



Probing Metal Ion Discrimination in a Protein Designed to Bind Uranyl Cation With Femtomolar Affinity

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The design of metal-binding sites in proteins that combine high affinity with high selectivity for the desired metal ion remains a challenging goal. Recently, a protein designed to display femtomolar affinity for UO_2^{2+} , dubbed “Super Uranyl-binding Protein” (SUP), was described, with potential applications for removing UO_2^{2+} in water. Although it discriminated most metal ions present in seawater, the protein showed a surprisingly high affinity for Cu^{2+} ions. Here, we have investigated Cu^{2+} binding to SUP using a combination of electron paramagnetic resonance, fluorescence and circular dichroism spectroscopies. Our results provide evidence for two Cu^{2+} binding sites on SUP that are distinct from the UO_2^{2+} binding site, but one of which interferes with UO_2^{2+} binding. They further suggest that in solution the protein’s secondary structure changes significantly in response to binding UO_2^{2+} ; in contrast, the crystal structures of the apo- and holo-protein are almost superimposable. These results provide insights for further improving the selectivity of SUP for UO_2^{2+} , paving the way toward protein-based biomaterials for decontamination and/or recovery of uranium.

Keywords: protein engineering, metalloproteins, metal ion selectivity, helical bundle proteins, EPR spectroscopy

INTRODUCTION

Proteins have evolved to bind metal ions with remarkable selectivity. This is achieved by controlling both the chemical nature and the geometry of the coordinating ligands at the metal binding site (Montes-Bayón and Blanco-González, 2016). As a result, proteins also exercise exquisite control over the reactivity of the metals they bind, for example fine-tuning properties such as Lewis acidity, oxidation state, and redox potential. However, biology utilizes a relatively small subset of metals and hence there has been considerable interest in designing proteins that bind non-biological metal ions, such as those in the lanthanide and actinide series (Le Clairche et al., 2003; Barak et al., 2006; Le Clairche and Vita, 2006; Wegner et al., 2009; Chakraborty et al., 2011; Plegaria et al., 2015; Starck et al., 2015; Brulfert et al., 2016). The design of proteins (and nucleic acids) that bind uranium has been of particular interest, given that this element is an essential component in nuclear weapons and nuclear reactors (Handley-Sidhu et al., 2010). Potential applications of uranium-binding proteins include biosensing and bio-remediation of uranium-contaminated environments that may result from the use of depleted uranium in munitions and from uranium processing associated with nuclear weapons and nuclear fuel manufacture (Bhalara et al., 2014; Newsome et al., 2014; Xie et al., 2019).

Recently, a small (80 residue) α -helical protein was described that had been engineered to bind uranyl cation, UO_2^{2+} , the predominant form of uranium in the environment, with remarkably high,

femtomolar affinity. This protein, dubbed Super Uranyl-binding Protein (SUP), also exhibits very high selectivity constants against other environmentally more abundant metal ions. These ranged from 10^3 to 10^7 for a series of the most common metal ions found in sea water (Zhang et al., 2014), suggesting that the protein might have potential for extracting UO_2^{2+} from seawater. SUP has also subsequently been incorporated into protein hydrogels (Kou et al., 2017a,b) and 2D protein multilayers (Zhang et al., 2018) with high UO_2^{2+} adsorption capacity. These materials could be used for the decontamination of depleted uranium, often found in groundwaters in former conflict areas or for the development of highly sensitive UO_2^{2+} biosensors.

In part, the selectivity of SUP derives from the atypical geometry of the uranyl cation which is a linear molecule. SUP was designed to bind UO_2^{2+} with pentagonal bipyramid geometry: five equatorial oxygen ligands to uranium are supplied by bidentate coordination of Glu17 and Asp68 and a water molecule, whereas the two axial positions are occupied by the oxo ligands of uranyl (Figure 1). An important stabilizing interaction is provided by Arg71, which supplies a hydrogen-bond to one of the axial oxo-ligands. However, MD simulations suggest a slightly different coordination sphere from that seen in the crystal structure, with Glu64 and Asp13 binding UO_2^{2+} in a monodentate fashion, while Glu17 and Asp68 bind UO_2^{2+} in an alternate monodentate/bidentate fashion (Odoh et al., 2014).

An oxygen-rich coordination sphere is very typical of UO_2^{2+} -protein complexes, due to the “hard” nature of the UO_2^{2+} cation and is in contrast to the binding of transition metal ions, which invariably include nitrogen or sulfur ligands (Van Horn and Huang, 2006; Carugo, 2018).

With this in mind, we were intrigued by the reported strong competition for SUP binding exhibited by Cu^{2+} . It was found that the presence of a $\sim 10^3$ -fold excess of Cu^{2+} completely prevented UO_2^{2+} from binding SUP (Zhang et al., 2014). Indeed Cu^{2+} was more effective at competing for binding than VO_2^{2+} , Pb^{2+} , or Mn^{2+} , which required between 10^4 - and 10^5 -fold excesses of metal ions to effectively displace UO_2^{2+} from the binding site. Cu^{2+} concentrations in seawater range between 0.004 and $1.6 \mu\text{M}$ (Campbell et al., 2014), indicating that Cu-binding could significantly impede the ability of SUP to bind UO_2^{2+} dissolved in the ocean.

In this study, we have re-evaluated the binding of Cu^{2+} to SUP and its ability to compete with UO_2^{2+} . Our results indicate that Cu^{2+} binds at two sites on the protein, but each is distinct from the UO_2^{2+} -binding site so that displacement of UO_2^{2+} by Cu^{2+} may occur by an indirect mechanism rather than simple competition. This view is supported by the observation of changes in the CD spectrum of SUP that occurs upon UO_2^{2+} binding, which suggest that conformational changes to SUP are involved in binding UO_2^{2+} .

RESULTS AND DISCUSSION

Structural Changes to SUP Accompanying UO_2^{2+} Binding

The crystal structure of SUP has been solved both with UO_2^{2+} bound and in the absence of metal ions (PDB 4FZO and 4FZP, respectively). The structures are almost superimposable, arguing that very little structural rearrangement occurs upon

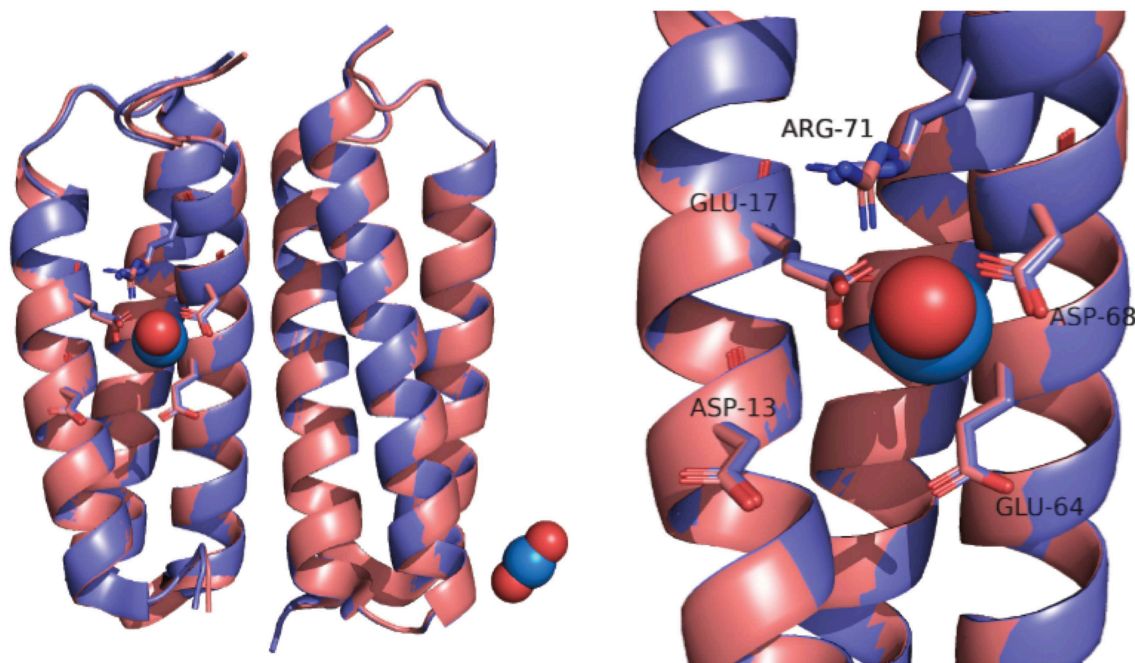
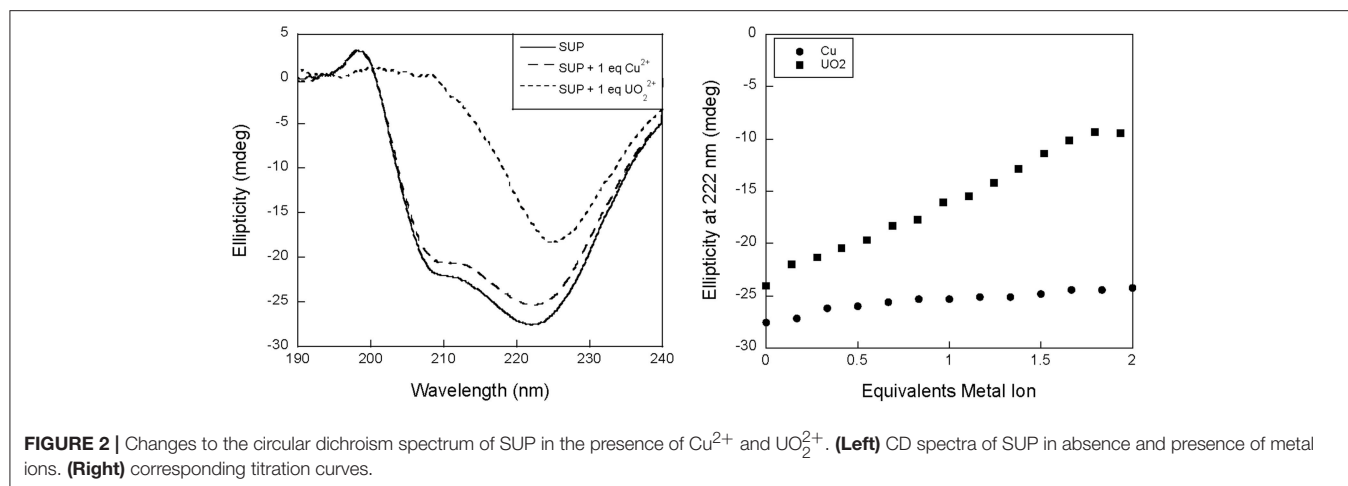


FIGURE 1 | Overlay of SUP crystal structures obtained with (pink) and without UO_2^{2+} bound (purple).



binding UO_2^{2+} (Figure 1). However, these structures could only be obtained at pH 4, and no structural information is available at physiological pH or at pH 8.9, for which UO_2^{2+} binding affinity was determined.

We therefore proposed to study UO_2^{2+} binding to SUP at pH 7.5 using circular dichroism (CD). Surprisingly, preliminary measurements revealed a significant change in the CD spectrum of SUP upon addition of metals. Apo-SUP possesses a CD spectrum characteristic of an extensively α -helical protein with minima at 208 and 222 nm; however, as shown in Figure 2, the addition of 1 equivalent of UO_2^{2+} to SUP resulted in an almost complete loss of the 208 nm band. This observation suggested that SUP may undergo changes to its structure upon binding metal ions. In particular, changes in the ratio of ellipticities at 222 and 208 nm are indicative of a change in the super-helical pitch of the helical bundle proteins such as SUP (Banerjee and Sheet, 2017).

The changes in the CD spectrum that accompanied UO_2^{2+} binding allowed us to measure the dissociation constant for this cation (Figure 3). Previous studies had used the colorimetric metal chelating agent Arsenazo III to determine UO_2^{2+} concentrations, which necessitated the separation of SUP- UO_2^{2+} from free UO_2^{2+} by ultrafiltration (Zhang et al., 2014). However, in our hands this assay proved difficult to reproduce and time consuming. In contrast, we were able to establish the dissociation constant for UO_2^{2+} quite straightforwardly by measuring the changes in ellipticity at fixed SUP and UO_2^{2+} concentrations as a function of increasing concentrations of Na_2CO_3 (Figure 3), which is a competitive chelator of UO_2^{2+} . Fitting the data as described in the Materials and Methods section yielded a K_d of ~ 0.4 fM at pH 7.9. This may be compared with the previously reported K_d of 7.4 fM obtained by the Arsenazo III method at pH 8.9 (Zhang et al., 2014). Importantly, Zhang et al. have previously reported a strong pH sensitivity of UO_2^{2+} binding affinity, with a reported 0.2 nM affinity at pH 6.0 measured by Arsenazo III method using DGA ligand (Zhang et al., 2014). The difference in the two K_d measurements likely rises from differences in the experimental conditions used

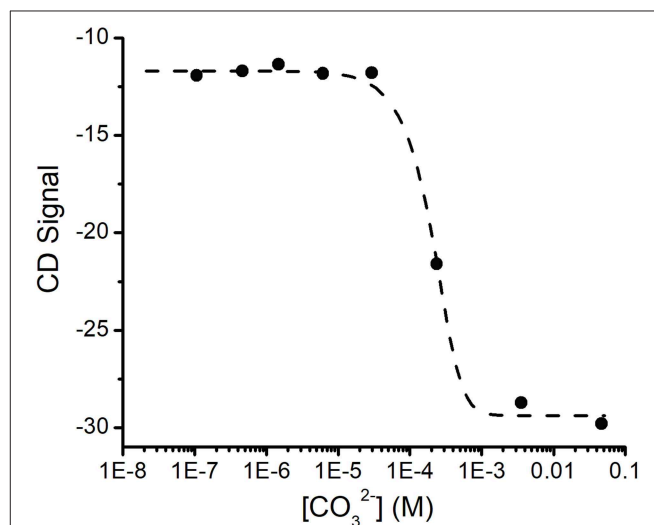
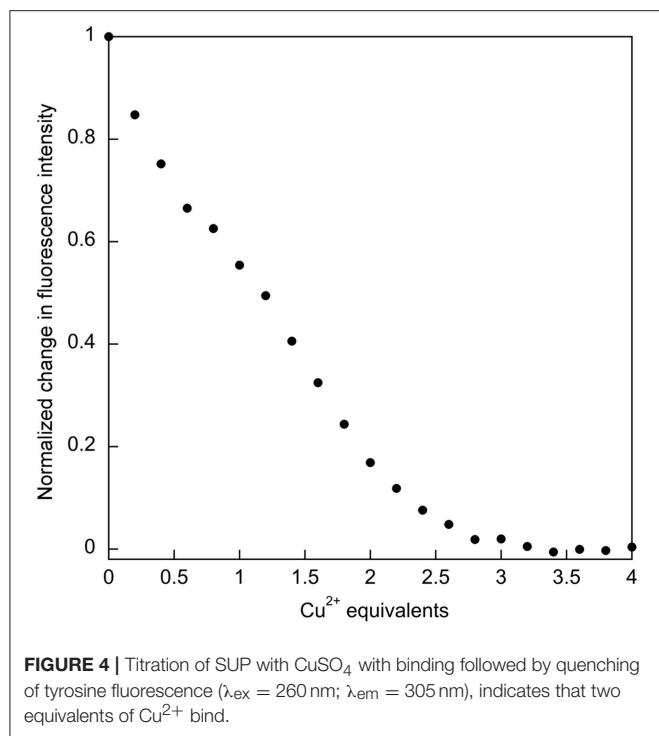


FIGURE 3 | Determination of the K_d for UO_2^{2+} binding SUP by competition with CO_3^{2-} . Increasing concentrations of CO_3^{2-} ion compete for UO_2^{2+} and the displacement of UO_2^{2+} was followed by changes in the CD spectrum of SUP. Experimental points were fitted using Dynafit (see Materials and Methods for details).

(notably pH), together with the fact that the calculated affinity of the protein for UO_2^{2+} is rather sensitive to small differences in CO_3^{2-} concentrations due to the presence of multiple, competing $\text{UO}_2^{2+}(\text{CO}_3^{2-})_n$ species. Therefore, given the different methods and conditions by which these K_d measurements were obtained, we consider the results to be in reasonable agreement.

Stoichiometry of Cu(II) Binding to SUP

The relatively high affinity of SUP for Cu^{2+} had previously been inferred from the ability of this metal ion to compete with UO_2^{2+} binding. It was reported that the presence of a 1,000-fold excess of Cu^{2+} was sufficient to prevent UO_2^{2+} from binding SUP (Zhang et al., 2014). This competitive effect of Cu^{2+} was much stronger



than, for example, VO²⁺ and Ca²⁺, ions that are expected to have coordination preferences similar to UO₂²⁺. We therefore decided to examine Cu²⁺ binding to SUP directly. We found that Cu²⁺ binding could be followed by monitoring changes in fluorescence due to the four Tyr residues in the protein. A 5 μM solution of the protein in HEPES buffer, pH 7.5, was titrated with increasing amounts of CuSO₄ and the fluorescence signal at 305 nm was recorded. The bi-phasic nature of the titration curve (**Figure 4**) suggested that SUP binds two equivalents of Cu²⁺ at sites that possess different affinities for the metal. This observation clearly implies that at least one of the Cu²⁺ binding sites must be distinct from the UO₂²⁺ binding site.

EPR Spectroscopic Studies on Cu²⁺ Binding to SUP

The oxygen-rich binding site for UO₂²⁺ would seem to be a poor binding site for Cu²⁺ which is a much softer cation. Indeed, Cu-binding sites in proteins invariably utilize at least one or more nitrogen or sulfur atoms as ligands to the metal (Andreini et al., 2008). EPR provides a sensitive method to probe the binding of copper to proteins as the A_{\parallel} and g_{\parallel} values are diagnostic for the ligands to the metal (Garribba and Micera, 2006). EPR spectra of Cu²⁺ were recorded in the presence of increasing stoichiometries of SUP in Tris-HCl buffer, pH 7.5 at 150 K. Comparison of spectra provided further evidence for two Cu²⁺ binding sites in SUP. Starting from free Cu²⁺ in solution (**Figure 5**, red curve), addition of 1 eq SUP gave rise to one set of hyperfine coupling bands, corresponding to the coordination of Cu²⁺ on SUP, denoted as site a (**Figure 5**, green curve). The addition of a second equivalent of SUP gives rise

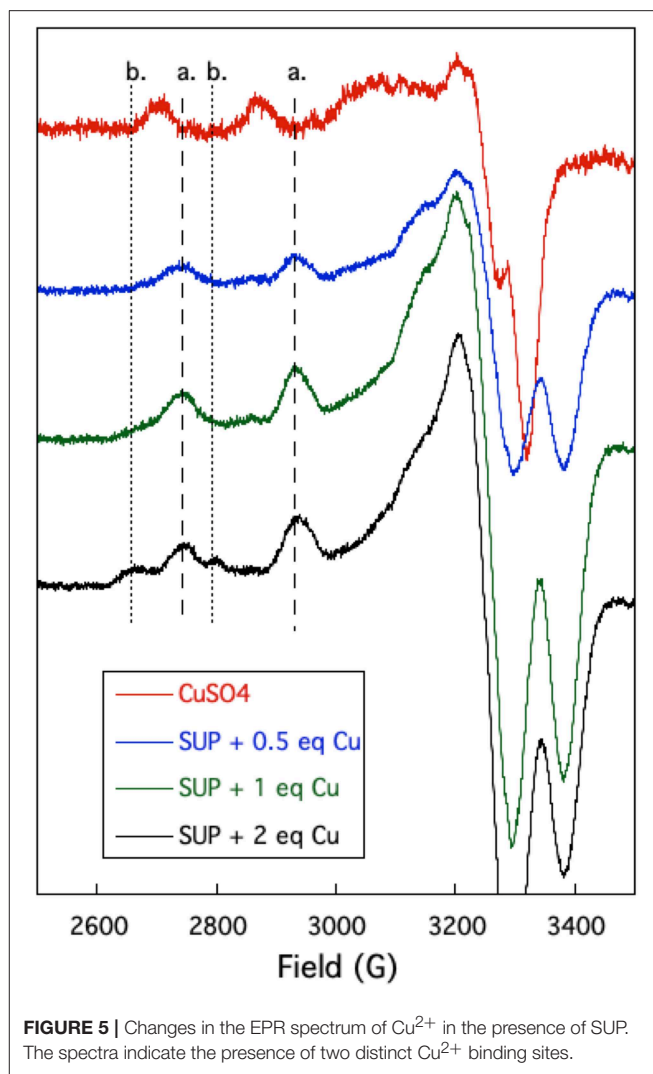
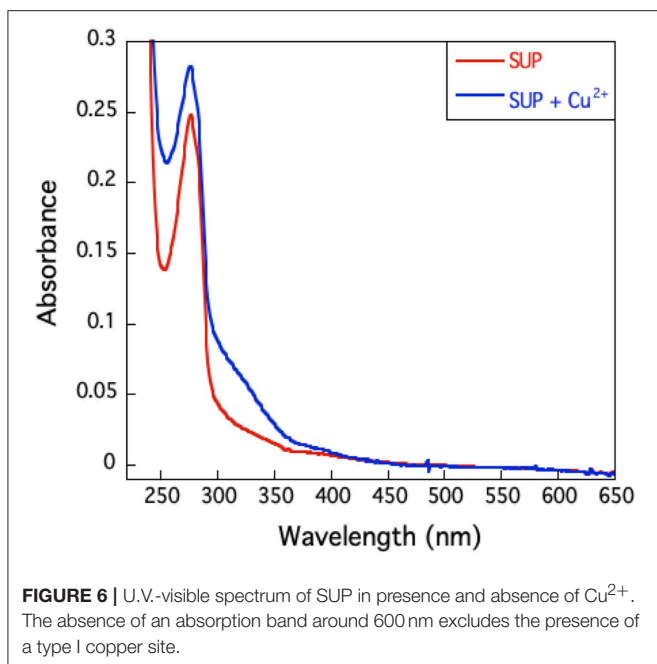


TABLE 1 | EPR parameters for the two binding sites of Cu²⁺ in SUP.

	A_{\parallel} (10^{-4} cm^{-1})	g_{\parallel}	xN_xO
Site a	177	2.207	4N
Site b	143	2.327	2N ₂ O

to a second set of hyperfine coupling bands, corresponding to a second binding site along SUP denoted as site b (**Figure 5**, black curve), which differs from site a and free Cu²⁺ signals. These data indicate that Cu²⁺ binds to site a first, and then b. The data are consistent with the two-site binding model indicated by the fluorescence studies.

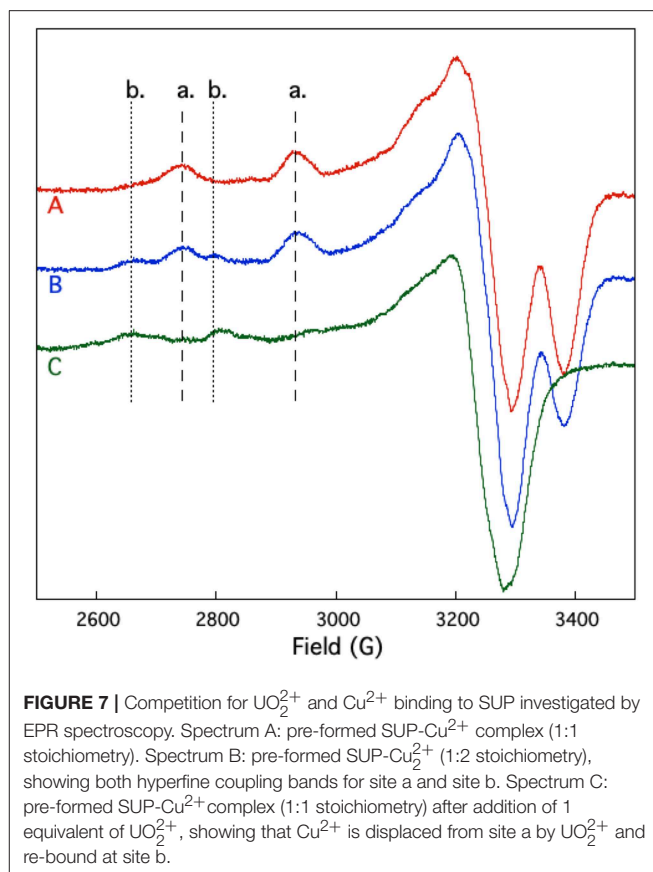
The hyperfine coupling constants and g values for site a and site b were calculated from the spectra and are given in **Table 1**. Comparison of the hyperfine coupling constants obtained for sites a and b with Peisach-Blumberg correlation tables (Peisach and Blumberg, 1974) indicates that the ligands constituting site a are all N-donors, whereas site b is consistent with a 2N₂O environment.



This is consistent with statistical survey data that shows that virtually all Cu(II) proteins use at least one N-donor ligand to bind Cu^{2+} , while only 21% of them use at least one O-donor ligand (Andreini et al., 2008). This further strengthens the hypothesis that Cu^{2+} is binding SUP through a different set of ligands from UO_2^{2+} . In their 2014 paper, Odoh et al. performed MD simulations on the UO_2^{2+} binding site in order to evaluate a possible binding of Cu^{2+} (Odoh et al., 2014). Their results concluded that Cu^{2+} could adopt a 6-O coordination, binding to Asp13 and 68, Glu17 and 64, and with one water molecule. However, a mismatch was detected between selectivity values obtained from this simulation and experimental data, showing that other parts of the protein might be to consider to fully model the SUP- Cu^{2+} interaction.

The UV-visible spectrum of SUP complexed with Cu^{2+} (Figure 6) shows no high intensity absorptions at wavelengths longer than 350 nm, which would be typical of a Type I copper protein. Taken together with the EPR hyperfine coupling, this indicates that SUP should be categorized as a Type II Cu protein with no sulfur ligation (Adman, 1991).

EPR spectroscopy was also used to study the competition between UO_2^{2+} and Cu^{2+} binding to SUP. If UO_2^{2+} shares coordinating residues with Cu^{2+} , addition of UO_2^{2+} to the pre-formed [SUP- Cu_2] complex should lead to a release of Cu^{2+} in solution, whereas preloading SUP with UO_2^{2+} should prevent Cu^{2+} from binding. The results of this experiment are shown in Figure 7. SUP loaded with one equivalent of Cu^{2+} exhibits an EPR spectrum in which copper is bound at the site. However, addition of one equivalent of UO_2^{2+} converts the spectrum to that of Cu^{2+} binding to the b site. These data indicate that UO_2^{2+} displaces Cu^{2+} from the high affinity a site,



causing it to move to the lower affinity b site. The copper a site either shares some metal-coordinating residues in common with the UO_2^{2+} site, or the two metal-binding sites are in sufficiently close proximity that UO_2^{2+} and Cu^{2+} are unable to bind simultaneously.

CONCLUSIONS

The anomalous affinity of SUP for Cu^{2+} ions, compared with other transition metal ions, may be explained by the fact that Cu^{2+} appears to bind at two sites on the protein. The initial studies of SUP measured the relative affinities of metal ions by examining their ability to displace UO_2^{2+} from the protein, rather than examining their binding directly. Based on our EPR data and the coordination environment of other well-characterized Cu-binding proteins (Rubino and Franz, 2012) it seems very unlikely that Cu^{2+} directly competes for the UO_2^{2+} binding site. Rather the data suggest that Cu^{2+} indirectly interferes with UO_2^{2+} binding. This could occur either by distorting the binding site or possibly competing for one of the protein side-chains that ligate UO_2^{2+} . These studies highlight an important problem designing proteins that bind metal ions with both high affinity and selectivity, i.e., that metal ions may bind adventitiously to the protein at unintended sites or recruit ligands from the designed binding site that interfere with binding of the intended metal ion.

Interestingly, we observed a significant change in the CD spectrum of SUP that accompanies UO_2^{2+} binding. This allowed us to measure the K_d for UO_2^{2+} binding far more easily than the previously published assay that involved separating protein-bound and free UO_2^{2+} and then determining free UO_2^{2+} using a chemical test. Also significant, the changes to the CD spectrum suggest that the UO_2^{2+} -SUP complex may have a different secondary structure from the apo-protein at pH 7.5, possibly associated with a change in the pitch of the alpha-helical bundle. This is of interest because a comparison of the crystal structures of SUP with and without UO_2^{2+} bound at pH 4 revealed very little alteration of the protein's structure (Zhang et al., 2014; **Figure 1**). Further studies into the solution structure of SUP are in progress to better define how UO_2^{2+} binding alters the structure of the protein. These studies may help to explain the remarkably high affinity of SUP for this unusual metal-oxo cation.

MATERIALS AND METHODS

Protein Expression and Purification

A *E. coli* codon-optimized gene encoding SUP with an N-terminal 6-His tag followed by a TEV protease cleavage site was commercially synthesized and introduced into the expression vector pET28a by standard methods. This construct was used to transform *E. coli* BL21 DE3 cells by standard methods. Cells were cultured in 1 L of LB medium containing kanamycin 50 mg/L and grown at 37°C until reaching OD_{600} 0.8–1. Protein expression was induced by addition of IPTG, 0.3 mM final concentration, and the cells allowed to grow overnight at 22°C.

Cells were harvested by centrifugation and the cell pellet was resuspended in lysis buffer (Tris-HCl pH 7.5 10 mM, NaCl 300 mM, TCEP 1 mM, protease inhibitor) using 4 mL of buffer per gram of cell paste. Urea was added to a final concentration of 2 M and the cells lysed by sonication, using 10 s pulses followed by a 20 s pause for a total time of 30 min. The lysate was then clarified by centrifugation (16,000 rpm, 45 min, 4°C) and slowly loaded onto a HisTrap column pre-equilibrated with buffer A (Tris-Cl pH 7.5 10 mM, NaCl 300 mM) at 4°C. The column was washed extensively with buffer A, followed by washing with 20% buffer B (Tris pH 7.5 10 mM, NaCl 300 mM, imidazole 500 mM) to remove non-specifically bound proteins. SUP was then eluted from the column by washing with 70% buffer B.

Fractions containing SUP were dialyzed against buffer A overnight and the 6-His-tag was then cleaved by addition of 10 μL TEV protease, followed by incubation at RT for 12 h. The His-tag and any uncleaved protein were removed by incubation with Ni-NTA beads overnight. TEV and remaining contaminant proteins were precipitated by heating the samples at 70°C for 30 min followed by centrifugation (12,000 rpm, 10 min, RT) to remove precipitated contaminants. Finally, the sample was concentrated and then desalted using a Superdex 200 10/30 column, equilibrated in SEC buffer (HEPES pH 7.5 20 mM, NaCl 100 mM) at 0.3 mL/min.

To concentrate the protein, if necessary, protein solutions were lyophilized to dryness, and the residual powder re-dissolved

in the desired volume of water and dialyzed overnight. The protein concentration was determined by absorbance at 280 nm ($\epsilon_{280} = 5,120 \text{ M}^{-1} \text{ cm}^{-1}$), and the identification of the protein was confirmed by LC-MS analysis. Samples were stored at 4°C until use.

EPR Spectrometry

Samples were prepared by mixing CuSO_4 (final concentration 200 μM) with increasing amounts SUP. The sample volume was adjusted to 200 μL by addition of buffer (Tris-HCl, 10 mM, pH 7.5, 300 mM NaCl) and samples were incubated at RT for 2 h. Ten percentage glycerol was added, and samples were transferred into EPR tubes and flash-frozen in liquid nitrogen. X-band EPR spectra were recorded on a Bruker EMX spectrometer at 150 K using an ER-4102-ST rectangular cavity. All measurements were replicated on at least two independent samples, showing identical spectra.

Fluorescence Spectrophotometry

A sample of SUP was diluted in HEPES buffer (20 mM, pH 7.4) containing 100 mM NaCl to a final concentration of 5 μM . Increasing amounts of a 1 mM stock solution of CuSO_4 were added to the sample, and fluorescence emission spectra were immediately recorded on a Cary Eclipse spectrometer at RT ($\lambda_{\text{exc}} = 260 \text{ nm}$, $\lambda_{\text{em}} = 290\text{--}400 \text{ nm}$, slits 5 nm) using a $10 \times 4 \text{ mm}$ quartz cuvette. Recording spectra after various equilibration times (min to h) did not show any variation in signal intensity, indicating that the binding of Cu^{2+} was rapid. Data presented is the average of three independent titrations.

Circular Dichroism

Circular dichroism spectra were recorded on a Jasco spectrophotometer using a 1 mm path length quartz cuvette. All measurements were made at room temperature. To a 30 μM solution of SUP in 10 mM Tris-HCl buffer, pH 7.5, containing NaCl 300 mM were added increasing amounts of CuSO_4 (1 mM stock) or $\text{UO}_2(\text{NO}_3)_2$ (0.414 mM stock). The solutions were carefully mixed to homogeneity before recording spectra. Spectra were recorded in triplicate and averaged.

To determine the K_d of SUP for UO_2^{2+} , a 30 μM solution of SUP and $\text{UO}_2(\text{OAc})_2$ was titrated with increasing amounts of Na_2CO_3 and the change in ellipticity at 222 nm recorded. Titration was repeated in triplicates, showing consistent results. A representative titration curve was fitted using the program Dynafit (Kuzmič, 1996) as a competition between the formation of the UO_2^{2+} -SUP complex and the uranyl carbonate complexes: $\text{UO}_2(\text{CO}_3)$, $\text{UO}_2(\text{CO}_3)_2^{2-}$, and $\text{UO}_2(\text{CO}_3)_3^{4-}$. The formation constants of the three carbonate forms were fixed at $1.45 \times 10^{-9} \text{ M}^{-1}$, $3.31 \times 10^{-16} \text{ M}^{-2}$, and $1.23 \times 10^{-22} \text{ M}^{-3}$, respectively, with the values being taken from reference (Zhang et al., 2014). The concentration of CO_3^{2-} at each titration point was adjusted for HCO_3^- formation by measuring the pH at each point and calculating the CO_3^{2-} concentration using a pK_a of 9.65 (Zhang et al., 2014).

DATA AVAILABILITY

All datasets generated for this study are included in the manuscript/supplementary files.

AUTHOR CONTRIBUTIONS

MH, ZC, and EM conceived the study and designed the experiments. MH performed the experiments. MH, KK, ZC, and EM analyzed the data. MH and EM wrote the paper.

REFERENCES

- Adman, E. T. (1991). Copper protein structures. *Adv. Prot. Chem.* 42, 145–197.
- Andreini, C., Banci, L., Bertini, I., and Rosato, A. (2008). Occurrence of copper proteins through the three domains of life: a bioinformatic approach. *J. Proteome Res.* 7, 209–216. doi: 10.1021/pr070480u
- Banerjee, R., and Sheet, T. (2017). Ratio of ellipticities between 192 and 208 nm (R_1): an effective electronic circular dichroism parameter for characterization of the helical components of proteins and peptides. *Proteins* 85, 1975–1982. doi: 10.1002/prot.25351
- Barak, Y., Ackerley, D. F., Dodge, C. J., Banwari, L., Alex, C., Francis, A. J., et al. (2006). Analysis of novel soluble chromate and uranyl reductases and generation of an improved enzyme by directed evolution. *Appl. Environ. Microbiol.* 72, 7074–7082. doi: 10.1128/AEM.01334-06
- Bhalara, P. D., Punetha, D., and Balasubramanian, K. (2014). A review of potential remediation techniques for uranium(VI) ion retrieval from contaminated aqueous environment. *J. Environ. Chem. Eng.* 2, 1621–1634. doi: 10.1016/j.jece.2014.06.007
- Brulfert, F., Safi, S., Jeanson, A., Martinez-Baez, E., Roques, J., Berthomieu, C., et al. (2016). Structural environment and stability of the complexes formed between calmodulin and actinyl ions. *Inorg. Chem.* 55, 2728–2736. doi: 10.1021/acs.inorgchem.5b02440
- Campbell, A. L., Mangan, S., Ellis, R. P., and Lewis, C. (2014). Ocean acidification increases copper toxicity to the early life history stages of the polychaete *Arenicola marina* in artificial seawater. *Environ. Sci. Technol.* 48, 9745–9753. doi: 10.1021/es502739m
- Carugo, O. (2018). Structural features of uranium-protein complexes. *J. Inorg. Biochem.* 189, 1–6. doi: 10.1016/j.jinorgbio.2018.08.014
- Chakraborty, S., Kravitz, J. Y., Thulstrup, P. W., Hemmingsen, L., DeGrado, W. F., and Pecoraro, V. L. (2011). Design of a three-helix bundle capable of binding heavy metals in a triscysteine environment. *Angew. Chem. Int. Ed.* 50, 2049–2053. doi: 10.1002/anie.201006413
- Garribba, E., and Micera, G. (2006). The determination of the geometry of Cu(II) complexes: an EPR spectroscopy experiment. *J. Chem. Educ.* 83:1229. doi: 10.1021/ed083p1229
- Handley-Sidhu, S., Keith-Roach, M. J., Lloyd, J. R., and Vaughan, D. J. (2010). A review of the environmental corrosion, fate and bioavailability of munitions grade depleted uranium. *Sci. Total Environ.* 408, 5690–5700. doi: 10.1016/j.scitotenv.2010.08.028
- Kou, S., Yang, Z., Luo, J., and Sun, F. (2017b). Entirely recombinant protein-based hydrogels for selective heavy metal sequestration. *Polym. Chem.* 8, 6158–6164. doi: 10.1039/C7PY01206C
- Kou, S., Yang, Z., and Sun, F. (2017a). Protein hydrogel microbeads for selective uranium mining from seawater. *ACS Appl. Mater. Interfaces* 9, 2035–2039. doi: 10.1021/acsami.6b15968
- Kuzmič, P. (1996). Program DYNAFIT for the analysis of enzyme kinetic data: application to HIV proteinase. *Anal. Biochem.* 237, 260–273. doi: 10.1006/abio.1996.0238
- Le Clainche, L., Plancque, G., Amekraz, B., Moulin, C., Pradines-Lecomte, C., Peltier, G., et al. (2003). Engineering new metal specificity in EF-hand peptides. *J. Biol. Inorg. Chem.* 8, 334–340. doi: 10.1007/s00775-002-0419-2
- Le Clainche, L., and Vita, C. (2006). Selective binding of uranyl cation by a novel calmodulin peptide. *Environ. Chem. Lett.* 4, 45–49. doi: 10.1007/s10311-005-0033-y

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- Montes-Bayón, M., and Blanco-González, E. (2016). “Metalloproteins,” in *Metalloomics: Analytical Techniques and Speciation Methods* (Wiley-Blackwell), 339–357.
- Newsome, L., Morris, K., and Lloyd, J. R. (2014). The biogeochemistry and bioremediation of uranium and other priority radionuclides. *Chem. Geol.* 363, 164–184. doi: 10.1016/j.chemgeo.2013.10.034
- Odoh, S. O., Bondarevsky, G. D., Karpus, J., Cui, Q., He, C., Spezia, R., et al. (2014). UO_2^{2+} uptake by proteins: understanding the binding features of the super uranyl binding protein and design of a protein with higher affinity. *J. Am. Chem. Soc.* 136, 17484–17494. doi: 10.1021/ja5087563
- Peisach, J., and Blumberg, W. E. (1974). Structural implications derived from the analysis of electron paramagnetic resonance spectra of natural and artificial copper proteins. *Arch. Biochem. Biophys.* 165, 691–708. doi: 10.1016/0003-9861(74)90298-7
- Plegaria, J. S., Dzul, S. P., Zuiderweg, E. R., Stemmler, T. L., and Pecoraro, V. L. (2015). Apoprotein structure and metal binding characterization of a *de novo* designed peptide, $\alpha 3DIV$, that sequesters toxic heavy metals. *Biochemistry* 54, 2858–2873. doi: 10.1021/acs.biochem.5b00064
- Rubino, J. T., and Franz, K. J. (2012). Coordination chemistry of copper proteins: how nature handles a toxic cargo for essential function. *J. Inorg. Biochem.* 107, 129–143. doi: 10.1016/j.jinorgbio.2011.11.024
- Starck, M., Sisommay, N., Laporte, F. A., Oros, S., Lebrun, C., and Delangle, P. (2015). Preorganized peptide scaffolds as mimics of phosphorylated proteins binding sites with a high affinity for uranyl. *Inorg. Chem.* 54, 11557–11562. doi: 10.1021/acs.inorgchem.5b02249
- Van Horn, J. D., and Huang, H. (2006). Uranium(VI) bio-coordination chemistry from biochemical, solution and protein structural data. *Coord. Chem. Rev.* 250, 765–775. doi: 10.1016/j.ccr.2005.09.010
- Wegner, S. V., Boyaci, H., Chen, H., Jensen, M. P., and He, C. (2009). Engineering a uranyl-specific binding protein from NikR. *Angew. Chem. Int. Ed.* 48, 2339–2341. doi: 10.1002/anie.200805262
- Xie, Y., Chen, C., Ren, X., Wang, X., Wang, H., and Wang, X. (2019). Emerging natural and tailored materials for uranium-contaminated water treatment and environmental remediation. *Prog. Mater. Sci.* 103, 180–234. doi: 10.1016/j.pmatsci.2019.01.005
- Zhang, C., Li, C. J., He, C., Liu, J., Zhang, L., Zhou, L., et al. (2014). A protein engineered to bind uranyl selectively and with femtomolar affinity. *Nat. Chem.* 6:236. doi: 10.1038/nchem.1856
- Zhang, X.-J., Wang, X.-W., Da, X.-D., Shi, Y., Liu, C., Sun, F., et al. (2018). A versatile and robust approach to stimuli-responsive protein multilayers with biologically enabled unique functions. *Biomacromolecules* 19, 1065–1073. doi: 10.1021/acs.biomac.8b00190

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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