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Insights into Uranyl Ion Binding to Ubiquitin from Molecular Modeling and Dynamics Simulations

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Uranium is toxic to the human body with mechanisms not fully understood. The interaction between uranyl ion (UO_2^{2+}) and ubiquitin (Ub) was predicted by molecular modeling, molecular dynamics (MD) simulation, and steered molecular dynamics (SMD) simulation. The structural and dynamics consequences, as well as protein unfolding behavior, upon uranyl binding to two surface residues of Ub, Glu18 and Asp21, were revealed at an atomic level. This in silico study exhibits a possibility of UO_2^{2+} binding to Ub, providing a possible mechanism of uranyl toxicity in vivo.

The widespread use of uranium for military and civilian purposes raises a public health concern of its high toxicity to humans. The harmful effects of uranium are observed in many organs such as lungs, liver, kidneys, bone, muscle, and brain.[1] Uranyl ion (UO_2^{2+}) is the most stable form of uranium under physiological conditions, and the toxicity of uranium, besides its radiation damage, might result from the ability of UO_2^{2+} to bind strongly to both nucleotides and proteins, thereby disrupting the native function of these biomolecules.[2] To reveal the mechanism underlying uranyl–protein interactions, the binding of UO_2^{2+} to serum proteins in the blood stream, in particular to albumin and transferrin, have been investigated recently.[3] A Protein Data Bank (PDB) survey shows that UO_2^{2+} binds to proteins mainly through carboxylic acid groups such as those of aspartate (Asp) and glutamate (Glu).[2a] We have also investigated the effects of UO_2^{2+} binding on the structure and function of a heme protein, cytochrome b_5 (cyt b_5).[4] To date, plentiful proteins are found to be the targets of UO_2^{2+},[2–5] while a full understanding the mechanism of uranyl toxicity requires more extensive studies.

Ubiquitin (Ub) is a conserved protein with 76 amino acids, which plays crucial roles in living cells by taking part in the degradation of misfolded proteins via the ubiquitin proteasome system (UPS).[6] Malard and co-workers performed a proteomic analysis of the response of human lung cells to uranium, which suggested a dysfunction of the UPS system or a regulatory pathway involving cytokeratin ubiquitinylation.[7] This is likely due to a consequence of interactions between UO_2^{2+} and Ub. Indeed, it was shown that metal interacting with Ub such as Cu^{2+} binding may cause UPS dysfunction.[8] On the other hand, Falini and co-workers recently performed a crystallographic analysis of metal ions binding to human Ub, including Cu^{2+}, Zn^{2+}, and heavy metals such as Cd^{2+} and Hg^{2+}.[9] Additionally, the finding that uranium is capable of crossing the blood–brain barrier[1c] and that Ub plays a key role in neurodegeneration[10] suggests an ability of UO_2^{2+} binding to Ub. While extensive studies have been devoted to probe both the UO_2^{2+}–protein and metal–Ub interactions, no structural information is available for a UO_2^{2+}–Ub complex that is likely to form in vivo.

With these in mind, we herein performed a molecular modeling study for uranyl binding to Ub. The in silico approach has been shown to be capable of providing structural insights into metal–proteins interactions that might otherwise be difficult to obtain experimentally.[11] By examining the metal binding sites in the crystal structure of Ub–metal complex (PDB Entry 3N30),[9] we found that a Zn^{2+} binding site of Glu18 with Asp21 nearby is a potential binding site for UO_2^{2+}, which shares the characteristics of uranyl binding as observed in well-resolved protein crystal structures.[2a] We, therefore, changed the Zn^{2+} ion to a UO_2^{2+} ion and performed a molecular modeling study for the UO_2^{2+}–Ub complex with NAMD 2.7 (Nanoscale Molecular Dynamics),[12] to examine the possibility by using a minimization–molecular dynamics (MD) simulation–minimization procedure described recently for modeling UO_2^{2+} binding to cyt b_5 and its variant.[4]

As analyzed using VMD 1.9 (Nanoscale Molecular Dynamics),[13] the equilibrated overall structure of the UO_2^{2+}–Ub complex overlaps well with the X-ray structure of Ub with Zn^{2+} ion removal after equilibration under the same condition, except for the flexible C-terminus (Figure 1, inset, left). Concurrently, slight conformational changes were observed for Glu18 and Asp21, suggesting that these two residues have suitable spatial positioning for UO_2^{2+} binding. A close view of the UO_2^{2+}-binding site shows that Glu18 and Asp21 coordinate to the U atom via one and two O atoms with a distance of 2.56 and 2.70, 2.78 Å, respectively (Figure 1, inset, right). The distances are shorter in comparison to the maximum values reported for carboxylate monodentate (2.61 Å) and bidentate (2.84 Å) ligands

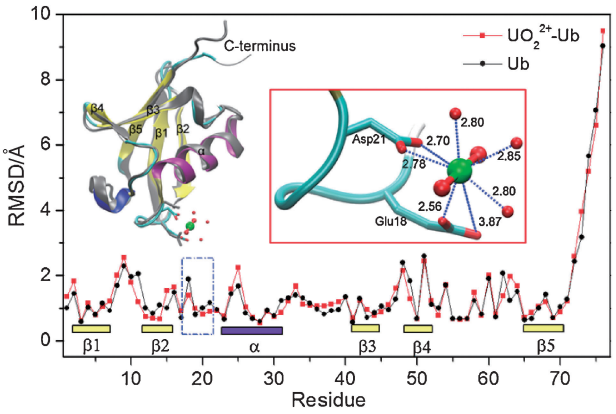

Figure 1. Average C_α RMSD over time of each residue for the UO_2^{2+}–Ub complex and Ub in MD simulation. Spatial alignment of UO_2^{2+}–Ub complex and Ub (gray) (inset, left), and the UO_2^{2+}-binding site (inset, right). The elements of secondary structure are highlighted, as well as Glu18 and Asp21. Water molecules are presented as spheres.

of uranyl ions in well-resolved protein crystal structures.[2a] Moreover, three water molecules are found to coordinate to the U atom with a distance of 2.80–2.85 Å, and a weak interaction between the other O atom of Glu18 and the U atom also exists. These observations indicate that Ub readily binds UO_2^{2+} ion.

In order to evaluate the dynamics properties of Ub as a result of uranyl binding, we performed a MD simulation study for both the UO_2^{2+}–Ub complex and Ub under periodic boundary conditions at 300 K for 5 ns using NAMD 2.7. The average C_α root-mean-square deviation (RMSD) over time of each residue is shown in Figure 1. It can be seen that in general UO_2^{2+}–Ub and Ub exhibit very similar mobility for the C-terminal half residues, while slightly different mobility for the N-terminal half residues. Glu18 and Asp21 were found to exhibit lower RMSD when UO_2^{2+} bound (Figure 1, in dashed box). On the other hand, Asn25, located close in space to Asp21, was found to exhibit higher RMSD with UO_2^{2+}–Ub complex. These observations suggest that uranyl binding to Ub causes a local dynamics alteration and retains the entire protein largely intact, which is in agreement with the spatial alignment (Figure 1, inset, left).

To further investigate the unfolding behavior of Ub as a result of uranyl binding, we performed a steered molecular dynamics (SMD) simulation study for both UO_2^{2+}–Ub and Ub using a constant velocity unfolding procedure.[14] The SMD simulation was carried out by fixing the C_A atom of Gly76 and applying an external force to the C_A atom of Met1, with a pulling speed of 0.5 Å ps^{-1} along the vector from the pulled atom to the fixed atom (Figure 2, inset, up). Each simulation was repeated four times, and an average force-extension profile was calculated based on the resultant five trajectories. As shown in Figure 2, the profiles for UO_2^{2+}–Ub and Ub are similar and exhibit two major peaks, which agrees well with previous observations.[15] The first peak with a force as high as about 1800 pN corresponds to separate β1, β2, and β5 from the rest of the protein. The second broad peak with a force around 900 pN is attributed to a disruption intermediate formed by β3, β4, and α, as well as a short 3_{10} helix (Figure 2, inset, down). The intermediate is similar to that observed in previous studies.[16] The similarity of the force-extension profile of UO_2^{2+}–Ub and Ub suggests that uranyl binding to the loop region of β2 and α exerts slight effect on the force-induced unfolding pathway of Ub.

In conclusion, molecular modeling and MD simulation reveal that two surface residues of Ub, Glu18 and Asp21, are capable of coordinating to a UO_2^{2+} ion, resulting in a slightly different dynamics property for the local region. Furthermore, although uranyl binding disturbs the unfolding behavior of Ub slightly, as revealed by SMD simulation, the UO_2^{2+}–Ub complex likely has a distinct conjugation behavior with UO_2^{2+} bound to the protein surface. Note that Ub conjugation is a crucial step in the UPS pathway.[6] The atomic and dynamic view of UO_2^{2+} binding to Ub in this in silico study sheds light on a possible mechanism of uranyl for cellular toxicity.

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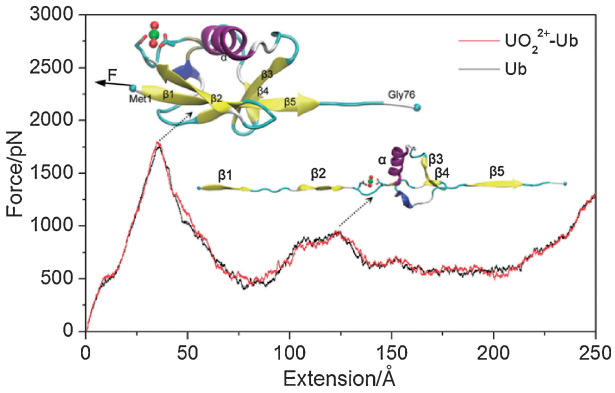

Figure 2. Average force-extension profiles for the UO_2^{2+}–Ub complex and Ub in SMD simulation. Snapshots according to two major peaks are shown as insets, as indicated by dashed arrows. The fixed atom, C_A of Gly76, and the pulling atom, C_A of Met1, are shown as spheres. The pulling direction of F is indicated by an arrow.

References

1 a) J. L. Domingo, *Reprod. Toxicol.* **2001**, *15*, 603. b) E. S. Craft, A. W. Abu-Qare, M. M. Flaherty, M. C. Garofolo, H. L. Rincavage, M. B. Abou-Donia, *J. Toxicol. Environ. Health, Part B* **2004**, *7*, 297. c) P. Lestaevel, P. Houpert, C. Bussy, B. Dhieux, P. Gourmelon, F. Paquet, *Toxicology* **2005**, *212*, 219.
2 a) O. Pible, P. Guilbaud, J.-L. Pellequer, C. Vidaud, E. Quéméneur, *Biochimie* **2006**, *88*, 1631. b) J. D. Van Horn, H. Huang, *Coord. Chem. Rev.* **2006**, *250*, 765. c) Y. Xiang, Y. Lu, *Nat. Chem.* **2011**, *3*, 697.
3 a) C. Vidaud, S. Gourion-Arsiquaud, F. Rollin-Genetet, C. Torne-Celer, S. Plantevin, O. Pible, C. Berthomieu, E. Quéméneur, *Biochemistry* **2007**, *46*, 2215. b) G. Montavon, C. Apostolidis, F. Bruchertseifer, U. Repinc, A. Morgenstern, *J. Inorg. Biochem.* **2009**, *103*, 1609. c) M. Hémadi, N.-T. Ha-Duong, S. Plantevin, C. Vidaud, J.-M. E. H. Chahine, *J. Biol. Inorg. Chem.* **2010**, *15*, 497. d) J. Michon, S. Frelon, C. Garnier, F. Coppin, *J. Fluoresc.* **2010**, *20*, 581.
4 D. Wan, L.-F. Liao, M.-M. Zhao, M.-L. Wu, Y.-M. Wu, Y.-W. Lin, *J. Mol. Model.* **2011**, in press. doi:10.1007/s00894-011-1097-1.
5 A. Dedieu, F. Bérenguer, C. Basset, O. Prat, E. Quéméneur, O. Pible, C. Vidaud, *J. Chromatogr., A* **2009**, *1216*, 5365.
6 a) C. M. Pickart, *Annu. Rev. Biochem.* **2001**, *70*, 503. b) M. H. Glickman, A. Ciechanover, *Physiol. Rev.* **2002**, *82*, 373.
7 V. Malard, O. Prat, E. Darrouzet, F. Bérenguer, N. Sage, E. Quéméneur, *Proteomics* **2005**, *5*, 4568.
8 D. Milardi, F. Arnesano, G. Grasso, A. Magrì, G. Tabbì, S. Scintilla, G. Natile, E. Rizzarelli, *Angew. Chem., Int. Ed.* **2007**, *46*, 7993.
9 F. Arnesano, B. D. Belviso, R. Caliandro, G. Falini, S. Fermani, G. Natile, D. Siliqi, *Chem.—Eur. J.* **2011**, *17*, 1569.
10 A. Ciechanover, P. Brundin, *Neuron* **2003**, *40*, 427.
11 Y.-W. Lin, *Proteins: Struct., Funct., Bioinf.* **2011**, *79*, 679.
12 L. Kalé, R. Skeel, M. Bhandarkar, R. Brunner, A. Gursoy, N. Krawetz, J. Phillips, A. Shinozaki, K. Varadarajan, K. Schulten, *J. Comput. Phys.* **1999**, *151*, 283.
13 W. Humphrey, A. Dalke, K. Schulten, *J. Mol. Graphics* **1996**, *14*, 33.
14 Y.-W. Lin, Z.-H. Wang, F.-Y. Ni, Z.-X. Huang, *Protein J.* **2008**, *27*, 197.
15 S. Falkovich, A. Darinskii, N. Balabaev, I. Neelov, *Macromol. Symp.* **2009**, *278*, 105.
16 a) A. Irbäck, S. Mitternacht, S. Mohanty, *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 13427. b) A. Irbäck, S. Mitternacht, *Proteins: Struct., Funct., Bioinf.* **2006**, *65*, 759. c) F. Gräter, H. Grubmüller, *J. Struct. Biol.* **2007**, *157*, 557. d) A. Das, C. Mukhopadhyay, *Proteins: Struct., Funct., Bioinf.* **2009**, *75*, 1024.

