A Rare Earth−DOTA−Binding Antibody: Probe Properties and Binding Affinity across the Lanthanide Series

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A Rare Earth-DOTA-Binding Antibody: Probe Properties and Binding Affinity across the Lanthanide Series

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Antibodies against metal chelates are useful for a variety of applications in chemistry, environmental science, and medicine because they can bind target molecules with high affinity and exquisite specificity.[1] An antibody that binds rare earth complexes selectively could be used as a docking station for a set of probe molecules, of particular interest for medical imaging and therapy.[2] The rare earths are rich in probe properties, such as the paramagnetism of Gd, the luminescence of Tb and Eu, and the nuclear properties of Lu and the group IIIB element Y. The chelating ligand DOTA (Figure 1) binds transition metals and rare earths with extreme stability under physiological conditions, leading to its use in vivo.[3] Considering these applications, we examined the monoclonal antibody 2D12.5, developed against the DOTA analogue Y-BAD conjugated to the immunogenic protein KLH through a 2-iminothiolane linker and selected to bind specifically to Y-NBD (Figure 1),[4] to determine the scope of its activity.

To assess the metal selectivity of antibody 2D12.5, we developed a competitive immunoassay to measure the binding constants of various metal-NBD complexes relative to the original Y^{3+} complex (Figure 2). Briefly, 2D12.5 was incubated at 37 °C in the presence of immobilized HSA-2IT-Y-BAD and a soluble metal-NBD competitor (Y-BAD was linked to human serum albumin via 2-iminothiolane). The metal-NBD concentration was varied from micromolar to picomolar to determine the relative binding affinity of 2D12.5 for each metal chelate in comparison to Y-NBD. Binding was measured by standard methods (Supporting Information).

We find that 2D12.5 binds not only Y-NBD but also NBD complexes of *all* of the lanthanides. Surprisingly, some metal chelates such as Gd-NBD bind more tightly than the original Y^{3+} complex; overall, the dissociation constants fall within a factor of 3 above or below the $K_d = 10$ nM value for Y-NBD (Supporting Information). Other antibodies that bind metal chelates do so with a strong preference for one or possibly two metals.[5] The NBD chelate of group IIIB ion Sc^{3+} binds to the antibody with a much lower affinity (<1%) than the strongest binding rare earth complexes, perhaps because Sc^{3+} has a much smaller ionic radius.[6,7]

The relative binding affinities determined for each rare earth NBD complex relative to Y-NBD are plotted as $\Delta\Delta G$ values in Figure 3. Out of 15 ions tested, we found six rare earth complexes with $\Delta\Delta G$ values more favorable for binding than the original Y^{3+} complex. The radii of the nonacoordinate trivalent lanthanide ions vary in small increments across the series from 1.21 (La^{3+}) to 1.03 Å (Lu^{3+}).[6] Our results show that when the shape of the NBD complex is perturbed by either increasing or decreasing the radius of the lanthanide ion, the stability of the protein−ligand complex changes in a regular fashion. The effect of the change in ionic radius on the standard ΔG of binding should be described approximately by an equation of the form

$$\frac{d\Delta G}{dr} = k|r - r_0|$$

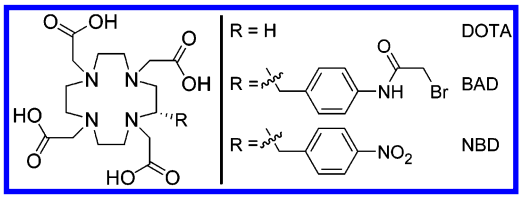

Figure 1. DOTA (1,4,7,10-tetraazacyclododecane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid) and two bifunctional analogues, BAD (((*S*)-2-(4-(2-bromo)-acetamido)-benzyl)-DOTA) and NBD ((*S*)-2-(4-nitrobenzyl)-DOTA).

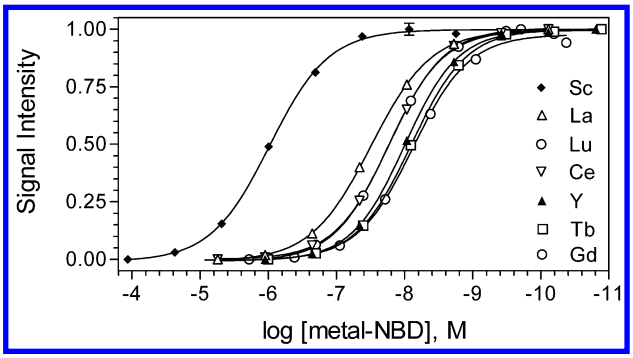

Figure 2. Relative binding of metal-NBD complexes to antibody 2D12.5. A representative set of competitive binding curves obtained from ELISA experiments. Error bars (representing the standard error of the mean) are shown but are generally smaller than the data points.

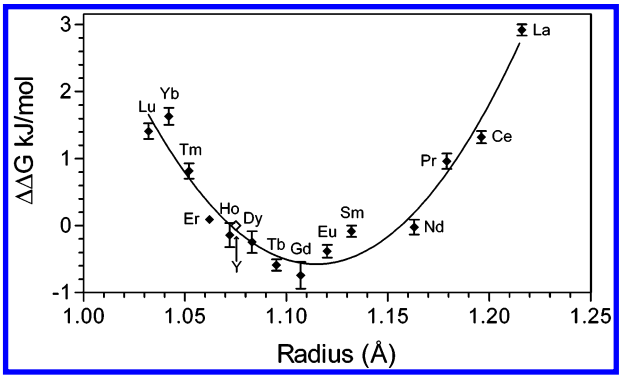

Figure 3. Dependence of the standard Gibbs free energy of binding on rare earth ionic radius shows thermodynamically elastic binding behavior between antibody 2D12.5 and rare earth-NBD complexes. $\Delta\Delta G$ values relative to Y-NBD (◇). Error bars represent standard error of the mean.

which integrates to

$$\Delta\Delta G = (1/2)k(r - r_0)^2$$

The behavior of $\Delta\Delta G$ as a function of ionic radius fits a parabola, as might be expected for a system that behaves in a thermodynamically elastic way, obeying Hooke's law over a small range of perturbations. The quantitative binding differences allow us to assess

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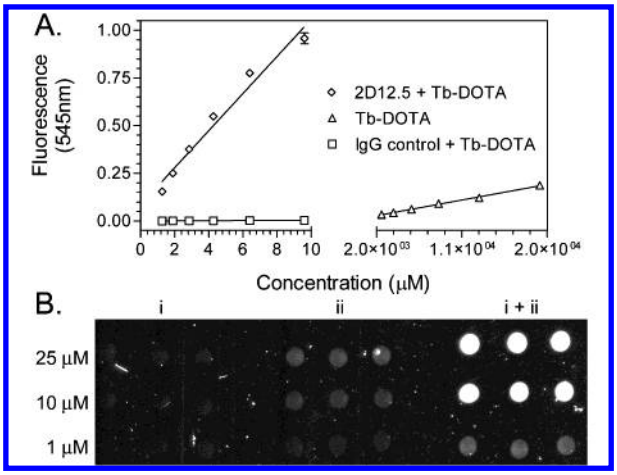

Figure 4. (A) Time-resolved 280 nm excitation yields a marked, linear enhancement in the green 545 nm emission of Tb-DOTA bound to antibody 2D12.5. The enhancement is not observed for Tb-DOTA and a nonbinding IgG control. (B) Steady-state 302 nm broadband excitation of droplets (prepared in triplicate) yields a similar result using a CCD camera imaging unit equipped with a 520 nm long pass emission filter. (i) Tb-DOTA, (ii) antibody 2D12.5.

the system's flexibility expressed as the force constant k, whose value is $\cong$50 N m^{-1}, comparable to a chemical bond. The optimal ionic radius r_0 predicted from the parabolic fit to the binding data is 1.115 Å, close to the strongest binders Tb^{3+}, Gd^{3+}, and Eu^{3+} (1.095, 1.107, and 1.120 Å).

Both Y-DOTA and Gd-DOTA are nonacoordinate in aqueous solution, with four nitrogens and four oxygens from DOTA plus a single coordinated water molecule, in a capped square antiprism arrangement (denoted **M**).[8] At equilibrium, rare earth DOTA complexes at either end of the lanthanide series differ in the layout of the acetate arms, and mixtures of isomers ranging from **M** to a distorted inverted antiprism (**m**) are observed for some. Large La-DOTA is almost exclusively isomer **m**, while small Lu-DOTA is predominantly isomer **M**. The ionic radius and geometry of each complex also affect the accessibility of a ninth coordination site for water. Although our studies evaluated complexes of NBD rather than DOTA, we suspect that the aforementioned conformational equilibria play a role in the different binding affinities observed here.

Antibody 2D12.5 has comparable but slightly lower affinity for Y-DOTA relative to Y-NBD. The chirality of the Y-NBD complex favors a single acetate helicity (Λ) and macrocycle helicity ($\delta\delta\delta\delta$). Y-DOTA exists in solution as enantiomers of the **M** isomer, having either Λ or Δ acetate helicity and $\delta\delta\delta\delta$ or $\lambda\lambda\lambda\lambda$ macrocycle helicity.[8,9] The lower affinity of 2D12.5 for Y-DOTA may be attributed to a preference of the antibody for a single enantiomer.

The broad specificity and high affinity of this antibody for rare earth-DOTA complexes make it particularly interesting for applications that take advantage of the unique characteristics of lanthanides. For example, UV excitation of the Tb-DOTA-2D12.5 complex leads to energy transfer from aromatic side chains of the antibody to bound Tb-DOTA, enhancing green Tb luminescence by approximately 4 orders of magnitude relative to unbound Tb-DOTA (Figure 4). The enhancement is comparable to that observed for Ca^{2+} binding proteins, which also transfer energy from aromatic side chains to Tb^{3+} ions bound in Ca^{2+} sites.[10] Simeonov et al. have recently described blue-fluorescent antibodies, potential sensors that change the emission of a stilbene ligand upon binding;[11] high affinity antibody sensors based on lanthanide luminescence exhibit millisecond emission lifetimes, making them potentially useful for a number of biological applications.[12]

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Supporting Information Available: Experimental details, along with a summary of the numerical results (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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