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# Novel Rhenium(I) Polypyridine Biotin Complexes That Show Luminescence Enhancement and Lifetime Elongation upon Binding to Avidin

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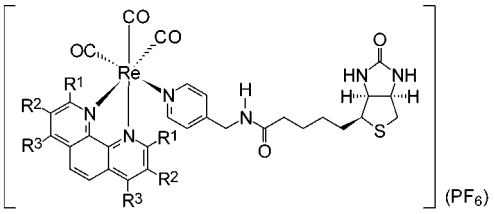
Since the noncovalent binding mode between avidin and biotin is the strongest biological interaction between a protein and a ligand, it plays an extremely important role in biochemistry, immunology, and bioanalytical chemistry.[1] Biotinylated biomolecules such as DNA, peptides, and proteins can be recognized by avidin conjugated with a marker such as a fluorescent label. Although a similar recognition and detection procedure can be carried out using avidin as the bridge and biotin−fluorophore conjugates as the reporter, the use of fluorescent biotin reagents is much less common. The major reason is that most conventional biotin−fluorophore conjugates, such as those of fluorescein, pyrene, tetramethylrhodamine, and Cy dyes, lose their fluorescence when they are bound to avidin, unless there are exceptionally long spacers between the biotin and the fluorophore units.[2] We anticipate that this problem can be solved using luminescent biotin−transition metal complex conjugates in view of their characteristic photophysical properties, in particular, the large Stokes shifts. In addition, the tunability of the emission energy and relatively long emission lifetimes of luminescent transition metal complexes with a charge-transfer excited state are also attractive properties for the purpose of a recognition assay. While there have been reports on the conjugation of the biotin moiety to different transition metal complexes,[3] the use of biotin−transition metal complex conjugates as luminescent probes has not been explored.

We report here that the metal-to-ligand charge-transfer (MLCT) emission of rhenium(I) polypyridines can be exploited to provide a new generation of luminescent biotin derivatives with emission properties remarkably different from those of traditional biotin−fluorophores. The complexes $[Re(N\text{-}N)(CO)_3(py\text{-}CH_2\text{-}NH\text{-}biotin)](PF_6)$ [N-N = 1,10-phenanthroline (**1**); 3,4,7,8-tetramethyl-1,10-phenanthroline (**2**); 2,9-dimethyl-4,7-diphenyl-1,10-phenanthroline (**3**); py-$CH_2$-NH-biotin = *N*-[(4-pyridyl)methyl]biotinamide] were prepared, in moderate yields, from the reactions of $[Re(N\text{-}N)(CO)_3(CH_3CN)](CF_3SO_3)$ with py-$CH_2$-NH-biotin in THF under refluxing conditions, followed by metathesis with $KPF_6$ and purification by column chromatography (alumina).[4] The structures of the complexes are shown in Chart 1.

Complexes **1**−**3** exhibit intense and long-lived greenish-yellow to yellow luminescence upon photoexcitation. The photophysical data are summarized in Table 1. The emission energy, band shapes, lifetimes, and luminescence quantum yields resemble those of typical $[Re(N\text{-}N)(CO)_3(py)]^+$ systems, and the emission is assigned to originate from an $^3$MLCT [d$\pi$(Re) → $\pi^*$(N-N)] excited state.[5]

The binding of the complexes to avidin has been investigated using the standard HABA (4′-hydroxyazobenzene-2-carboxylic acid) assay.[6] The results reveal that **1**−**3** bind to the avidin molecule with the same stoichiometry as unmodified biotin (4:1). Luminescence titrations using the rhenium(I) complexes as titrants show that complex **1** displays a higher emission intensity in the presence of avidin. At [**1**]:[avidin] = 4:1, the emission intensity is ca. 1.4 times that of the complex in the absence of avidin (Table 1). The increase in emission intensity is ascribed to the binding of the complex into the biotin-binding sites of avidin because no increase in emission intensity is observed when excess unmodified biotin is present (Table 1). These observations are of great interest because most biotin−fluorophore conjugates suffer from severe emission quenching upon binding to avidin due to resonance-energy transfer (RET), unless exceptionally long spacers such as poly(ethylene glycol) are present between the fluorophore and the biotin units.[2] This provides sufficient separation between the fluorophores bound to the same avidin molecule to eliminate this effect. It is also well-known that biomolecules labeled with organic fluorophores do not become more highly fluorescent with higher degrees of labeling.[7] The absence of emission quenching in the present case is due to the insignificant overlap between the absorption and emission spectra of **1**, which renders quenching by RET unfavorable. The enhancement in emission intensity is in line with the observations that when luminescent transition metal complexes are conjugated to protein molecules, the emission is enhanced and oxygen-quenching becomes much less effective.[8] Interestingly, more

***Chart 1.*** Structures of Complexes **1**−**3**




(**1**): $R^1 = R^2 = R^3 = H$
(**2**): $R^1 = H$; $R^2 = R^3 = CH_3$
(**3**): $R^1 = CH_3$; $R^2 = H$; $R^3 = C_6H_5$

***Table 1.*** Photophysical Data for Complexes **1**−**3** at 298 K

| complex | medium | $\lambda_{em}$/ nm[a] | $\tau_o$/ $\mu$s[a] | $\Phi$[a] | $I$ ($\tau$/$\mu$s)[b,c] | $I$ ($\tau$/$\mu$s)[b,d] | $I$ ($\tau$/$\mu$s)[b,e] |
|---|---|---|---|---|---|---|---|
| **1** | $CH_3CN$ | 552 | 1.37 | 0.079 | 1.00 (0.56) | 1.42 (0.90) | 0.98 (0.55) |
| | $CH_2Cl_2$ | 536 | 2.69 | | | | |
| **2** | $CH_3CN$ | 518 | 7.32 | 0.072 | 1.00 (1.23) | 2.25 (2.96) | 1.04 (1.25) |
| | $CH_2Cl_2$ | 510 | 7.81 | | | | |
| **3** | $CH_3CN$ | 550 | 6.78 | 0.079 | 1.00 (1.84) | 2.98 (2.70) | 0.96 (1.90) |
| | $CH_2Cl_2$ | 540 | 7.22 | | | | |

[a] In degassed solvents. [b] Relative emission intensities in aerated 50 mM potassium phosphate buffer pH 7.4. [c] [Re] = 15.2 $\mu$M, [avidin] = 0 $\mu$M, [unmodified biotin] = 0 $\mu$M. [d] [Re] = 15.2 $\mu$M, [avidin] = 3.8 $\mu$M, [unmodified biotin] = 0 $\mu$M. [e] [Re] = 15.2 $\mu$M, [avidin] = 3.8 $\mu$M, [unmodified biotin] = 380.0 $\mu$M.

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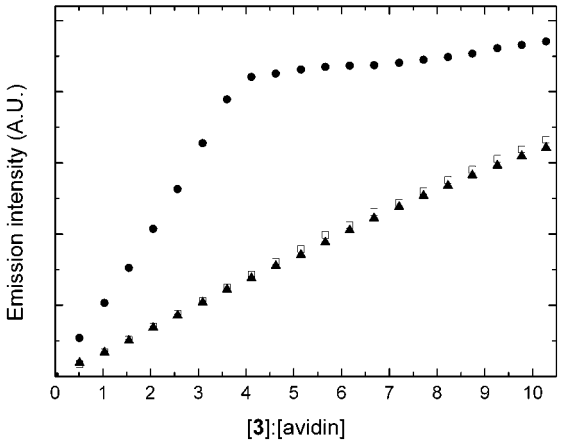


***Figure 1.*** Luminescence titration curves for the titrations of (i) 3.8 $\mu$M avidin (●), (ii) 3.8 $\mu$M avidin and 380.0 $\mu$M unmodified biotin (▲), and (iii) a blank phosphate buffer solution (□) with complex **3**.

significant luminescence enhancement has been observed for **2** and **3**. At [Re]:[avidin] = 4:1, the emission intensities of **2** and **3** are increased by factors of ca. 2.3 and 3.0, respectively (Table 1). Emission titration curves for **3** are shown in Figure 1.[4] The emission lifetimes have also been found to increase by ca. 1.5−2.4-fold when **1**−**3** are bound to avidin (Table 1). Since the lifetimes of the excited complexes are sensitive to the hydrophobicity of the environment, evidenced by the fact that the lifetimes of all three complexes increase from $CH_3CN$ to $CH_2Cl_2$ (Table 1), it is conceivable that the enhancement in emission intensities and lifetimes result from the hydrophobicity associated with the binding pockets of avidin. This is in agreement with the finding that **2** and **3**, being more hydrophobic than **1**, exhibit more significant increases in emission intensities and lifetimes after binding to avidin. We, however, notice that at [Re]:[avidin] > 4:1, the titration curves are not exactly parallel to (i) those of the solutions without avidin and (ii) those with avidin in the presence of excess unmodified biotin. Therefore, the possibility of nonspecific interactions between the free complexes and bound complexes or rhenium-bound avidin cannot be excluded.

The binding properties of **1**−**3** to avidin with respect to unmodified biotin have been studied by competitive association and dissociation assays.[9] The assays show that ca. 12−23% avidin molecules remain bound by the complexes in the presence of biotin.[4] Dissociation constants ($K_d$) of ca. $3.2 \times 10^{-10}$, $5.2 \times 10^{-11}$, and $4.0 \times 10^{-9}$ mol dm$^{-3}$ have been determined for the avidin complexes of **1**−**3**, respectively, from luminescence titration experiments.[10]

In view of the long emission lifetimes of these rhenium(I) polypyridine biotin complexes, we have investigated the possibility of their utilization as probes for biotin and biotinylated species based on the RET principle.[11] As an example, a degassed buffer solution of **3** (12.0 $\mu$M) exhibits emission quenching ($I/I_o = 0.37$) in the presence of avidin (6.0 $\mu$M) that has been modified with the nonfluorescent energy-acceptor dye QSY-7 isothiocyanate (Molecular Probes).[4] The dye QSY-7 is chosen because its absorption maximum (560 nm) overlaps significantly with the emission maximum of **3** (556 nm) in phosphate buffer. It appears that RET plays an important role in this emission quenching since the lifetime decreases from 6.02 to 4.90 $\mu$s and a similar decrease in the emission lifetime is not observed when unmodified biotin (300.0 $\mu$M) is initially present ($\tau = 5.94$ $\mu$s). On the basis of the spectral data of the donor and acceptor, a Förster distance of 44.5 Å is estimated.

In conclusion, these novel luminescent rhenium(I) polypyridine biotin complexes offer remarkable advantages over traditional biotin−fluorophores as probes for avidin and can be utilized in homogeneous assays for biotin and biotinylated biomolecules. Related studies of other luminescent biotin−transition metal complex conjugates are in progress.

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**Supporting Information Available:** Synthetic procedures and characterization data for the ligand py-$CH_2$-NH-biotin and complexes **1**−**3**, electronic absorption spectral data for **1**−**3**, luminescence titration curves for **1** and **2**, details of competitive association and dissociation assays for the interactions between complexes **1**−**3** and avidin, and procedure for the conjugation of QSY-7 isothiocyanate to avidin (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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