Recognition of Monosaccharides with Energy-transfer Luminescence Using Residual Coordination Sites of Lanthanide(III)–4-Aminobenzyl-EDTA Complex in Aqueous Solution

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In this paper, we present the luminescent lanthanide complexes, Tb^{3+}– and Dy^{3+}–4-aminobenzyl-EDTA as receptors for monosaccharides. The monosaccharides are recognized through their coordinate bonds and hydration bonds in alkaline aqueous solution by distinctive energy-transfer luminescence enhancement of the center metal ions. The stability constants of the ternary complexes formed with several monosaccharides, which are particularly sensitive in the case of *N*-acetylneuraminic acid (Neu5Ac), were measured. A high stability constant for Neu5Ac was observed with an affinity of about 5000.

The development of artificial receptors for sugar chains is of particular interest due to the biological importance of sugar.[1] A number of receptors for carbohydrates have been developed to date, with most of them employing either the interaction between boronic acid and *cis*-diol, or the hydrogen bond between nitrogen in the receptor and oxygen in the carbohydrate for their recognition. Many of these receptors have been shown to be effective in nonaqueous solution. Our goal in this study was to construct a luminescent enhancement system for monosaccharides in aqueous solution with the coordination and hydrogen-bond interaction approach.

In this study, we employed simple lanthanide(III) (Ln^{3+}) complexes with an aromatic EDTA derivative, which provided the center lanthanide ion with both its emissive and binding roles, for the recognition of the following monosaccharides (D-arabinose (Ara), D-fructose (Fru), D-galactose (Gal), D-glucose (Glc), D-mannose (Man), D-ribose (Rib), D-xylose (Xyl) as a nonionic monosaccharide and *N*-acetylneuraminic acid (sialic acid, Neu5Ac) as an anionic example). While sialic acids play very important roles in modulating and mediating the physiological and pathological processes in these monosaccharides,[2] there are few specific receptors for sialate, to our knowledge. Since the reagent, 4-aminobenzyl-EDTA (abbreviated as H_4L), is hexadentate and the coordination number of Ln^{3+} is generally 8–10, the Ln complex has 2–3 residual coordination sites[3] available for binding with the monosaccharide molecule. The first coordination sphere could provide different ternary complexing stability constants for various monosaccharides due to their stereochemically specific environment. Although a similar approach to recognizing monosaccharide molecules was made using the residual coordination sites of dinuclear copper complexes,[4,5] there are some disadvantages. First, spectrophotometric detection is not nearly as good as fluorometric detection with respect to sensitivity and selectivity. Second, the center metal ion in the receptors is expected to be labile for dissociation reactions. When other chelating molecules in samples coexist, labile complexes are not appropriate owing to interference with the measurement through fast ligand-exchange reactions. The good reasons to use these complexes however, are that they form inert complexes with Ln^{3+},[6] and that they emit a characteristic luminescent signal. The L complexes with Tb^{3+} and Dy^{3+} show a metal-centered emission based on the f–f transition ($\lambda_{ex} = 245$ or 289 nm and $\lambda_{em} = 542$ or 579 nm for Tb^{3+} ($\phi = 0.014$) or Dy^{3+}, see Figure 1) due to the energy-transfer (ET) process from the T_1 level of the aromatic group in the ligand to the f orbital of the center Ln^{3+}. The ET mechanism and the advantages for probes, including their large stokes shift and long lifetime, have been reported for a number of Ln complexes in detail.[7]

When monosaccharides were added to alkaline aqueous solution (pH 11.8) including the Ln^{3+}–L complex, the Ln-based luminescence intensity increased with increasing saccharide concentration (Figure 1). It is well known that the harmonic tone of the O–H oscillator of the water molecules in the first hydration shell quenches the Ln^{3+} emission via radiationless deactivation.[7] Since the Ln^{3+}–L complex has 2–3 water molecules in the first hydration shell, the metal-centered emission is rather weak. When added monosaccharide would bind to the lanthanide complex (Ln^{3+}–L·monosaccharide), the emission of Tb^{3+} and Dy^{3+} increases because the water molecules bound to the metal ion are excluded. The increase in luminescent intensity observed depended on a variety of monosaccharides, strongly indicating that the ternary complex of the Ln^{3+}–L·monosaccharide formed. Examples for Tb^{3+}–L·Rib and Dy^{3+}–L·Neu5Ac systems are depicted in Figure 1. To ascertain the formation of the ternary

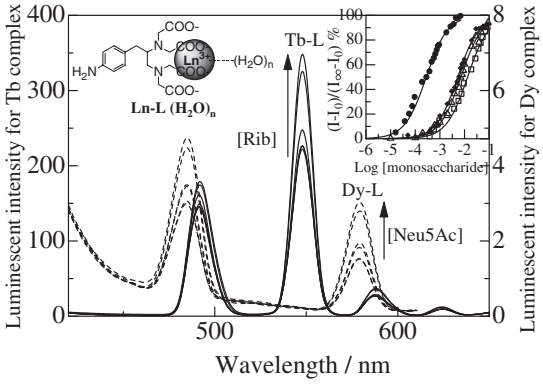

Figure 1. The Ln-based luminescence enhancement with the addition of monosaccharides. [Ln] = [L] = 1.0×10^{-5} M, [Rib] = 0, 0.05, 0.1, 1.0, 10 mM; [Neu5Ac] = 0, 0.01, 0.05, 0.1, 0.5, 1.0 mM; pH 11.8 (NaOH). 298 K, $\mu = 0.1$ (KCl). The insertion is the luminescent enhancement ($(I - I_0)/(I_\infty - I_0)$ (%)) as a function of the monosaccharide concentrations. ○, Tb–L·Rib; □, Tb–L·Glc; ●, Dy–L·Neu5Ac; ◆, Dy–L·Fru. The solid lines are the least-square fittings of the equilibrium calculation ($R^2 = 0.991$–0.999).

complex, in which a monosaccharide actually binds to the residual coordination sites, a ligand-exchange reaction of the Eu^{3+}–L·chelidamate ternary complex with Rib was conducted. The Eu^{3+}–L·chelidamate provides a metal-centered emission at 619 nm originating from the $^5D_0$–$^7F_2$ transition of Eu^{3+}, since the excitation at 277 nm of chelidamate, which is directly bound to the center metal, caused the ET to center-metal ion (the ET from L does not work for Eu^{3+}). When Neu5Ac was added to the solution of the Eu^{3+}–L·chelidamate complex, a significant decrease in luminescent intensity was observed.[8] This strongly indicated that a ligand-exchange process from Eu^{3+}–L·chelidamate (emissive) to Eu^{3+}–L·Neu5Ac (nonemissive) took place. From this, it was found that monosaccharides directly bound to the first coordination sphere on the metal.

The pH dependence of the luminescent intensity of Tb^{3+}–L complex in the presence of various monosaccharides was investigated.[8] While the fluorescence intensity was constant at pH 7–10, at pHs of around 11–12, the intensity for solutions including monosaccharides drastically increased in contrast to that for the Tb–L, and was most remarkable in the case of Neu5Ac. This is most probably due to the deprotonation of monosaccharides with pK_a values around 12.[9] The order of the intensity at pH 11.8 is Neu5Ac $\gg$ Rib > Fru > Xyl $\approx$ Gal $\approx$ Man > Glc > Ara > Tb–L complex (Figure 2). On the other hand, when monosaccharides were added to the Dy^{3+}–L complex, the luminescent intensity of the energy transfer also increased. The order of the intensity is as follows; Neu5Ac $\gg$ Fru > Xyl $\approx$ Rib > Gal $\approx$ Glc > Man > Ara > Dy–L at pH 11.8 (Figure 2). The order of the pK_a values for monosaccharides reported (Ara(12.43) > Glc(12.35) > Gal(12.35) > Xyl(12.29) > Rib(12.21) > Man(12.08) > Fru(12.03) > Neu5Ac(2.6 for carboxylate),[9,10] does not correspond to that of their luminescent intensity. This implies that there is another factor which governs the selectivity besides the basicity of the monosaccharides. Above pH 12, the intensity decreased. Similar behavior was frequently observed for other Ln complexes because of the hydrolysis.

The stability constants for ternary complexation (Tb and Dy–L·monosaccharide complex formation) were investigated[8] with regard to the dependence of luminescence intensity on the monosaccharide concentration (insertion in Figure 1). The sigmoidal responses obtained confirm the formation of 1:1 com-

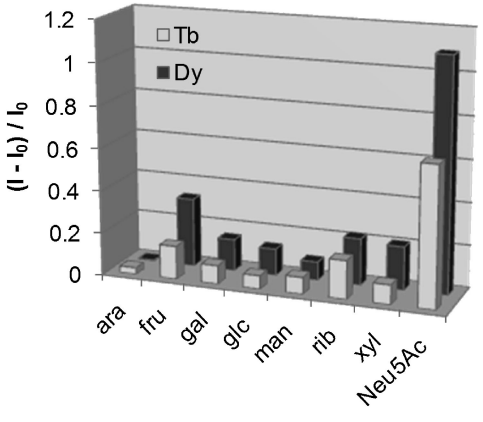

Figure 2. Enhancement factor ($(I - I_0)/I_0$) of Ln-based emission with the addition of monosaccharides (1.0×10^{-3} M) at pH 11.8 (NaOH).

plexes of the Ln–L-monosaccharide. The values of the conditional stability constants, K_{Neu5Ac}', K_{Rib}', K_{Fru}', and K_{Glc}' (M^{-1}) at pH 11.8 were determined as $10^{3.69}$, $10^{2.15}$, $10^{2.18}$, and $10^{1.88}$ for Tb complex, and $10^{3.55}$, $10^{2.28}$, $10^{2.11}$, and $10^{1.99}$ for the Dy complex, respectively. It is noted that the values for Neu5Ac were much larger (at more than one order) than the other monosaccharides. The magnitude of stability in this system is comparable to other superior artificial receptors,[1] especially for Neu5Ac, though it is inferior to carbohydrate-binding proteins.[11]

The Tb and Dy complex provide slightly different selectivity judging from the stability constants. This implies that selectivity can be controlled by substitution of the center metal owing to the change of the number and properties of the residual coordination sites; heavier lanthanide complexes have smaller residual space due to lanthanide contraction,[3] which enables the stereochemical control of the residual coordination sphere in angstrom order. Compared with the rather small change of K values, the change of luminescent intensity is quite drastic. This indicates that not only thermodynamic stability but also the orientation of hydroxy group, which plays the role of O–H oscillator to quench the emission, governs the emissive property. The change of the intensity with the addition of Neu5Ac-methyl ester in Tb–L complexes was only half of the change for Neu5Ac.[8] This strongly suggests the carboxyl group in Neu5Ac takes part in the coordination, though the coordination of Neu5Ac was not observed at pH 6–10, at which the carboxyl group was dissociated (p$K_a = 2.6$).[10] Accordingly, both the alkoxide and the carboxylate seem to be involved in the selective binding to Neu5Ac. The relationship between the selectivity and the chemical structure of the complexes remains unclear at this stage.

Finally, the findings in this work that Ln–polyaminocarboxylate complexes function as receptors for monosaccharides in aqueous solution, and that the complexes with high affinity to biologically significant anionic monosaccharide, Neu5Ac, provide a structural framework to design new artificial receptors.

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