Ru(II)–carbohydrate dendrimers as photoinduced electron transfer lectin biosensors†

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Three tris(bipyridine)ruthenium dendrimers bearing either six or eighteen mannose units have been synthesized; encapsulation of the Ru(bipy)$_3$ core alters the rate of energy and electron transfer as well as the lectin biosensing abilities.

Significant effort has been directed at utilizing the unique combination of optical and electrochemical properties of ruthenium tris(bipyridine) in various materials and biological applications.[1] Photo-excitation of ruthenium tris(bipyridine) results in electron and/or energy transfer processes. These events have been exploited to generate biosensors, establish artificial photosynthesis systems and to induce the generation of free radicals to photoxidize DNA, RNA and cell surfaces.[2] In order to target specific biological events, ruthenium complexes need to be equipped with recognition molecules. One possible strategy involves the fusion of ruthenium(II) complexes with a carbohydrate to target specific cells and tissues *via* lectin–carbohydrate interactions. Lectins are carbohydrate-binding proteins that mediate important biological processes such as cell growth, the inflammatory response and viral infections.[3] Since monosaccharide–lectin binding affinities are usually quite weak, carbohydrate dendrimers[4] that increase the avidity and surround the ruthenium(II) core have been explored. Homogenous branching of the dendrimers produces a microenvironment that encapsulates the ruthenium(II) core and modifies its photochemical and electrochemical properties.[1,5] Recently, the way in which dendrimers encapsulate the core and alter its properties has been studied in detail. Electron transfer on ferrocenyl redox cores was shown to depend on the molecular orientation.[6] Similar results were observed with divalent viologen and Ru(bipy)$_3$ complexes,[7] as well as tetravalent Fe_4S_4[8] and porphyrin core redox units.[9] However, a systematic investigation of the core activities for different carbohydrate densities during biosensing processes has not been reported.

Here, we report on investigations concerning the rate of electron and energy transfer from the Ru(II)–carbohydrate dendrimers to *N*,*N*′-4,4′-bis(benzyl-3-boronic acid)-bipyridinium dibromide (BBV, Fig. 1) and tetramethyl piperidine (TEMP), respectively. Additionally, as an example of a potential application, we present the study of the electron transfer mechanism of these dendrimers used to detect lectins. We demonstrate that increasing the dendrimer density around the Ru(bipy)$_3$ unit does indeed influence lectin sensing by photoinduced electron transfer (PET) between the ruthenium(II) core and BBV molecules and, in general, we show that covalent attachment of carbohydrates to ruthenium tris(bipyridyl) based dendrimers paves the way to new molecules capable of sensing lectins with very low detection limits.

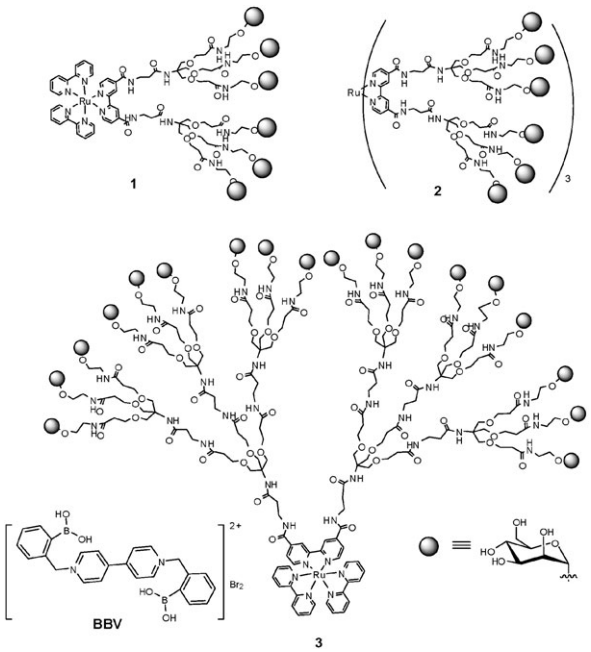

Fig. 1 Structures of Ru(II)-complexes **1**, **2** and **3** and **BBV**.

Mannose-capped dendrimers **1–3** (Scheme 1) were prepared from bipyridine sugar derivatives **6** and **13** and reaction with $RuCl_3$ or *cis*-Ru(bipy)$_2$Cl$_2$ respectively. Deprotection of the *tert*-butoxycarbonylamino group of **5** and coupling to bipyridine 4,4′-dicarboxylic acyl chloride yielded bipyridine derivative **6**. Refluxing **6** with *cis*-Ru(bipy)$_2$Cl$_2$ and $RuCl_3$ in ethanol followed by deacetylation yielded dendrimers **1** and **2** respectively. Reaction of tri-mannose substituted **5** with tripod active ester **4** furnished **12**. Then, **12** was reacted with bipyridine 4,4′-dicarboxylic acyl chloride, followed by complexation with *cis*-Ru(bipy)$_2$Cl$_2$ and deacetylation to obtain dendrimer **3**.

The rate of electron transfer in complexes **1–3** was investigated using PET between photo-excited Ru(II)-templates and a quencher. Several quenchers were reported for the MLCT (metal–ligand charge transfer) excited state of the $[Ru(bpy)_3]^{2+}$

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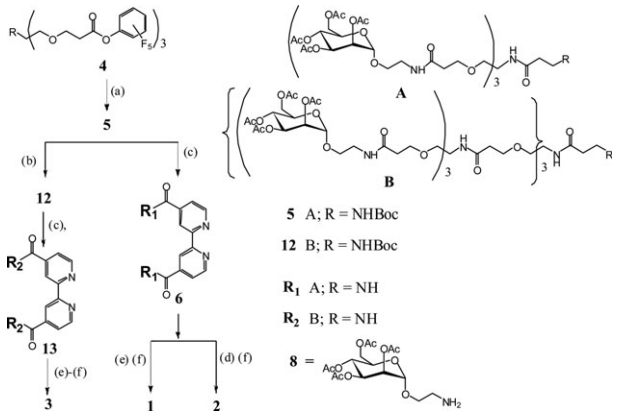

Scheme 1 Synthesis of Ru(II)-dendrimers **1**, **2** and **3**. (a) Triethylamine (TEA)/DCM/**8**; (b) **4**, TEA, DCM; (c) TFA, 2,2'-bipyridine 4,4'-dicarboxylic acyl chloride, DCM, TEA; (d) $RuCl_3$, EtOH; (e) *cis*-$Ru(bipy)_2Cl_2$, EtOH; (f) NaOMe, MeOH.

model.[5] Among them, methyl viologen dication (MV^{2+}) shows a high quenching constant.[9] Moreover, MV^{2+} does not possess excited states below the MLCT level of the $[Ru(bipy)_3]^{2+}$ chromophoric unit, but is readily reduced. As a consequence, quenching can only take place by electron transfer. Since the boronic acid derivative of MV^{2+} (BBV) has a high affinity for sugar, BBV has been used extensively in the development of sugar sensors.[10] All compounds exhibited different quenching constants (k_q), that were obtained with the Stern–Volmer equation,

$$\tau_o/\tau = I_o/I = 1 + k_q\tau_o[Q] \qquad (1)$$

where, τ_o and I_o are the excited-state lifetime and quantum yield in the absence of quencher, and τ and I are the same quantities measured in the presence of a certain concentration of quencher [Q]. The experimental values of I_0, τ_o and k_q of complexes **1**, **2** and **3**, together with the standard $Ru(bipy)_3$ are given in Table 1. In the absence of quencher, there is a difference in the quantum yield between the complexes with six (**1**) and eighteen mannose units (**2** and **3**) that must be related to the different density of dendritic encapsulation. Moreover, the difference in carbohydrate densities has a dramatic effect on the quenching constant, which decreases from complex **1** to **3** by almost an order of magnitude. These results indicate that a high degree of carbohydrate density around the ruthenium core allows for efficient encapsulation and modification of the core properties.

To evaluate the rate of energy transfer by **1–3**, we studied the formation of molecular oxygen in the singlet state upon photoexcitation of the ruthenium tris(bipyridine) complexes (Fig. 2). Tetramethyl piperidine (TEMP) was used as a trap for singlet oxygen to form a stable species (TEMPO) easily detected by EPR (see eqn (2)).[2]

Table 1 Photophysical data of complexes **1–3**. Quantum yield and lifetime were measured by excited complex **1–3** at λ_{max} = 450 nm and emission at λ_{max} = 645 nm

Compound	λ_{max}/nm	k_q/M^{-1} s^{-1}	τ_o/μs	I_o
1	645	9.8×10^8	0.61	0.072
2	648	1.8×10^8	1.31	0.102
3	648	1.1×10^8	1.26	0.112
$Ru(bipy)_3$	613	2.5×10^9	0.54	0.062

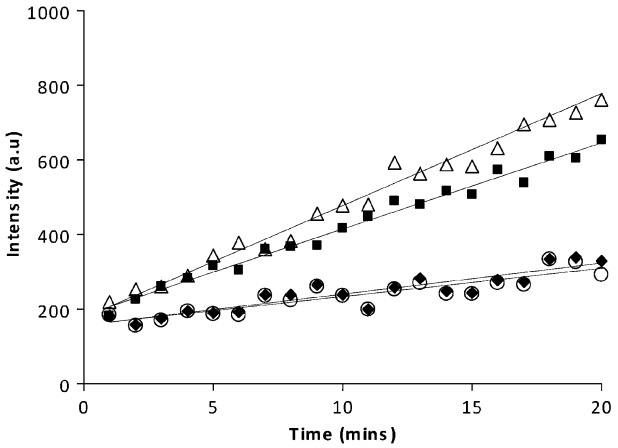

Fig. 2 Kinetic profile of singlet oxygen formation upon irradiation of complexes **1** (■), **2** (♦), **3** (○) and $Ru(bipy)_3$ (△).

$$Ru(II) \rightarrow Ru(II)^*$$

$$Ru(II)^* + {}^3O_2 \xrightarrow{\text{energy transfer}} Ru(II) + {}^1O_2 \qquad (2)$$

$${}^1O_2 + TEMP \rightarrow TEMPO$$

Continuous irradiation of the Ru-complexes (2 mM) in the presence of TEMP (100 mM) yielded a nitroxide triplet in the EPR spectrum (see ESI†) typical for the TEMPO radical. The rate of appearance of the TEMPO signal decreases from **1** to **3**, in support of the notion that carbohydrate encapsulation of the Ru(II)-template stops effective energy transfer to dissolved oxygen.

After gaining insight into how the energy and electron transfer processes are affected by the dendrimer structure, we studied how selective and sentitive these processes can be by illustrating the capabilities of dendrimers **1–3** as lectin biosensors. Concanavalin A (ConA) and *galanthus nivilis agglutinin* (GNA) that recognize mannose were selected as lectins.[11] Both, ConA and GNA are tetramers that contain one and three mannose binding sites per subunit respectively. The sensors are based on the specific and strong binding between mannose-dendrimers and the lectins (Fig. 3). This tight interaction segregates the quencher (BBV) from the Ru(II)-complex, thus reconstituting the fluorescent signal (Fig. 4).

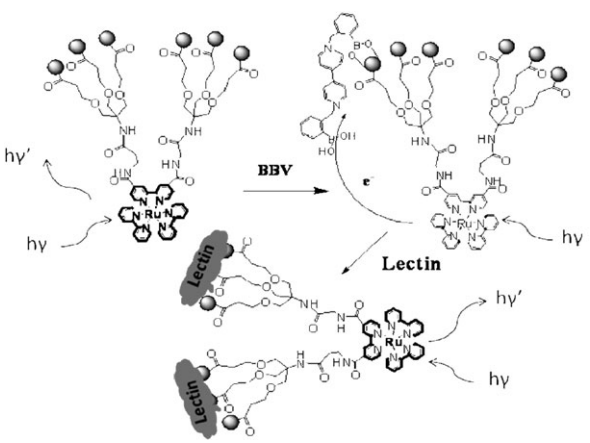

Fig. 3 Schematic representation of the Ru(II)–carbohydrate dendrimer lectin sensor.

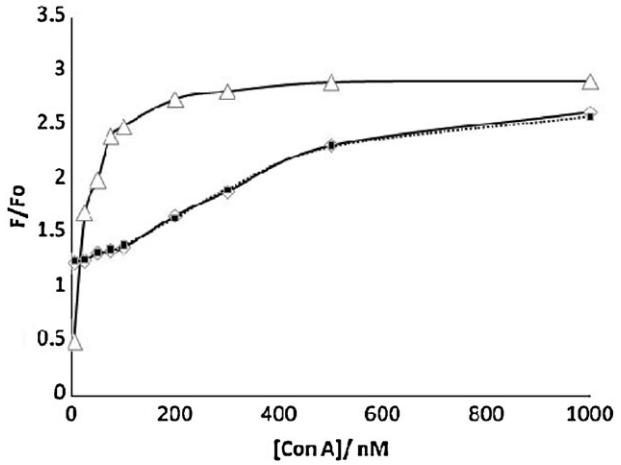

Fig. 4 Rate of fluorescence gain from Ru(II)–carbohydrate–BBV upon addition of lectin; **1** (△), **2** (◊) and **3** (■) at λ_{max} = 645 nm.

Using a donor/acceptor mixture of complex **1** and BBV, a spontaneous gain in fluorescence upon the addition of 75 nM of ConA and a further slow increase in the signal at 200–1000 nM was observed. In contrast, for 0 to 100 nM of ConA, complexes **2** and **3** displayed much more modest gains in fluorescence compared to complex **1** but a steady and linear increment upon the addition of 100–600 nM. Similar experiments with the higher valency lectin GNA were performed (see ESI†). The detection limits for the Ru-complexes were calculated based on these results (Table 2) and we found that complex **1** is noticeably more sensitive than other sensors described in the literature.[12] The eighteen-mannose dendrimers (**2** and **3**) have a higher detection limit whereas the range of linear response is broader. Complex **1** has the best compromise between encapsulation and good quenching properties for sensitive lectin sensing. While complexes **2** and **3** bind much more strongly to lectins than complex **1**, the effective encapsulation of the Ru(II)-core by the carbohydrate dendrimer results in low quenching and weak PET, and thus is a less sensitive biosensor.

In conclusion, we have synthesized three new carbohydrate dendrimers with a $Ru(bipy)_3$ core unit. The electron and energy transfer rates of the photoexcited state were established using the quencher BBV and monitoring the formation of singlet oxygen. The behavior of complexes **2** and **3** was typical of an encapsulated $Ru(bipy)_3$ core unit with one order of magnitude decrease in the quenching rate compared to the $Ru(bipy)_3$ complex. The quenching constant decreases with increasing number and size of the dendritic branches. Similar results were also observed for the energy transfer process. The influence in the rate of photoinduced electron transfer was also visible in the lectin sensing process. Complex **1** showed more sensitive detection compared to complexes **2** and **3**. These results indicate that PET biosensing is more effective with the small dendrimer **1**. Dendrimers **2** and **3** are sensitive biomarkers to study lectin–carbohydrate interactions, owing to their high quantum yield and high carbohydrate density. The application of **2** and **3** to optical biosensing on microarrays and for *in vitro* imaging is currently under investigation.

Table 2 Detection limit of lectins using different Ru–mannose dendrimers

Compound	ConA/nM	GNA/nM
1	28 ± 3	25 ± 4
2	340 ± 12	328 ± 9
3	347 ± 14	331 ± 12

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