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New Molecular Motif for Recognizing Sialic Acid Using Emissive Lanthanide–Macrocyclic Polyazacarboxylate Complexes: Deprotonation of a Coordinated Water Molecule Controls Specific Binding

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Supporting Information

ABSTRACT: A new molecular motif—lanthanide–macrocyclic polyazacarboxylate hexadentate complexes, Ln^{3+}-ABNOTA—was found to specifically bind to sialic acid with strong emission enhancement and high affinity. The selectivity toward sialic acid over other monosaccharides was one of the highest among artificial receptors. Also, the novel binding mechanism was investigated in detail; binding selectivity is controlled by interactions between sialic acid and both the central metal and a hydroxyl group produced by deprotonation of a coordinated water molecule in the Ln^{3+} complex.

N-Acetylneuraminic acid (Neu5Ac; Figure 1) is an anionic monosaccharide and one of the important sialic acids in living

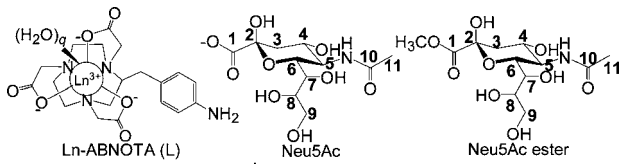

Figure 1. Chemical structures of the Ln^{3+}-L complex, Neu5Ac, and Neu5Ac ester.

organisms. It frequently occupies the terminal positions of carbohydrate chains in glycoproteins, glycolipids, and cell membranes; thus, it is involved in many biological and pathological phenomena.[1,2] Because the sugar chains containing Neu5Ac are known to be overexpressed on the surface of tumor cells,[1] it is a potential tumor marker. Therefore, an artificial receptor motif, capable of selectively binding and recognizing Neu5Ac, is of great importance in the field of coordination chemistry[3] and in terms of the development of novel diagnostic reagents, sugar-chain probes in a living organism, and separation reagents for various sugar sequences.

Many receptors that recognize monosaccharides have been developed, while few motifs selective for Neu5Ac have been found thus far.[4] The general strategy for the molecular design of saccharide-binding motifs is to employ both hydrophobic and hydrogen bonding.[5] While artificial receptors reported to date have been shown to be effective in organic solutions or mixed solvents,[5] few have been developed that respond to monosaccharides in a purely aqueous environment.[6−8] An alternative strategy is to employing a phenylboronic acid (PBA) motif,[9] which provides a selective response to saccharides in an aqueous solution through reversible covalent binding between PBA and *cis*-diol. However, the stability constants for PBA derivative complexes with monosaccharides are lower ($\sim 10^2$ M^{-1})[10] than those with sugar-binding proteins (ca. 10^4 M^{-1}), and the recognition is limited to neutral sugars.[11] While emission enhancement is desirable for detecting saccharides, the quenching response of fluorescence tends to be used for most of the motifs.[12] Thus, it has been a challenging task to develop an emission-enhancing molecular motif specific to Neu5Ac with strong interaction.

In this study, we developed water-soluble lanthanide (Ln) complexes with some residual coordination sites on the central metal ions [Ln^{3+}-ABNOTA (L) in Figure 1] as promising molecular motifs. To our knowledge, these provide the highest selectivity toward Neu5Ac among artificial monosaccharide acceptors reported thus far. The ability of these complexes to recognize saccharides (the structures of which are shown in Figure S1 in the Supporting Information, SI) and an elucidation of the recognition mechanism are presented here.

The principle of the emission enhancement for monosaccharides is as follows:[8] the L complexes with Tb^{3+} and Dy^{3+} themselves showed long-lived emissions based on the f–f transition via the energy-transfer process from the lowest T_1 level of the light-absorbing group to the f orbital of Ln^{3+} after excitation to the S_1 level.[13] The Ln^{3+}–L complexes possess two or three residual coordination sites because Ln^{3+} provides octa- or nonadentate coordination, while L demonstrates hexadenticity.[14] The emission of the Ln^{3+} complexes, which have some water molecules in their first coordination sphere, is rather weak due to the radiationless deactivation processes via energy transfer to the harmonic overtones of the OH oscillator.[13] In the presence of monosaccharide, if the ternary Ln^{3+}–L–monosaccharide complexes form in the first coordination sphere accompanied by substitution of the coordinated water molecules with the monosaccharide, the emission intensity increases because of a concomitant increase in the energy-transfer efficiency arising from exclusion of the OH oscillators.

When Neu5Ac was added to the Ln^{3+}–L complex solution at pH 10.2, the emission enhancement of the Dy^{3+} complex was up to 221% greater than that of the free complex (Figure 2). The

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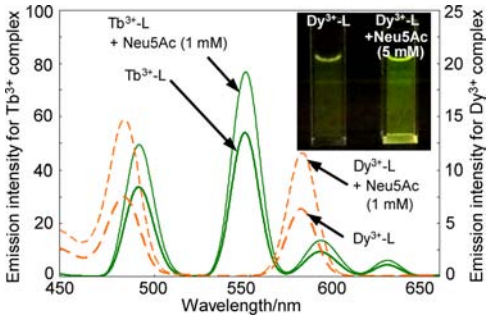

Figure 2. Ln-based emission enhancement with the addition of Neu5Ac. $[Ln^{3+}–L]$ = 1.0 μM, pH 10.2 (no buffer solution). Inset: photograph of emission of the Dy^{3+}–L complex (10 μM) without (left) and with Neu5Ac (5 mM) (right).

complexes were characterized by electrospray ionization mass spectrometry and NMR and fluorescence spectroscopy, as shown in Figures S2–S4 in the SI. The emission enhancement could be confirmed even with the naked eye for solutions greater than 0.4 mM Neu5Ac, as seen in Figure 2, inset. (A photograph of the emission enhancement for a Tb^{3+} complex is shown in Figure S5 in the SI.) The pH dependence of the emission intensity for the ternary complexes was investigated for the Dy^{3+} complex, as shown in Figure 3 (while the results for the Tb^{3+}

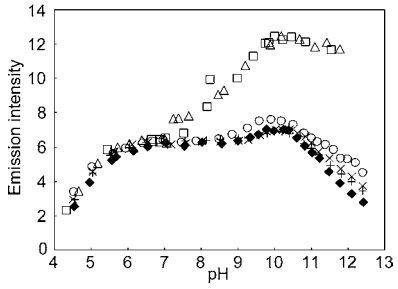

Figure 3. Emission intensity of the Dy^{3+}–L–monosaccharide complexes as a function of the pH: ♦, Dy^{3+}–L; ×, glucose (10 mM); +, mannose (10 mM); ○, ribose (10 mM); Δ, Neu5Ac (1 mM); □, Neu5Ac methyl ester (1 mM). $[Dy^{3+}–L]$ = 1.0 μM.

complex are shown in Figure S6 in the SI). The intensity for the solution containing Neu5Ac dramatically increased at pH 8–10 compared with that of free Ln^{3+}–L complexes (Δ in Figure 3). Selectivity toward 1 mM Neu5Ac was observed over other monosaccharides present at 10 times higher concentration (10 mM), as seen in Figure 3. Essentially the same pH dependence was observed for 1 mM Neu5Ac methyl ester (□ in Figure 3), which strongly indicated that the carboxyl group in Neu5Ac was not involved in its binding mode. The enhancement factors upon the addition of 1 mM mono- or disaccharides are shown in Figure S7 in the SI. Selective enhancement for mono- and diNeu5Ac was observed.

Detection limits (DLs) of Neu5Ac based on 3σ of a blank (n = 9) were determined as 1.9×10^{-5} and 1.5×10^{-5} M for Tb^{3+} and Dy^{3+} complexes, respectively ($[Ln^{3+}–L]$ = 1 μM; [Neu5Ac] = 0–80 μM; pH 10.3). Because the obtained DLs were lower than those of Neu5Ac motifs reported previously[4] and lower even than the Neu5Ac concentration level (~0.2 mM) in the blood of healthy adults,[15] the potential of this motif as an analytical agent is clearly indicated.

The stability constants for ternary complexation (Ln^{3+}–L–saccharide complex formation) were determined by a titration method (Figure S8 in the SI). The conditional stability constants K' of Tb^{3+}–L–saccharide at pH 10.3 were $10^{3.39}$, $10^{3.03}$, and $10^{1.77}$ M^{-1} for Neu5Ac, diNeu5Ac, and ribose, respectively, and K' for Dy^{3+}–L–Neu5Ac complexation was $10^{2.99}$ M^{-1}. The measured stability constant for Neu5Ac was significantly larger than that for ribose, which means the enhancement selectivity toward Neu5Ac originates from the thermodynamic stability. It should be noted that these values exceed those of PBA receptors in aqueous solution by more than an order of magnitude.[9,10] Such selectivity of the molecular motif toward anionic Neu5Ac over other neutral sugars is rare, and its recognition mechanism is of great importance.

With respect to the recognition mechanism, the deprotonation reaction was expected to be involved because emission of Ln^{3+}–L–Neu5Ac significantly depends on the pH around 8–10 (Figures 3 and S6 in the SI) and also deprotonation of the coordinated water molecule in the Ln–polyaminocarboxylate complexes under weak and strong alkaline conditions has been reported.[16] To experimentally confirm this deprotonation, the electrophoretic mobility of the free Tb^{3+}–L was measured using capillary electrophoresis (CE; see the SI). The pH dependence of the electrophoretic mobility of the Tb^{3+}–L complex (shown in Figure S9 in the SI) resulted in a sigmoidal curve with more negative mobility at higher pH. This suggests deprotonation of the coordination water molecule in the complex. The pK_a value of free Tb^{3+}–L was determined to be 9.44 ± 0.06. Because the deprotonation complex $[Tb^{3+}–L^{3-}–OH]^-$ is predominant at pH 10, it can be deduced that the species of the ternary complex with Neu5Ac is $[(Tb^{3+}–L^{3-}–OH^-)–Neu5Ac]^{2-}$ at pH 10. The pK_a value was also determined by analysis of the pH dependence (from pH 8–11) of the emission intensity of the Tb^{3+}–L–Neu5Ac complex. This analysis relied on the overall complex formation reaction scheme including deprotonation: $Tb^{3+}–L^{3-}$ + Neu5Ac → $[(Tb^{3+}–L^{3-}–OH^-)–Neu5Ac]^{2-}$ + H^+ (see the text and Figure S10 in the SI). As a result, a pK_a value of 9.4 ± 0.2 for Tb^{3+}–L, which is almost identical with the above-mentioned pK_a obtained by CE, and a log K of 3.5 ± 0.1 for the ternary complex formation were obtained (8.7 ± 0.1 and 3.0 ± 0.2 for the Dy^{3+} complex, respectively). Thus, it can be asserted that the deprotonation reaction of a coordinated water molecule controls complex formation with Neu5Ac. That is, the anionic Ln–L recognized anionic Neu5Ac.

The luminescence lifetimes of the Tb^{3+}–L–Neu5Ac complex were measured to allow the number of coordinated water molecules in the Ln^{3+} complexes to be estimated (Figure S11 in the SI). The number of coordinated water molecules on the central Tb^{3+} ion in the complexes, q, was obtained from eq 1 for luminescence lifetimes in D_2O and H_2O solutions.[17]

$$q = 4.2(\tau_{H_2O}{}^{-1} - \tau_{D_2O}{}^{-1}) \quad (1)$$

The results are summarized in Table 1. Because the difference in the q values between the free Tb and ternary complexes with Neu5Ac at pH 10.3 was near unity (−1.04), it can be concluded

Table 1. Number of Coordinated Water Molecules in the Tb^{3+}–L Complex at pH 10.3 (No Buffer Solution)[a]

	τ/ms[b]	q	Δq
free Tb^{3+}–L	1.46 ± 0.02	1.34	
Tb^{3+}–L–Neu5Ac	2.28 ± 0.04	0.29	−1.04

[a]A large excess of Neu5Ac was added in order to quantitatively form the Tb^{III}–L–Neu5Ac complex. [b]See Figure S11 in the SI.

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that Neu5Ac coordinates to the inner sphere of the metal center in a monodentate fashion. Judging from the enhancement factors measured upon the addition of model substances [*N*-methylacetamide (NMA) and glycerol; see Figure S12 in the SI], the negatively charged oxygen atom in the acetamide group[18] is very likely to coordinate to the Ln^{3+} complexes accompanied by the exclusion of coordinated water.

^{1}H NMR spectra of Eu^{3+}−L−Neu5Ac and Neu5Ac ester complexes were also measured to gain additional insight into the coordination configuration. No signal for Neu5Ac was observed in the Eu^{3+}−L−Neu5Ac ternary complex because of the strong paramagnetic effect of Eu^{3+} (Figure 4c). This indicates that all of

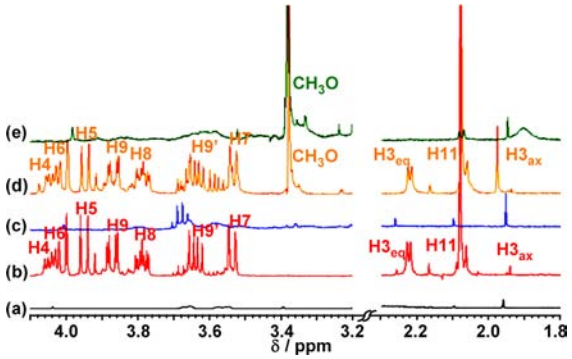

Figure 4. ^{1}H NMR spectra of Eu^{3+}−L (a; [Eu^{3+}−L] = 8.2 mM, pH 10.4), Neu5Ac (b; [Neu5Ac] = 5 mM, pH 11.0), Eu^{3+}−L−Neu5Ac (c; [Eu^{3+}−L] = 8.2 mM, [Neu5Ac] = 5 mM, pH 10.5), Neu5Ac ester (d; [Neu5Ac ester] = 5 mM, pH 10.5), and Eu^{3+}−L−Neu5Ac ester (e; [Eu^{3+}−L] = 8.2 mM, [Neu5Ac ester] = 5 mM, pH 10.5). Labeling numbers correspond to those in Figure 1.

the protons in the complex are within close enough proximity to Eu^{3+} to be highly shifted and broadened. On the other hand, the distinct signal of the methoxy group remained in the Eu^{3+}−L−Neu5Ac ester complex (Figure 4e). This strongly suggests that the (esterified) carboxyl group in the Neu5Ac (methyl ester) structure was the most distant group from the central Eu^{3+} in the complex. In order to form such a configuration, the acetamide and/or glycerol groups must be closest to Eu^{3+}.

According to these results, a new mechanism for the recognition of Neu5Ac is proposed (Figure 5), which is based

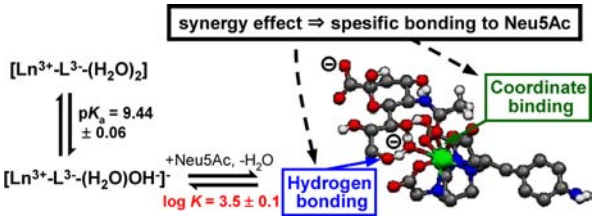

Figure 5. Proposed mechanism of the recognition of Neu5Ac.

on the synergistic effect of two types of interactions. One is the monodentate coordination binding of the acetamide oxygen atom in Neu5Ac to the first coordination sphere on the central metal ion, and the other is highly likely to be hydrogen bonding between the hydroxide group generated by deprotonation in the Ln^{3+} complex and the glycerol group in Neu5Ac in the second coordination sphere. This mechanism is indicated because no significant emission enhancement occurred either by NMA or the glycerol group alone, as unique to Neu5Ac, or by deprotonation of the Ln motif. It should be emphasized that this is the first report explaining the binding mechanism of sialic acid via a highly arranged coordination environment with deprotonation of a coordinated water molecule. This new molecular motif to selectively bind to Neu5Ac suggests a high potential for new artificial receptors and separation reagents.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental details and supplementary spectroscopic figures. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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