandatory to observe that some of the uranium sites in the Protein Data Bank are of dubious quality. On the one hand, there are several uranium atoms that are disconnected from the protein and from any other atom. This is clearly absurd since in protein crystals an isolated atom would reside in regions occupied by liquid water and would therefore be free to move and consequently it would undetectable in the electron density maps. On the other side, there are uranium atoms that lack the two oxygen atoms with which they form the uranyl cation (UO_2^{2+}). 41 of the 52 PDB files that contain uranium report the presence of uranyl cations, named IUM according to the PDB rules. In the other PDB files, uranium is deposited as a monoatomic cation, named U1 according to the PDB syntax. However, urnayl was used in the crystallization medium for all these structures and it is therefore probable that uranyl was also present in the crystals, since this is essentially the only chemical form for uranium that is stable in oxygenated water. In one PDB file (3dgc) both IUM and U1 uranium sites are reported and, although no information is available on the uranium salt used in the crystallization medium, it is reasonable to suppose that it was a uranyl containing salt. Several uranium sites were discarded either because monatomic atoms or because lack of the uranyl oxygen atoms and only 42 uranium sites were kept and further analyzed (10% of the total) (Table 1). This reduces enormously the data abundance but it

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