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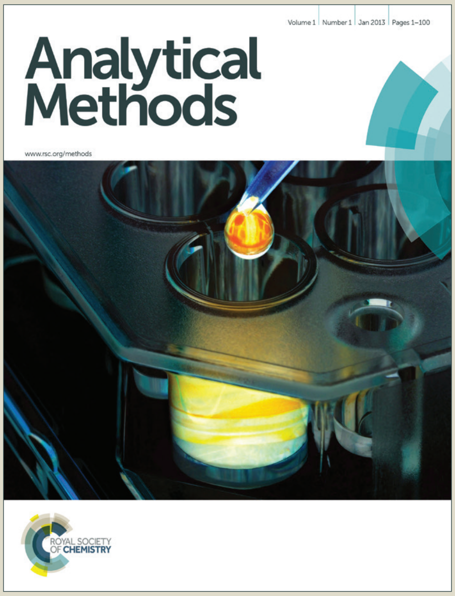

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ARTICLE TYPE

Novel electrochemical sugar recognition system using ruthenium complex and phenylboronic acid assembled on gold nanoparticles

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The construction of a novel electrochemical sugar recognition system using tris(acetylacetonato)ruthenium complex and phenylboronic acid assembled on gold nanoparticles is described. This system showed excellent sensitivity compared to those by the assembly on the dendrimer and the optical recognition.

The recognition of molecules or ions with high sensitivity and selectivity requires the combination of various materials and methodologies. We previously reported a sugar recognition system that combines two methodologies (electrochemistry and self-assembly) and two materials (a ruthenium complex and a dendrimer).[1] Using that system, we demonstrated that the assembly of the ruthenium complex on the dendrimer is very important for the sensitivity and selectivity for sugar recognition. However, the sensitivity was not so high (*ca.* 1×10^{-4} mol dm^{-3}), although the D-glucose sensitivity could be obtained. In the current study, a gold nanoparticle (**GNP**) was used instead of the dendrimer to improve the sensitivity and selectivity of the detection system. Assembly on the **GNP** was expected to provide higher sensitivity because more ruthenium complexes can assemble on a **GNP** than on a dendrimer, and the Au-S bond is stronger than the electrostatic interactions between the dendrimer and ruthenium complex. Furthermore, it should be possible to control the selectivity by controlling the length of the alkyl chains of the two probes.

GNPs have recently been widely used for the optical recognition of a variety of molecules and ions (*e.g.*, sugars,[2] biological substances such as DNA and dopamine,[3-9] TNT,[10] cations[11,12] such as Ag and Cr, and anions[13] such as phosphate). Highly sensitive visual recognition is due to optical changes between aggregation and dispersion. **GNP**s have also been used for the electrochemical recognition of biological substances in which recognition systems were constructed as modified electrodes associated with **GNP**s.[14,15] However, although several reports have been published where a metal complex was used as a molecular recognition site for other molecules and ions,[16] there have been no examples of the electrochemical recognition of sugars using a metal complex assembled on a **GNP**.

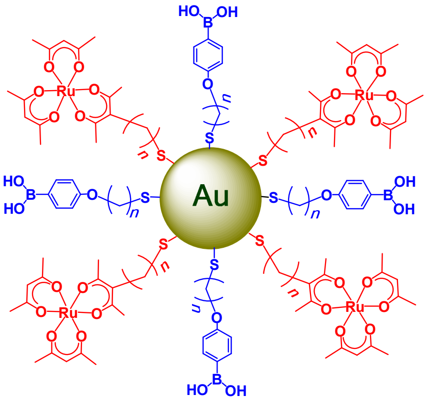

Fig. 1 A schematic of tris(acetylacetonato)ruthenium, an electro active probe, and phenylboronic acid, assembled as a sugar recognition probe on a **GNP**

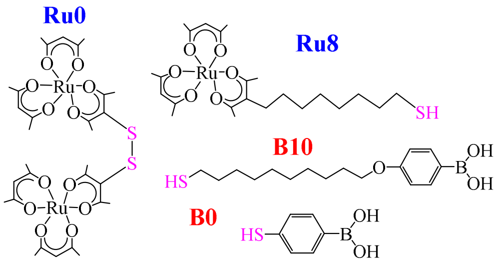

Fig. 2 Two ruthenium complexes (**Ru0** and **Ru8**) and two phenylboronic acids (**B0** and **B10**) for assembly on **GNPs**

We report herein a novel electrochemical sugar recognition system constructed by separately assembling a redox-active ruthenium complex probe and a phenyboronic acid probe on a **GNP**. A schematic of tris(acetylacetonato)ruthenium and phenylboronic acid assembled on a **GNP** is shown in Fig. 1. Two ruthenium complexes (**Ru0** and **Ru8**)[17] and two phenylboronic acids (**B0** and **B10**) with different alkyl chain lengths were prepared (Fig. 2) and assembled on **GNP**s. Hence, four kinds of **Ru/B/GNP** complexes were constructed and used for sugar recognition: **Ru0/B0/GNP**, **Ru0/B10/GNP**, **Ru8/B0/GNP**, and **Ru8/B10/GNP**. The length of **Ru8** and **B10 is** calculated to be almost same, ca. 2 nm.[18] The dinuclear ruthenium complex $[\{Ru^{III}(acac)_2\}_2(dtba)]$ (**Ru0**, Hacac: acetylacetone, H_2dtba: 3,3'-dithiobis(acetylacetone) was synthesized as previouslyreported.[18] $[Ru^{III}(acac)_2(acac\text{-}(CH_2)_8\ SH)]$ (**Ru8**, Hacac-$(CH_2)_8$SH: 3-(1-octanethiol)-2,4-pentandion) was synthesized from $[Ru^{III}(acac)_2(acac\text{-}(CH_2)_8I)]$. **GNP**s were prepared from $HAuCl_4$ by the reduction of sodium citrate according to the general method.[19] The average diameter of the **GNP**s determined by DLS (dynamic light scattering) was ca. 14 nm in aqueous solution at 25°C. The optimum modification ratio, n_{Ru}:n_{BA} (n_{Ru} and n_{BA} denote the amount of Ru complex and phenylbronic acid assembled on the **GNP**s), was determined by UV-vis spectral measurements.[20] When n_{Ru}:n_{BA} was decreased, the peak at 620 nm, indicating the aggregation state, increased for **Ru0/B0/GNP** (Fig. S1). In

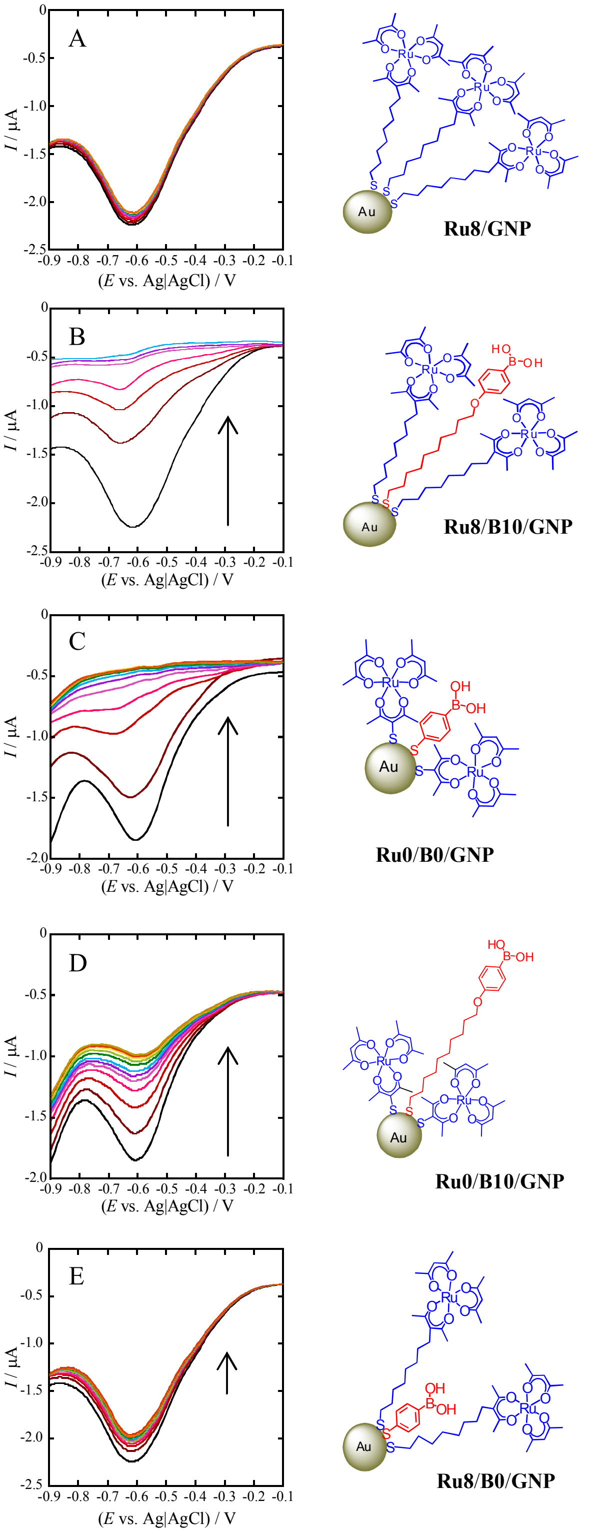

Fig. 3 DPV of **Ru8/GNP** (A), **Ru8/B10/GNP** (B), **Ru0/B10/GNP** (C), **Ru0/B0/GNP** (D), and **Ru8/B0/GNP** (E) complexes in 0.1 mol dm^{-3} NaClO$_4$-(H$_2$O:EtOH = 3:1) at glassy carbon electrode (ϕ = 3 mm) under Ar with addition of D-glucose. [D-glucose]: A; 0 – 250 μmol dm^{-3}, B; 0, 0.5, 1.0, 2.5, 5.0, 7.5, 10 μmol dm^{-3}, C; 0, 0.5, 1.0, 2.5, 5.0, 7.5,10,15, 25, 50, 75, 100, 150 μmol dm^{-3}, D; 0, 0.5, 1.0, 2.5, 5.0, 7.5,10,15, 25, 50, 75, 100, 150, 250, 500 μmol dm^{-3}, E; 0, 0.5, 1.0, 2.5, 5.0, 7.5,10,15, 25, 50, 75, 100, 150, 250 μmol dm^{-3}

addition, a time dependence for the absorbance was observed. Therefore, the optimum ratio was determined to be 9:1 (Ru:BA). All measurements were conducted 10 min after preparing the sample solution.

Using differential pulse voltammetry (DPV), a glassy carbon electrode (GCDE, ϕ = 3 mm), and 0.1 mol dm^{-3} NaClO$_4$-(H$_2$O:EtOH = 3:1 v/v%), the **Ru8/GNP** complex provided a reduction wave with a peak potential of E_p = -0.61 V *vs*. Ag|AgCl (3 mol dm^{-3} NaCl aq.) (Fig. 3A). The reproducibility of peak current value is within ca. 5 %.This reduction wave should attribute to the reduction of ruthenium complex corresponding to the Ru^{III}/Ru^{II} couple, as the free ruthenium complexes ([Ru(acac)$_3$], **Ru0**, and **Ru8**) showed reduction waves at -0.56, -0.60, and -0.55 V, respectively, in the same medium. No reduction wave was observed using a **GNP** solution.

The addition of a solution of D-glucose to a solution of **Ru8/B10/GNP** complex caused a decrease in reduction current and a slight negative peak potential shift (Fig. 3B). No significant decrease in reduction current was observed with **Ru8/GNP** complex upon the addition of D-glucose (Fig. 3A); therefore, the decrease in current was likely due to the recognition of D-glucose by boronic acid on the **GNP**. The **Ru0/B0/GNP** and **Ru0/B10/GNP** complexes provided similar current depressions as **Ru8/B10/GNP** (Fig. 3C and 3D); however, the **Ru8/B0/GNP** complex did not show a significant decrease in current upon the addition of D-glucose (Fig. 3E), which was likely due to steric blocking of D-glucose to **B0** by **Ru8**.

When D-fructose and D-galactose were added to the solution of **Ru/B/GNP** instead of D-glucose, essentially the same behaviour was observed for all the **Ru/B/GNP** complexes. Fig. 4A shows the molar concentration dependence of the ratio of the current decrease $\{(Ip^0 - Ip)/Ip^0$, where Ip and Ip^0 denote the peak current of the DVP in the presence and the absence of sugar, respectively$\}$ for the **Ru8/B10/GNP** complex and three types of sugar. The order of the ratio of current decrease is D-glucose > D-fructose > D-galactose. The phenyboronic acid on **Ru8/B10/GNP** showed high response for D-glucose, although simple phenyboronic acid exhibited D-fructose selectivity.

In this system, the decrease in current does not provide direct information regarding sugar recognition, since the ruthenium complex is an electro-active site and not a sugar recognition site; therefore, the real binding constant cannot be obtained from this experiment. However, the apparent binding constant, $K_{\mathrm{app.bind}}$, can be calculated by the following equation derived by Damos *et al*., assuming a Langmuir isotherm.[21]

$$[\mathrm{Sugar}] / \Delta I_{\mathrm{p}} = 1/ (K_{\mathrm{app.bind}} \cdot \Delta I_{\mathrm{p.max}}) + [\mathrm{Sugar}] / \Delta I_{\mathrm{p.max}}$$

where [Sugar] is the bulk concentration of sugar, ΔI_p is the difference in the current in the presence and absence of the sugar ($=Ip - Ip^0$), and $\Delta I_{\mathrm{p.max}}$ is the maximum value of ΔI_p. The plot of $\{[\mathrm{Sugar}]/(\Delta Ip/Ip^0)\}$ *vs*. [Sugar] for the **Ru8/B10/GNP** complex and three types of sugar gave a straight line, as shown in Fig. 4B. The value of $K_{\mathrm{app.bind}}$ was able to be determined from the intercept obtained by the least-squares method. Similar analyses for the other **Ru/B/GNP** complexes also gave straight lines, except for the **Ru8/B0/GNP** complex. All the apparent binding constants are provided in Table 1. There were no large differences of $K_{\mathrm{app.bind}}$ among three kinds of **Ru/B/GNP** except **Ru0/B10/GNP**. However, the **Ru8/B10/GNP** and **Ru0/B0/GNP** complexes showed better responsibility than the other two complexes.

In this system, the reduction current of ruthenium complexes decrease with addition of sugar. This means that the electron transfer between the sensing electrode and ruthenium complexes on **GNP** is inhibited by some reasons. There are following two

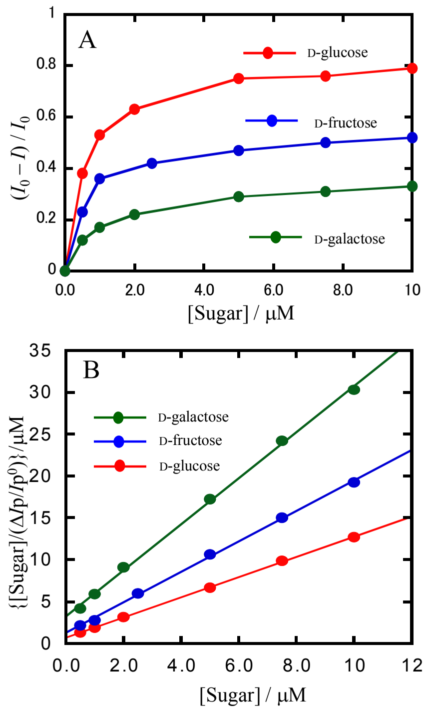

Fig. 4A plot of $\{(Ip^0 - Ip)/Ip^0$ *vs*. [Sugar] (A) and plot of {[Sugar] /$(\Delta Ip/Ip^0)\}$ *vs*. [Sugar] (B) for the **Ru8/B10/GNP** complex

possibilities. 1) Shielding of ruthenium complexes by the aggregation between **GNP**s due to the addition sugar, 2) shielding of ruthenium complexes by sugar bound with phenyl boronic acid. In order to inspect the mechanism behind sugar recognition, UV-vis spectra were measured before and after the addition of D-glucose to the solution of **Ru8/B10/GNP**. However, no significant shifts in the spectra were observed, indicating that the decrease in current is not primarily caused by aggregation of the **GNP**s. In addition, the observed shift in the current followed the Langmuir isotherm described above. Therefore, the decrease in current should be due to shielding of ruthenium complexes by sugar bound with phenyl boronic acid.

Table 1. Apparent binding constants ($K_{\mathrm{app.bind}}$) of four Ru/**B/GNP** complexes and three types of sugars

	$K_{\mathrm{app.bind}}/\mathrm{mol^{-1}\,dm^3}$			
	Ru8B10	**Ru8B0**	**Ru0B10**	**Ru0B0**
D-fructose	$1.5_0\times10^6$	N.D.	$5.7_2\times10^5$	$1.3_9\times10^6$
D-glucose	$1.7_5\times10^6$	N.D.	$7.0_2\times10^5$	$1.2_4\times10^6$
D-galactose	$8.2_9\times10^5$	N.D.	$1.1_3\times10^6$	9.52×10^5

Conclusions

We synthesized four **Ru/B/GNP** complexes (**Ru0/B0/GNP**, **Ru0/B10/GNP**, **Ru8/B0/GNP**, and **Ru8/B10/GNP**), and prepared a novel electrochemical sugar recognition system. The reduction current at tris(acetylacetonato)ruthenium immobilized on **GNP** decreased upon the addition of sugar. The change in current followed an equation derived from the Langmuir isotherm. The **Ru8/B10/GNP** complex showed the best sensitivity, of *ca*. 1 µmol $\mathrm{dm^{-3}}$. This means that the sensitivity could be improved by 100 times compared to the assembly on the dendrimer.[1] Furthermore, this sensitivity is better than those obtained by optical recognition and electrochemical recognition using modified electrode assembled by phenylboronic acid or ferrocene boronic acid (50 µmol $\mathrm{dm^{-3}}$ in the maximum).[2] Furthermore, this system should be applicable to the recognition of various molecules and ions simply by changing the recognition site, *e.g*., dipycolylamine for recognition of metal ions, crown ether for alkaline metal ions. Although these are now under investigation, excellent electrochemical recognition system could be constructed.

Notes and references

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† Electronic Supplementary Information (ESI) is available: Synthesis method of ruthenium complex, current shifts by addition of sugars. See DOI: 10.1039/b000000x/

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