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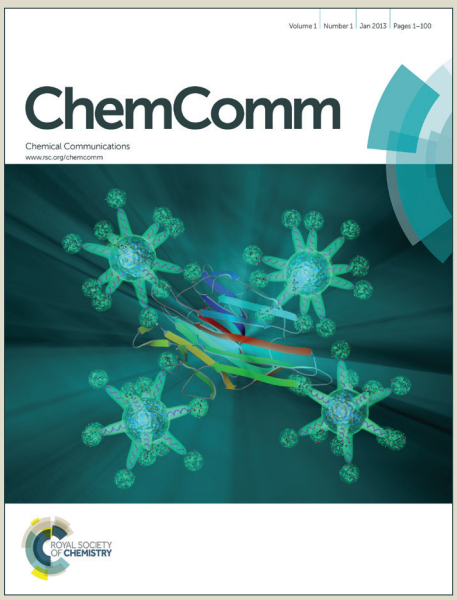

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Fluorescent recognition of uranyl ions by a phosphorylated cyclic peptide

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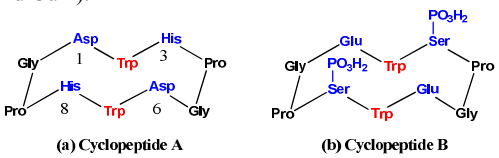
Fluorescent recognition of uranyl ions was achieved with a phosphorylated cyclic peptide, which can be used as a fluorescent sensor for the detection of uranyl ions with high selectivity and sensitivity.

Naturally occurring uranium is important for nuclear fission. With the growing human demand for non-fossil energy, the use of uranium as nuclear fuel has been increasing.[1] However, uranium is radioactive and chemically toxic. Its widespread application increases the risk of human exposure, and poses environmental problems from the storage of radioactive uranium waste.[2] Therefore, the development of new analytical methods for uranium, materials for its separation, effective antidotes, and more efficient remediation methods for pollution have become the focus of radiochemistry.[2,3]

For uranium, the most stable and common ionic form is the linear trans uranyl ion UO_2^{2+}, which contains a dioxo unit and a hexavalent uranium.[4] Through a long period of evolution, recognition molecules (mainly proteins) highly sensitive to and selective for toxic metal ions have occurred naturally. The high selectivities and affinities of these molecules are highly promising for uranium's separation, detection and biological regeneration. The good biocompatibilities are beneficial for designing efficient detoxification agents. Indeed, some target proteins found in the human body (such as albumin, transferrin, fetuin and osteopontin protein) show high affinity for uranyl ion.[5–8] In recent studies from He et al., protein engineering techniques, such as imitating Ni-binding sites (NikR)[9] and *de novo* design (SUP: 4FZO and 4FZP),[10] have been successfully used to develop recognition proteins with high affinity and selectivity for uranyl ions. Nonetheless, for practical application, mimicking these proteins to design small peptide ligands may be a better approach.[11] In this context, Delangle et al. recently reported their systematic studies on the promising complex of uranyl with structured cyclic peptide scaffolds with four acidic residues.[12] However, the good affinity of carboxyl group with a variety of metal ions results in the difficult recognition of uranyl ion from different competition ions in nature.[13,14] Therefore, the specific complex/recognition of uranyl ion in presence of competition ions remains requisite whereas challenging.[15]

In this paper, results showed that the uranyl ion produces fluorescence quenching of phosphorylated cyclodecapeptides, while the other metal ions (including Th^{4+}, lanthanides, transition metals, and main group elements) have little influence on the intensity of fluorescence. This type of cyclic peptide is highly selective for and sensitive to uranyl ions and it has strong anti-interference capability, so they can serve as a novel fluorescent sensor of uranyl ions. To our knowledge, this is the first report on the fluorescent recognition of uranyl ion using polypeptides, and will hopefully lead to the development of more powerful biocompatible chelating agents and biomaterials for uranium's separation and detoxification.

In this study, structured cyclodecapeptides are served as model peptides for two reasons: 1) The strong conformational restriction enable the significantly enhanced stability of cyclic peptides (compared to the linear polypeptide chains). Meanwhile, low flexibility resulting from ring restriction improves their binding affinity for various receptors.[16] 2) Two Pro-Gly sequence induced β-turns, and thus the backbone exhibited an antiparallel β-sheet conformation. As a result, the conformation of peptide shows two independent planes. On the top plane the amino acid side chain can coordinate with uranyl to construct the equatorial plane, and on the bottom plane the tryptophan can be used as a fluorescent probe.[17] In accordance with these ideas, we first mimicked the binding sites of uranyl-specific binding protein from NikR by introducing two aspartic acids (positions 1 and 6 on cyclic peptide A, Fig. 1a) and two histidines (positions 3 and 8). Subsequently, we investigated the fluorescent response of cyclic peptide A to uranyl ion. The tryptophan fluorescence was quenched at 360 nm with the addition of uranyl ion, indicating the presence of uranyl-cyclic peptide A complex (Fig. S1 in ESI). Unfortunately, similar fluorescence quenching phenomenon was also observed with the addition of other metal ions (such as Th^{4+}, Nd^{3+}, and Cu^{2+}).



(a) Cyclopeptide A (b) Cyclopeptide B

Fig. 1 Structure of cyclic peptides

In order to improve the selectivity for uranyl ion, the cyclic peptide B (Fig. 1b) was then designed according to the following strategies: 1) Histidine was replaced with phosphorylated serine to achieve stronger binding ability and selectivity of uranyl ion with phosphate groups.[8d, 18] 2) Aspartic acid was replaced with glutamic acid, because the weaker acidity and flexible side chains can provide stronger binding capability.[12] Fig. 2 shows the fluorescent response for the titration of cyclic peptide B with uranyl nitrate, and the addition of uranyl ion could lead to a large quenching of tryptophan fluorescence at pH 6.0. Meanwhile, the addition of one equivalent of uranyl led to an endpoint, indicating the possible formation of 1:1 UO_2-B complex. This proposal was further supported by both the continuous variation (Job's plot, Fig. S2 in ESI) and the Hildebrand-Benesi equation.[19] The association constant in this complex was calculated as 2.4×10^5 (Fig. S3 in ESI), higher than that at more acidic condition (pH 4.0, 1.3×10^5, Fig. S4 in ESI), and much higher than that of

uranyl-cyclic peptide A (1.2×10^4, Fig. S3 in ESI).

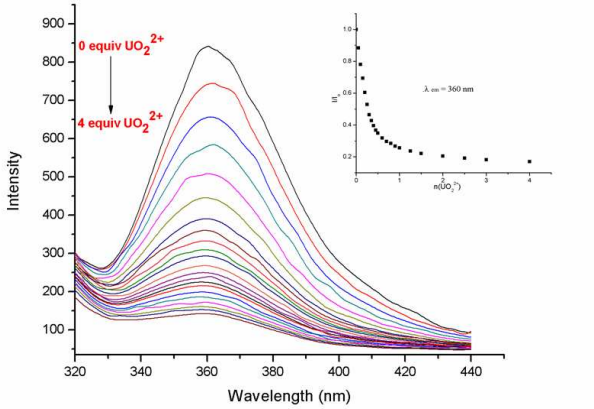


Fig. 2 Fluorescent titration of B (ca. 21 μM) with UO_2^{2+} (0–4 equiv) at pH 6.0, MES buffer (20 mM), with excitation at 285 nm. Inset: variation of the intensity at the peak maximum (360 nm) with UO_2^{2+}.

Interestingly, when one equivalent of other metal ions (e.g. Th^{4+}, Ho^{3+}, Pr^{3+}, Er^{3+}, Sm^{3+}, Ce^{3+}, Tb^{3+}, Yb^{3+}, Gd^{3+}, La^{3+}, Tm^{3+}, Y^{3+}, Lu^{3+}, Nd^{3+} , Cr^{3+}, Co^{2+}, Zn^{2+}, Ni^{2+}, Pb^{2+}, Cu^{2+}, Ag^{+}, Cd^{2+}, Al^{3+}, Mn^{2+}, Li^{+}, K^{+}, Mg^{2+}, or Ca^{2+}) was added to cyclic peptide B, the fluorescence emission at 360 nm was not significantly affected ($(I_0 - I)/I_0$ <5 %, Fig. 3). These observations are in strong contrast to the fluorescence quenching upon the addition of one equivalent of uranyl ions (quenching efficiency: $(I_0 - I)/I_0$ = 75%, λ = 360 nm). In other words, cyclic peptide B displays a specific fluorescent response for uranyl ions. Herein, it is worth noting that cyclic peptide B exhibited good selectivity for uranyl ions compared with either vanadyl ions VO^{2+} (which showed linear structure and valence similar to uranyl ions), Th^{4+} or lanthanides usually interfere with uranyl sensors (Fig. 3b).

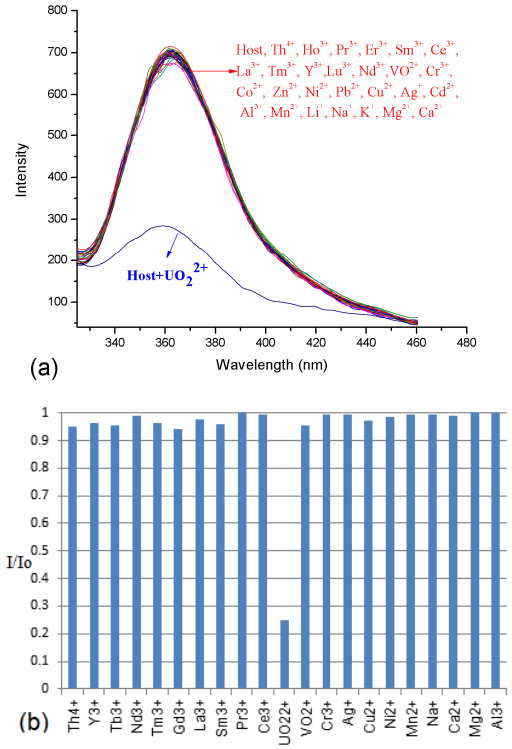


Fig. 3 (a) Fluorescent responses of B (ca. 21 μM) to metal ions (1 equiv) at pH 6.0, MES buffer (20 mM), with excitation at 285 nm; (b) The bar graphs of the fluorescence intensity at λ= 360 nm.

The addition of the complex with various metal ions (Fig. 4a, main group elements and transition metal ions; Fig. 4b, lanthanides) to the cyclic peptide B did not show significant quenching on the fluorescence emission, whereas the addition of uranyl ion significantly quenched the fluorescence. These observations further demonstrate the high selectivity of cyclic peptide B for uranyl ion, and suggest that the cyclic peptide B might be used as a highly promising uranyl sensor. To verify this sensor, the following experiments were then carried out.

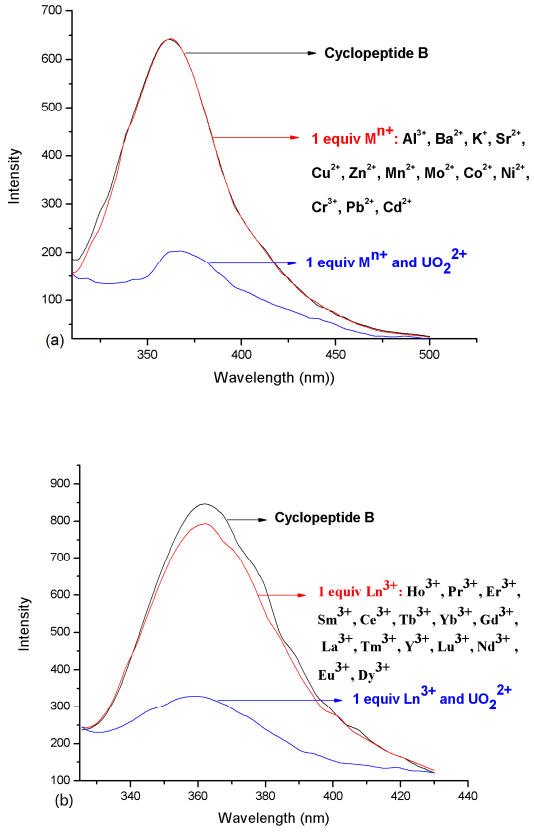


Fig. 4 (a) Fluorescent responses of B (ca. 21 μM) with mixed metal ions (1 equiv) at pH 6.0, MES buffer (20 mM), with excitation at 285 nm; (b) with Ln^{3+}.

The interference of the foreign metal ions on uranyl ion and the fluorescent response of cyclic peptide B was studied with competition experiments (Table 1). The results showed that almost all of the lanthanides, common transition metals, alkali metals, and alkaline earth metals exhibited no interference, indicating the good anti-interference capability of this fluorescent sensor. In addition, with cyclic peptide B as uranyl fluorescent sensor, the estimated detection limit was 0.36 μM (Fig. S5 in ESI).[20]

Table 1 Fluorescent responses of B to mixtures of UO_2^{2+} and other ions [a]

Added species	I_1/I_0 (%)	Added species	I_1/I_0 (%)	Added species	I_1/I_0 (%)

Th^{4+}	96.6	La^{3+}	98.5	Zn^{2+}	100.0
Y^{3+}	97.3	Sm^{3+}	97.3	Co^{2+}	100.1
Tb^{3+}	96.3	Pr^{3+}	100.0	Hg^{2+}	98.6
Lu^{3+}	95.9	Ce^{3+}	99.8	Cu^{2+}	100.2
Ho^{3+}	99.4	Mn^{2+}	99.6	VO^{2+}	99.1
Er^{3+}	97.3	Pb^{2+}	96.7	Na^{+}	99.4
Yb^{3+}	98.9	Cr^{3+}	99.4	K^{+}	98.7
Nd^{3+}	100.3	Ag^{+}	100.1	Mg^{2+}	100.2
Tm^{3+}	97.4	Cd^{2+}	98.3	Ca^{2+}	99.4
Gd^{3+}	96.1	Ni^{2+}	99.3	Al^{3+}	100.1

[a] Conditions: B (ca. 21 μM), UO_2^{2+}(1 equiv), other metal ion (1 equiv), pH 6.0, MES buffer (20 mM), with excitation at 285 nm.

To simulate the natural conditions, we then used fluorescence spectroscopy to measure the uranyl concentration in the aqueous uranyl-containing solution (prepared from river water). The results showed that cyclic peptide B fluorescent sensor could reliably detect uranyl ions with the mean error within ±10% (Table 2). In this way, this sensor could be suitably used in monitoring contaminated environments and uranium-related environmental remediation.[21]

Table 2 Results of UO_2^{2+} determination in water samples

Sample	Added UO_2^{2+} (μM)	Found (μM)[a]	Recovery (%)
River water 1	2.5	2.3 (± 0.01)	92
River water 2	5.0	4.7 (± 0.02)	94
River water 3	10.0	10.8 (±0.04)	108

[a] Average of three determinations

Finally, we put effort in understanding the structure of the uranyl-cyclic peptide B complex. The ultraviolet-visible (UV-Vis) spectra data of the uranyl-cyclic peptide B interaction confirmed the formation of uranyl-cyclic peptide B complex in aqueous solution. As shown in Fig. 5, the addition of uranyl ion to cyclic peptide B solution resulted in a red shift and drop in the intensity of peptide bond absorption peak (220 nm) and a drop in the intensity of the tryptophan absorption peak (280 nm). We suggest that this phenomenon is caused by conformational changes introduced by the coordination of uranyl ion with cyclic peptide B.

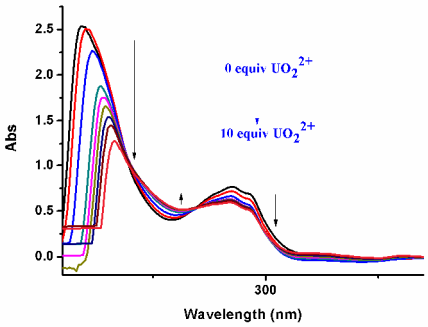


Fig. 5 UV spectra of B (0.1 mM in Mes buffer solution, pH = 6.0) with UO_2^{2+} (0–10 equiv)

This proposal is also supported by our preliminary DFT calculations on the proposed UO_2-B complex. As shown in Fig. 6, this complex accepts a typical U(IV) structure with five oxygen atoms coordinating on the equatorial plane: two from carboxylate of Glu, two from P=O of phosphate, and one from H_2O. Compared to the widely reported structures of U(VI) complexes,[13] all the U-O (carboxylate, phosphate and water) bond distances in Fig. 6 lie in the typical range of U-O single bond length (2.2-2.6 Å). According to the optimized structure, two reasons are mainly responsible for the high affinity of cyclic peptide B with uranyl ion: 1) appropriate coordination plane ~5.0 Å above the peptide backbone, thanks to the similar side chain length of Ser and Glu; 2) compact hydrogen bonding interactions constituted by the carboxylic acid, phosphate acid and amide bonds, which provide extra stability to the complex. Nonetheless, due to the complexity of the concerned uranyl-cyclic peptide B system, more efforts are necessary to gain deep understandings on the related peptide structure-binding ability relationships.

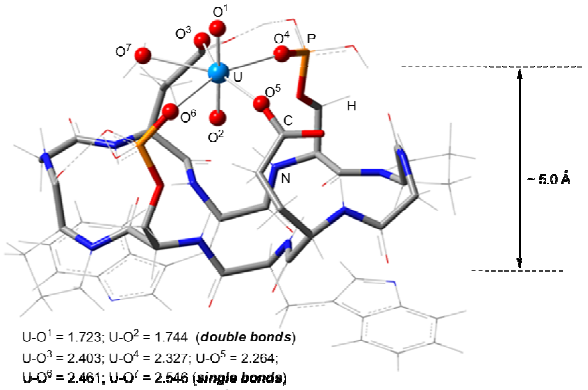


Fig. 6 The DFT predicted UO^{2+}-cyclic peptide B structure

In summary, this study provides the first case of fluorescent identification of uranyl ions using a polypeptide. The cyclic peptide B and uranyl ions formed a 1:1 complex, and phosphorylated functional groups and pre-organized structure are key structural parameters. Cyclic peptide B is highly selective to uranyl ions (compared with the other competition metal ions such as VO^{2+} and Th^{4+} etc). In addition, B was successfully used as a fluorescent sensor in the detection of uranyl ion in river water. The structure of this uranyl-specific binding polypeptide will hopefully facilitate the future design of new materials for the separation of uranyl ion. These studies are currently ongoing in our laboratory.

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Notes and references

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† Electronic Supplementary Information (ESI) available: Experimental details, MS data, calculation details are provided. See DOI: 10.1039/b000000x/.

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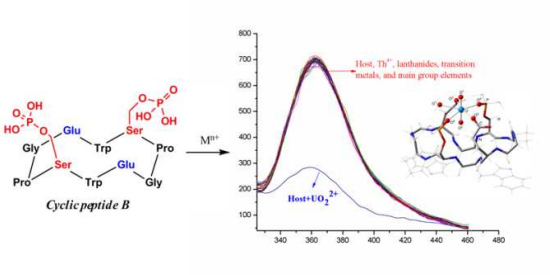

Fluorescent recognition of uranyl ions was achieved with a phosphorylated cyclic peptide, which can be used as a fluorescent sensor.