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Selective Recognition of Americium by Peptide-Based Reagents

Salih Özçubukçu,[†] Kalyanaswer Mandal,[‡] Seraphine Wegner,[†] Mark P. Jensen,*[,§] and Chuan He*[,†]

[†]Department of Chemistry and Institute for Biophysical Dynamics, [‡]Department of Biochemistry and Molecular Biology, The University of Chicago, Illinois 60637, United States

[§]Chemical Science and Engineering Division, Argonne National Laboratory, Argonne, Illinois, 60439, United States

Supporting Information

ABSTRACT: The separation of lanthanides from minor actinides such as americium and curium is an important step during the recycling process in the treatment of nuclear waste. However, the similar chemistry and ionic size of lanthanide and actinide ions make the separation challenging. Here, we report that a peptide-based reagent can selectively bind trivalent actinides over trivalent lanthanides by means of introducing soft-donor atoms into a peptide known as a lanthanide-binding tag (LBT). Fluorescence spectroscopy has been used to measure the dissociation constant of each metal/peptide complex. A 10-fold selectivity was obtained for Am^{3+} over the similarly sized lanthanide cation, Nd^{3+}, when the asparagine on the fifth position of a LBT was mutated to a cysteine and further functionalized by a pyridine moiety.

The treatment of used nuclear fuel is an important step toward reducing the environmental impacts of nuclear power. Light actinides (An), such as uranium or plutonium, are readily separated for recycle or prepared for long-term storage,[1] but the most effective use of geological repositories also requires minor actinides such as americium (Am) and curium (Cm) to be transmuted into short-lived radionuclides in closed nuclear-fuel cycles. However, the presence of fission-product lanthanides (Ln) in the Am/Cm destined for transmutation will greatly decrease the efficiency of this process because some of the lanthanides are strong neutron absorbers.[1] The fission product lanthanides are also 100 times more abundant than Am and Cm in used nuclear fuel.[2] Therefore, Am and Cm must be separated from the lanthanides to be transmuted effectively.

The chemistries of lanthanides and the transplutonium actinides are very similar, making efficient separation quite challenging.[3] The trivalent lanthanides and actinides are considered hard Lewis acids in the Pearson hard and soft acids and bases formalism. They form the strongest complexes with ligands containing hard-donor atoms, especially oxygen, and the coordination chemistry of the Ln^{3+} and An^{3+} cations with hard-donor ligands is usually nearly indistinguishable[3] because they are both trivalent hard cations of similar ionic radii[4] and the metal/ligand bonding in An^{3+} and Ln^{3+} is predominantly electrostatic. It is generally observed that An^{3+} interacts with softer donor atoms such as sulfur and nitrogen more strongly than the equivalent Ln^{3+}, an effect usually attributed to a moderately more covalent interaction between An^{3+} and the soft-donor atoms relative to Ln^{3+}. Hence, there is intense interest in developing soft-donor ligand systems that allow selective separation of An^{3+} from Ln^{3+}.[5] The most efficient reagents currently available for separating Am and Cm from the lanthanides are based on dialkyl- or diaryldithiophosphinic acids or aromatic amines. As an example, bis(2,4,4-trimethylpentyl)dithiophosphinic acid, is among the most selective ligands known for An^{3+}/Ln^{3+} separation with an Am/Eu separation factor ($S_{Am/Eu}$) of more than 5000 under ideal conditions.[6] Ligands with aromatic amine donors, such as the bis(triazinylpyridines) or tris(2-pyridylmethyl)ethylenediamine, can also achieve An/Ln selectivities of more than 100.[7]

Imperiali and co-workers have developed a series of peptides called lanthanide-binding tags (LBTs) that selectively bind lanthanide ions with high affinities. By optimizing the structure of EF-hand motifs of calcium-binding proteins,[8] they produced LBTs with dissociation constants (K_d) in the low nanomolar range.[9] LBTs have a wide range of applications in biochemistry such as protein-structure determination and the investigation of protein trafficking and metal/protein interactions.[10] This preorganized ligand template provides a tunable system that could allow the systematic study of bonding differences between trivalent actinides and lanthanides and the development of new bonding motifs for minor actinide/lanthanide separations.

In this study, we redesigned LBTs to systematically incorporate nitrogen- and sulfur-based ligands in the metal-binding pocket in an attempt to create selectivity for An^{3+} over Ln^{3+} and gain insight into the chemistry of An/Ln separations. The eight-coordinate lanthanide complexes of the original LBTs are formed by the oxygen ligands in the peptide structure. In order to introduce softer ligands on the peptides we decided to modify the third and fifth positions on the LBT because of their lower steric hindrance and greater structural flexibility[9c] (Figure 1). The controlled introduction of softer ligands can be accomplished through the unique reactivity of cysteine toward electrophiles. As a general tool for peptide functionalization, a cysteine amino acid is introduced to the third and fifth positions, which allows us to anchor different ligands to the peptide framework via S-alkylation.

The syntheses of the peptides were performed by a solid-phase peptide synthesis method based on Boc chemistry using a PAM resin as the solid support. We have synthesized LBTs with asparagine 3 (N3) and aspartic acid 5 (D5) mutated to cysteine (Scheme 1) and purified them by reverse-phase high-performance liquid chromatography (HPLC) on a semipreparatory C18 column. Preparation of the other peptides is described in the Supporting Information.

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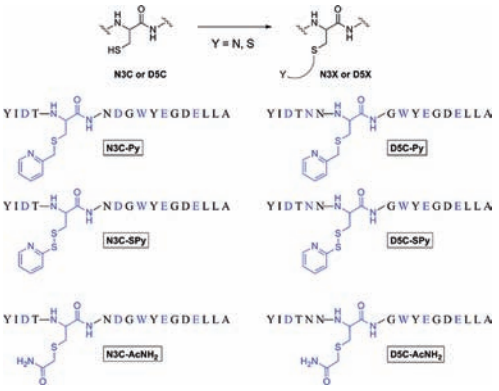
-1	1	3	5	7	9	12	Position
Y	I	D	T	N	N	D	G W Y E G D E L L A LBT
Y	I	D	T	X	N	D	G W Y E G D E L L A N3X
Y	I	D	T	N	N	X	G W Y E G D E L L A D5X
Y	I	D	T	X	N	X	G W Y E G D E L L A N3X-D5X

Figure 1. Design of a new peptide for selective actinide binding from the LBT. The amino acids in the LBT shown in blue bind lanthanides.

Scheme 1. Modification of Cysteine-Mutated Peptides



To determine the thermodynamic selectivity of the peptides for trivalent actinides and lanthanides, the binding affinities of the peptides for Tb^{3+}, Nd^{3+}, Eu^{3+}, and Am^{3+} were measured by spectrofluorometric titration by monitoring the fluorescence from the Tb^{3+} $^5D_4 \rightarrow {}^7F_5$ transition at 545 nm.[11] The lanthanide cations used were selected for specific properties. The lanthanide and actinide contractions cause Nd^{3+} and Am^{3+} to have almost equal ionic radii (Nd^{3+}, 1.107 Å; Am^{3+}, 1.106 Å, with coordination number 8).[4b] Because they have the same size and charge, the electrostatic contribution to binding should be the same for both cations, and any difference is attributable to the slight differences in the covalency of Am^{3+} and Nd^{3+}. The smaller lanthanide cations Eu^{3+} and Tb^{3+} will feature stronger electrostatic interactions with the ligands. Europium also was chosen because, like Am^{3+}, it has an f^6 configuration, and the ready availability of radioactive europium isotopes makes it the lanthanide most commonly used for measurement of Am/Ln separation factors. Terbium was used because of its favorable optical properties. While the hydrated Tb^{3+} ion shows minimal fluorescence emission, the binding of Tb^{3+} to the peptide could be observed as an increase in fluorescence because the binding of Tb^{3+} to the peptide both excludes water from the primary coordination sphere and sensitizes the emission due to the tryptophan residue in the peptide sequence.[12] The binding constants of the metal/peptide complexes were measured at pH 7 in 0.01 M HEPES/0.1 M NaCl at 23 °C. Equilibrium was attained within seconds of mixing. The binding affinity of Tb^{3+} to the modified peptides was measured by the direct titration method. Solutions containing 0.5−1.0 μM peptide were titrated with various concentrations of Tb^{3+} ions (Figure 2A), correcting the emission intensity at 545 nm for the weak background emission of unbound Tb^{3+}. The resulting titration curve was fit to the Hill equation. From the fitting, $n = 1$ for all of the peptides studied, which indicates a 1:1 Tb/peptide stoichiometry for each peptide. The dissociation constants were also computed from the Hill equation, as shown in Table 1.

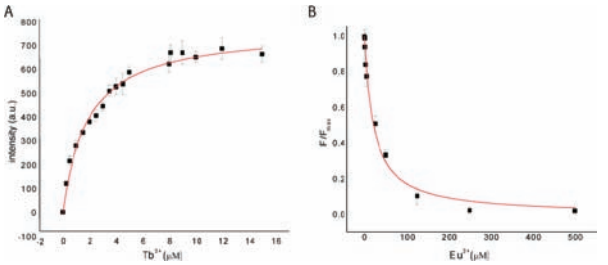


Figure 2. (A) Direct titration of 1.0 μM D5C with Tb^{3+}. (B) Competitive titrations of 1.0 μM D5C and 20 μM Tb^{3+} with Eu^{3+} as a competing metal. Details are given in the Supporting Information.

Table 1. Conditional Dissociation Constants (K_d) of Each Peptide with Various Lanthanides and Americium(III) in Micromoles/L (10^{-6} M) Obtained from Triplicate Measurements

entry	peptide	Tb^{3+}	Eu^{3+}	Nd^{3+}	Am^{3+}
1	N3C	4.85 ± 0.30	6.53 ± 0.40	1.82 ± 0.07	0.87 ± 0.02
2	N3C-Py	3.45 ± 0.34	3.51 ± 0.28	2.50 ± 0.11	1.38 ± 0.03
3	N3C-SPy	4.14 ± 0.29	2.18 ± 0.07	7.44 ± 0.53	1.13 ± 0.06
4	N3C-$AcNH_2$	5.75 ± 0.33	3.07 ± 0.10	6.47 ± 0.44	1.80 ± 0.08
5	D5C	1.82 ± 0.16	5.72 ± 0.53	4.56 ± 0.21	2.69 ± 0.23
6	D5C-Py	1.63 ± 0.10	0.41 ± 0.05	2.43 ± 0.13	0.23 ± 0.02
7	D5C-SPy	1.62 ± 0.09	1.02 ± 0.07	1.16 ± 0.03	0.60 ± 0.05
8	D5C-$AcNH_2$	2.68 ± 0.21	0.98 ± 0.08	1.03 ± 0.04	0.84 ± 0.06
9	N3C-D5C	3.20 ± 0.12	1.16 ± 0.07	1.10 ± 0.06	0.42 ± 0.03
10	LBT	0.0543 ± 0.006	0.0610 ± 0.006	0.27 ± 0.01	0.045 ± 0.01

The binding affinities of Eu^{3+}, Nd^{3+}, and Am^{3+} to the modified peptides were measured by competitive titrations.[13] In this method, a constant concentration of peptide and an excess of Tb^{3+} are titrated with various concentrations of competing metal ions. Metal ions that competitively replace the bound Tb^{3+} from the peptide cause significant decreases in the Tb^{3+} emission. A sample titration with Eu^{3+} is depicted in Figure 2B. The emission of the bound Tb^{3+} was quantified as F/F_{max}, the ratio of the emission in the presence and absence of a competing metal. The curves were fit to the Hill equation to obtain an apparent dissociation constant. Because the peptides were almost saturated with Tb^{3+} at the starting point of the competition, the true dissociation constant can be calculated using eqs 2 and 3 in the Supporting Information. In the case of Am, the titrations were also analyzed by the program *SQUAD*.[14] Only 1:1 metal/peptide complexes were detected. The dissociation constants of the peptide/metal ion complexes are shown in Table 1. These are conditional dissociation constants. To be consistent with previous studies,[9c] the K_d values were determined without considering the metal hydroxide species that begin to form near pH 7. The hydrolysis constants are similar for these lanthanides and Am, and they will have only a small (<10%) effect on the relative K_d values of each metal under these conditions.

For LBTs, the conditional K_d values obtained with Tb^{3+}, Eu^{3+}, and Nd^{3+} are comparable to those previously reported.[9c] For the peptides containing soft-donor ligands, the lanthanide affinities drop 4−100-fold when the hard-donor ligand sites of the original LBT are selectively replaced with lower-affinity soft-donor sites.

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It is also possible that the greater steric bulk of the soft-donor substituents play a role in reducing the modified peptides' affinities for lanthanides. However, Am^{3+} binding to the peptide is stronger than the binding of the size-equivalent Ln^{3+} cation, Nd^{3+}, in every case, and is often 2−3-fold and seldom 5−10-fold stronger than any of the three Ln^{3+} tested. Only D5C resulted in a lower affinity for Am^{3+} compared to the significantly smaller Tb^{3+} cation (Table 1, entry 5). In contrast, the single amino acid substitution in D5C-Py gave 10-fold selectivity for Am^{3+} over Nd^{3+} (entry 6), suggesting greater interactions between Am^{3+} and the soft-donor ligands. Although N3C showed moderate selectivity for Am^{3+} over lanthanides (entry 1), pyridine substitution in N3C-Py led to a reduced affinity for Am^{3+} and lower selectivity. However, in the case of N3C-SPy and N3C-$AcNH_2$, 4−7-fold selectivity was obtained for Am^{3+} over Nd^{3+} (entries 3 and 4). Clearly, soft donors in position 5 give better Am binding and selectivity than those in position 3.

When both positions 3 and 5 were mutated into cysteine in the same peptide (N3C-D5C, entry 9), a higher affinity for Am^{3+} was obtained compared to that of N3C and D5C. Although this peptide gives better selectivity (3-fold) for Am^{3+} over Nd^{3+} compared to N3C and D5C, it is still not superior to D5C-Py.

Interestingly, D5C-SPy, which is only one sulfur atom different than D5C-Py in the pyridine arm (entry 7), exhibits 3-fold less affinity than D5C-Py for Am^{3+} (entry 6). This result indicates that nonbonding atoms in close proximity are also playing roles to modulate the electronics of the donor atoms and affecting the binding affinities.

One surprising result is that N3C showed stronger binding to Nd^{3+} compared to that of Tb^{3+} and Eu^{3+} (entry 1), whereas LBT binding to Nd^{3+} was significantly weaker compared to that of Tb^{3+} and Eu^{3+} (entry 10). This might be due to a change of the binding sites when the third position is mutated to cysteine.

Unexpectedly, Am^{3+} binds LBTs almost as strongly as Tb^{3+} and Eu^{3+} and is more strongly bound than Nd^{3+} (entry 10). This observation does not fit the general tendency of the size dependence of actinide and lanthanide ions. We would expect that the affinities of LBTs for Am^{3+} and for its size-matched lanthanide, Nd^{3+}, would be almost equal because the LBT has only oxygen as its donor atom. However, repeating the measurement several times did not change the result that the LBT gave some selectivity for Am^{3+} over the similarly sized lanthanide Nd^{3+} but not over smaller sized lanthanides Tb^{3+} and Eu^{3+}.

In conclusion, we have demonstrated that a one amino acid change in the fifth position to cysteine and further functionalization with a 2-methylenepyridine group (D5C-Py) makes the LBT 10-fold more selective for Am^{3+} over the size-equivalent lanthanide Nd^{3+}. The size and electronic properties of the substituted group and the position of cysteine play important roles for An versus Ln affinity and selectivity. These results show that an LBT can be a useful template to systematically introducing soft-donor atoms in order to study the coordination chemistry of actinides and lanthanides. These natural peptides, with further improved selectivity, may be incorporated into biological entities for selective actinide enrichment. With this well-defined coordination scaffold, we are also in the process of introducing additional modifications such as dithiophosphinic acids to further understand the chemistry of An binding.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information. The synthesis and HPLC purification profile of peptides, methods for determining the K_d values, and details of the Am purification. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: chuanhe@uchicago.edu (C.H.), mjensen@anl.gov (M.P.J.).

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