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Uranyl–Peptide Interactions in Carbonate Solution with DAHK and Derivatives

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Metal–peptide complexes in a 1:1 ratio between the uranyl cation (UO_2^{2+}) and the peptides, DAHK or GGH, are observed in the gas phase (ESI-MS). Solution state studies with the same peptides and variants, DGHG, AcDGHG, and DAHKSE-$CONH_2$, indicate that peptide–carboxylato donors can coordinate to the uranyl bis-carbonato complex. UV–vis and fluorescence spectra of uranyl carbonate exhibit significant changes or quenching upon addition of peptide. NMR titration data were used to determine conditional association constants, log $K = 2.2 \pm 0.4$ and log $K = 3.1 \pm 0.4$, for the $[UO_2(CO_3)_2(GGH)]$ and $[UO_2(CO_3)_2(DAHK)]$ species, respectively. Uranyl asymmetric stretching frequencies for uranyl/DAHKSE–$CONH_2$ ($\nu_3 = 914$ cm^{-1}) and uranyl/DAHK ($\nu_3 = 908$ cm^{-1}) complexes and other infrared spectral features are also consistent with peptide–carboxylato coordination.

Remarkably, the biocoordination behavior of the uranyl cation is relatively unexplored.[1−4] While the adverse effects associated with uranium nephrotoxicity have been well established,[1,4,5] researchers have not pinpointed the specific molecular interactions involved with uranyl transport and chemical toxicity in vivo. In addition, there have been few quantitative studies on thermodynamic stability of uranyl and other actinyl ions with proteins and other bioligands except for reports relating uranyl−transferrin (Tf)[6,7] and neptunyl−Tf binding.[8] A recent study has also highlighted the potential for the uranyl cation to potentiate hydrolysis reactions in the backbone of the DNA molecule.[9]

These few data and our interest in heavy metal−peptide coordination chemistry led us to study the interaction of uranyl with a model transport moiety for this cation derived from human serum albumin (HSA). In studies using blood plasma from workers in uranium mines in Hungary, researchers proposed uranyl coordination to protein components of blood serum.[10,11] Their findings included a stability constant for the uranyl−"albumin" (or protein) complex to be around 9.9 under physiological conditions. Beyond this report, little has been done to evaluate the molecular speciation of uranyl in vivo.

Under physiologic conditions in serum (pH $\sim$ 7.4, [carbonate] $\sim$ 0.027, [salt] $\sim$ 0.13, [phosphate] $\sim$ 0.002),[12] the predominant forms of uranyl ($[UO_2^{2+}] \sim 1 \times 10^{-6}$) are the carbonate species represented by the following equilibrium:

$$[UO_2(CO_3)_2]^{2-} + CO_3^{2-} \rightleftharpoons [UO_2(CO_3)_3]^{4-} \qquad (1)$$

The tris-carbonato species is predominant, but some of the bis-carbonato complex will be present in vivo. At physiological pH and carbonate concentration, ligands with moderate formation constants should compete favorably with a third carbonate to form complexes with the uranyl cation. Generally, uranyl speciation in serum is expected to be the uranyl carbonate species in equilibrium with a uranyl−protein species.[13,14] This protein is presumably Tf or HSA, or may be another protein or peptide fragment; whether these interactions are specific or nonspecific has not been determined.

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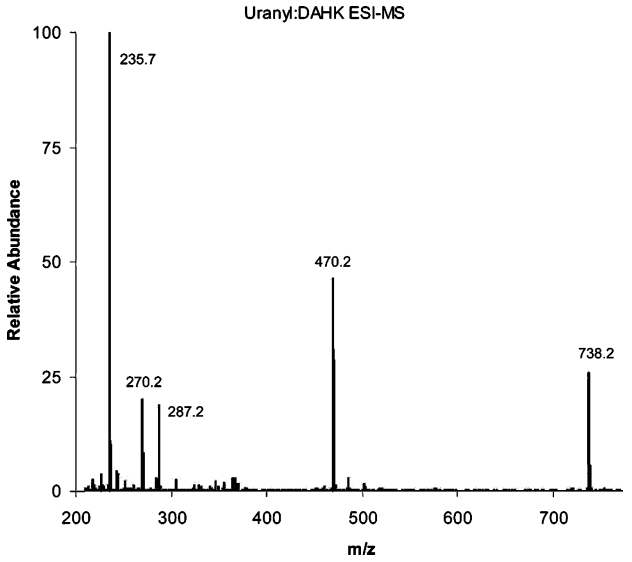

Figure 1. ESI-MS of uranyl/DAHK. Significant peaks (*m*/*z*) include the 1:1 adduct (738.2), singly and doubly charged peptide ligand (470.2 and 235.7), uranyl monocation (270.2), and uranyl hydroxide cation (287.2).

The amino terminal region of the serum albumins of a number of mammalian species exhibits high affinity for transition metal divalent cations (especially Ni and Cu).[15] This "amino−terminal metal binding motif" has a general sequence of XXH and coordinates these metal ions with a formation constant of around 10−12. With the biomedical data, and the literature on metal peptide complexation of the M[XXH] variety, we hypothesized that coordination of uranyl cation in HSA might be associated with a modified donor set of the binding motif. Specifically, the *N*-terminus of HSA has the sequence DAHKSE, potentially providing carboxylate donors from Asp and Glu, and electrostatic or H-bonding interactions with the side chains of Lys and His. Another model is a purely electrostatic model of binding between the $UO_2(CO_3)_n$ complex anion ($n = 2$ or 3) and charged peptide side chains. Mixed inner- and outer-sphere interactions present a final possibility for mediating uranyl−protein interactions.

Preliminary experiments probed the gas-phase behavior of uranyl/peptide samples.[16−18] Thus, a 2:1 ratio of uranyl ion plus the peptide (DAHK or GGH) was infused into an ion trap mass spectrometer via electrospray ionization (ESI MS), which gave rise to the anticipated $[UO_2/DAHK]^+$ 1:1 complex at *m*/*z* 738 (Figure 1). Also present in the spectrum were signals for uranyl monocation, uranyl−hydroxide cation, and singly and doubly charged peptide ligand. Separately, the $[UO_2/GGH]^+$ 1:1 complex was found at *m*/*z* 538.9. Further, collision induced dissociation of the parent $[UO_2/DAHK]^+$ complex gave rise to a mass spectrum that included an expected $[ML - H_2O]^+$ fragment,[19] and significant fragments associated with the gas-phase dissociation of ligand functional groups, e.g., loss of a Lys or Asp residue, Asp-Ala fragment, or imidazole (His) side chain. In the MS/MS experiment, demetalation of uranyl from the $[UO_2/DAHK]^+$ complex was negligible.

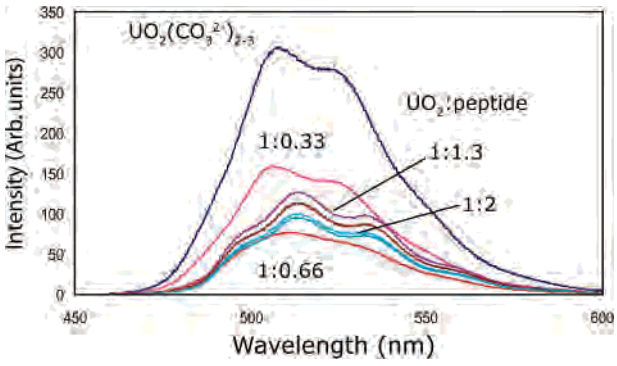

Figure 2. Quenching of uranyl carbonate emission spectra with addition of peptide, DAHK (UO_2/carbonate/DAHK 1:3:[0.33, 0.66, 1, 1.3, 1.6, and 2 equiv.]).

The above experiments do not offer bonding details, but the observed fragmentation in the MS/MS experiment points to ample coordinating groups for the uranyl cation, Asp, Glu, C-terminal, or His side chain donors and possibly amino donor groups. Metal replete solutions appear to be a necessary condition for the observation of low to moderate stability metal−peptide complexes using the electrospray ionization method. We estimate from these data a formation constant for coordination of GGH or DAHK of log K_f less than 6.

The second line of investigation with the UO_2/DAHK system involved solution phase spectroscopies: absorbance, fluorescence, and NMR. The addition of 0.95−10.0 equiv of UO_2 to solutions of peptides (DAHK, DGHG, or AcDGHG) at neutral pH induces an increase in the absorbance bands in the UV and visible regions of the spectrum. In addition, titration of UO_2/DAHK with 0.1 M NaOH increases the uranyl absorption feature at 430 nm and a shoulder at 335 nm; these observations are qualitatively assigned to peptide−carboxylato coordination to uranyl. Similar experiments with solutions of the tripeptide, GGH, did not illicit similar changes in the spectrum, suggesting that the Asp residue is an essential feature in solution interactions.

Dilute, degassed (Ar) uranyl carbonate solutions that mimic potential physiological conditions (~pH 7) with carbonate ratios, UO_2/CO_3 1:6 or 1:3, give strong fluorescence emission spectra. The addition of DAHK or Ac-DGHG to these dilute solutions results in a dramatic quenching of the emission band, indicating uranyl−peptide or uranyl−carbonate−peptide interactions (Figure 2). Quantitative addition of DAHK peptide to a UO_2/CO_3^{2-} 1:3 solution led to a quenching of the characteristic uranyl carbonate emission signal at around 500 nm, and the formation of a new emission feature was centered at 520 nm. Ligand coordination appears to be the mechanism for quenching, as the solutions are dilute

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and essentially free of air. This experiment supports the hypothesis that peptide caboxylate ligands compete with carbonate to form ternary uranyl/carbonate/peptide complexes (vide infra). In separate experiments, uranyl carbonate emission spectra are quenched with the addition of 0.1 equiv of any of the proteins, human serum albumin, human transferrin, or chicken egg white lysozyme.

Changes in peptide side chain signals in NMR experiments are a sensitive probe of coordination of transition metals to peptide ligands, e.g., [Ni(GGH)].[15,20] Solutions of UO_2/peptide/CO_3 mixtures (peptide = DAHK, DGHG, AcDGHG, DAHKSE−$CONH_2$) give rise to small changes in signals for His imidazole protons and only slightly larger shifts of Asp and other side chains' β protons. Variation of the carbonate buffer ratio (1−10 equiv) occasionally led to precipitation of uranyl peptide adducts, and excess carbonate led to the formation of uranyl carbonate exclusively as indicated by absorbance measurements (vide supra). An NMR titration[21] at constant pH, with stoichiometric additions of uranyl carbonate, allows for the determination of an association constant for the addition of peptide ligand (presumably carboxylate) to the uranyl biscarbonate species:

$$[UO_2(CO_3)_2]^{2-} + L^n \rightleftharpoons [UO_2(CO_3)_2L]^{n-2} \qquad (2)$$

Experimental data were fit to the formation of a 1:1 complex of uranyl/peptide and refined using a least-squares method in the program hypNMR.[22,23] Stepwise conditional association constants (0.1 M sodium carbonate) for the species $[UO_2(CO_3)_2(GGH)]$ and $[UO_2(CO_3)_2(DAHK)]$ were determined to be $\log K = 2.2 \pm 0.4$ and $\log K = 3.1 \pm 0.4$, respectively. These values are in accord with association constants for uranyl coordination with amino acid carboxylates. A speciation diagram (Figure 3) using the above constant for uranyl−DAHK and the conditions used in the fluorescence experiment indicates substantial formation of a ternary uranyl−carbonate−peptide species.

Finally, uranyl−peptide complexes that precipitated from carbonate solution (approximately 1:3:6 uranyl/peptide/carbonate) were analyzed by IR spectroscopy. The asymmetric stretch, ν_3, of O=U=O is found at 908 or 914 cm^{-1} for the uranyl/DAHK and the uranyl/DAHKSE−$CONH_2$ precipitate, respectively. When compared to the same

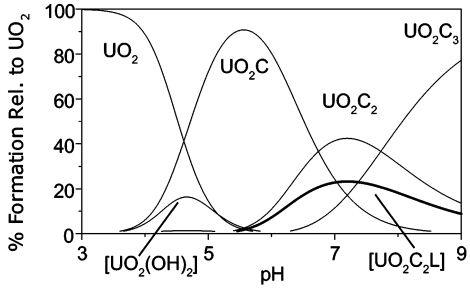

Figure 3. Speciation diagram for uranyl/carbonate/DAHK 1:2:1 under conditions similar to fluorescence experiment (L = peptide; C = carbonate). $[UO_2^{2+}] = 0.45$ mM; $[CO_3^{2-}] = 1.35$ mM; [DAHK] = 0.45 mM.

stretching frequency for uranyl nitrate (~940 cm^{-1}), acetate (~920 cm^{-1}), or carbonate (~893 cm^{-1}), the observed band is consistent with carboxylate coordination from a peptide ligand. The spectrum also exhibits features consistent with peptide amide and amine groups, e.g., 1649 and 1536 cm^{-1}; uranyl nitrate complexes may be ruled out.

In conclusion, a qualitative assessment of uranyl binding to peptides indicates that unique and important species can form in vitro with peptide ligands in the presence of carbonate. A preliminary determination of the relevant association constants for these species indicates binding may occur at physiological pH; the presence of these species is supported by fluorescence quenching experiments. Extending this analysis to conditions that mimic blood serum,[12] our data suggest that nonspecific binding interactions with protein are a mechanism for the speciation and transport of uranyl in the serum (see Supporting Information). This does not preclude specific and high stability coordination of the uranyl cation to proteins[6,7] or results from studies with whole serum.[10,11] Taken together, the results of this study indicate that nonspecific binding of uranyl to carboxylate donors may be one component of expected uranyl−protein interactions in vivo.

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Supporting Information Available: Experimental details, tables, and figures. This material is available free of charge via the Internet at http://pubs.acs.org.

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