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Utilization of the Highly Environment-Sensitive Emission Properties of Rhenium(I) Amidodipyridoquinoxaline Biotin Complexes in the Development of Biological Probes

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We report the synthesis and characterization of luminescent rhenium(I) amidodipyridoquinoxaline biotin complexes $[Re(CO)_3(dpqa)(L)](PF_6)$ (dpqa = 2-(*n*-butylamido)dipyrido[3,2-*f*:2′,3′-*h*]quinoxaline; L = 4-(biotinamidomethyl)pyridine (py-4-CH_2-NH-biotin) (**1**), 3-(*N*-((2-biotinamido)ethyl)amido)pyridine (py-3-CO-NH-en-NH-biotin) (**2**), 4-(*N*-((6-biotinamido)hexanoyl)aminomethyl)pyridine (py-4-CH_2-NH-cap-NH-biotin) (**3**)), and their biotin-free counterpart $[Re(CO)_3(dpqa)(py)](PF_6)$ (py = pyridine (**4**)). Upon irradiation, these complexes exhibited intense triplet metal-to-ligand charge-transfer (^{3}MLCT) ($d\pi(Re) \rightarrow \pi^*(dpqa)$) emission in fluid solutions at 298 K and in alcohol glass at 77 K. However, the emission became much weaker in aqueous buffer, probably due to the interactions of water molecules with the amide substituent of the dpqa ligand. These properties render the complexes good candidates as luminescent probes for hydrophobic media, such as the substrate-binding sites of proteins. The avidin-binding properties of the new biotin complexes have been studied by 4′-hydroxyazobenzene-2-carboxylic acid (HABA) assays, emission titrations, and competitive association and dissociation assays. Most importantly, the complexes showed a profound increase in emission intensities upon binding to avidin. Additionally, we found that the fluorescence of anthracene was quenched by these rhenium(I) complexes, and the ^{3}MLCT emission of the complexes was also quenched by anthracene. On the basis of these findings, new homogeneous assays for biotin using these complexes, avidin, and anthracene-labeled avidin have been designed.

Introduction

The extremely high binding affinity of biotin to the glycoprotein avidin (first dissociation constant, K_d = ca. 10^{-15} M) renders this ligand-protein system a valuable tool for a wide variety of bioanalytical applications.[1−3] Biotinylated biomolecules are commonly detected and quantitated by avidin labeled with various reporters. Since avidin contains four biotin-binding sites, biotinylated biomolecules can also be recognized by biotin-reporter conjugates when avidin is used as a bridge. However, this strategy does not apply to conventional biotin-fluorophore conjugates because they lose their fluorescence upon binding to avidin due to fluorescence resonance-energy transfer, unless long spacers are present between the biotin and fluorophore units.[4]

Many rhenium(I) polypyridine complexes have been employed as ion sensors,[5−8] intercalators and photocleavage agents for nucleic acid,[9,10] reporters of rigidity,[11] anisotropic

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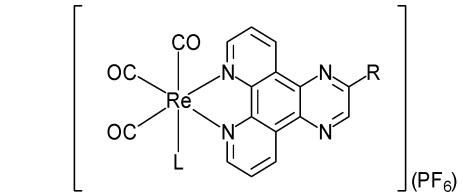
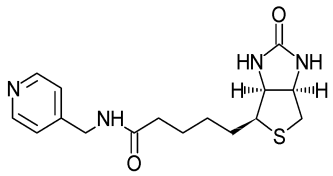
probes for proteins,[12] and luminescent labeling reagents and probes for biological molecules.[13] We have recently reported luminescent polypyridine biotin complexes of rhenium(I)[13c,e,f] and other metal centers[14] that display increased emission intensities and lifetimes upon binding to avidin. The absence of self-quenching is a result of the large Stokes' shifts of the emission of these transition metal complexes. To achieve higher detection sensitivity, our current target is to design new biotin conjugates that would show substantial luminescence amplification upon binding to avidin, or ideally speaking, "light switches" for this protein. One of the strategies is to identify systems that show very weak emission in aqueous solution but intense luminescence in more hydrophobic media, such as the substrate-binding sites of proteins. In view of the interesting DNA-induced emission of a ruthenium(II) amidodipyrido[3,2-*f*:2′,3′-*h*]quinoxaline (amido-dpq) complex reported by Kelly and co-workers,[15] we anticipate that related rhenium(I) amidodipyridoquinoxaline complexes are very promising candidates as sensitive biological probes.

Here we report the synthesis and characterization of luminescent rhenium(I) amidodipyridoquinoxaline biotin complexes $[Re(CO)_3(dpqa)(L)](PF_6)$ (dpqa = 2-(*n*-butylamido)dipyrido[3,2-*f*:2′,3′-*h*]quinoxaline; L = 4-(biotinamidomethyl)pyridine (py-4-CH_2-NH-biotin) (**1**), 3-(*N*-((2-biotinamido)ethyl)amido)pyridine (py-3-CO-NH-en-NH-biotin) (**2**), 4-(*N*-((6-biotinamido)hexanoyl)aminomethyl)-pyridine (py-4-CH_2-NH-cap-NH-biotin) (**3**)) and their biotin-free counterpart $[Re(CO)_3(dpqa)(py)](PF_6)$ (py = pyridine (**4**)). The structures of these complexes and their dpq (dipyrido[3,2-*f*:2′,3′-*h*]quinoxaline) analogues **1a**−**4a**[13f] are shown in Chart 1. The photophysical and electrochemical properties of the dpqa complexes have been studied. The avidin-binding properties of the biotin complexes **1**−**3** have been examined by 4′-hydroxyazobenzene-2-carboxylic acid (HABA) assays, emission titrations, and competitive association and dissociation assays. Additionally, we have designed new homogeneous assays for biotin using these complexes, avidin, and avidin molecules that had been labeled with anthracene.

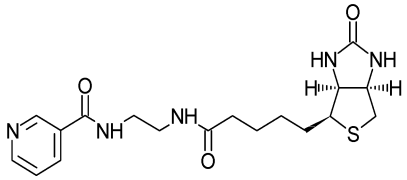
Chart 1. Structures of Complexes **1**−**4** and **1a**−**4a**



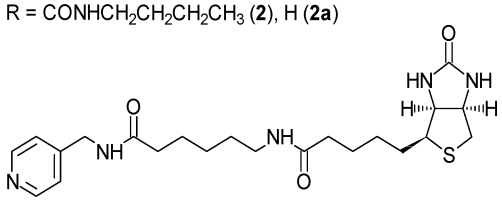
L =



py-4-CH_2-NH-biotin
R = $CONHCH_2CH_2CH_2CH_3$ (**1**), H (**1a**)



py-3-CO-NH-en-NH-biotin
R = $CONHCH_2CH_2CH_2CH_3$ (**2**), H (**2a**)



py-4-CH_2-NH-cap-NH-biotin
R = $CONHCH_2CH_2CH_2CH_3$ (**3**), H (**3a**)

py
R = $CONHCH_2CH_2CH_2CH_3$ (**4**), H (**4a**)

Experimental Section

Materials and Synthesis. All solvents were of analytical reagent grade. $Re(CO)_5Br$ (Aldrich), 6-aminocaproic acid (Acros), *N*-hydroxysuccinimide (Acros), *N*,*N*′-dicyclohexylcarbodiimide (Acros), KPF_6 (Acros), biotin (Acros), and HABA (Sigma) were used as received. 4-(Aminomethyl)pyridine (Acros) was purified by distillation under nitrogen. All buffer components were of molecular biology grade. PD-10 columns and YM-30 centricons were purchased from Pharmacia and Amicon, respectively. 2-Methoxycarbonyldipyrido[3,2-*f*:2′,3′-*h*]quinoxaline,[16] py-4-CH_2-NH-biotin,[13c,e,f] py-3-CO-NH-en-NH-biotin,[13f] and py-4-CH_2-NH-cap-NH-biotin[13e,f] were synthesized according to reported procedures.

Dpqa. A mixture of 2-methoxycarbonyldipyrido[3,2-*f*:2′,3′-*h*]-quinoxaline (216 mg, 0.74 mmol) and *n*-butylamine (3.7 mL, 37 mmol) in 20 mL of CH_2Cl_2 was refluxed under nitrogen for 12 h. The mixture was evaporated to dryness to give a pale yellow solid. The solid was then dissolved in $CHCl_3$, and the solution was washed with H_2O, dried over anhydrous magnesium sulfate, and evaporated to dryness. Recrystallization of the crude product from CH_2Cl_2/diethyl ether afforded dpqa as pale yellow crystals. Yield: 177 mg

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(72%). ^{1}H NMR (300 MHz, acetone-d_6, 298 K, TMS): δ 9.72−9.69 (m, 2 H, H4′ of pyrido ring and H3 of quinoxaline ring of dpqa), 9.54 (d, 1 H, J = 8.2 Hz, H4 of pyrido ring), 9.27 (d, 1 H, J = 4.1 Hz, H6′ of pyrido ring), 9.24 (d, 1 H, J = 4.4 Hz, H6 of pyrido ring), 9.08 (s, 1 H, CON*H*), 7.95 (dd, 1 H, J = 7.9 and 4.0 Hz, H5′ of pyrido ring), 7.90 (dd, 1 H, J = 8.5 and 4.8 Hz, H5 of pyrido ring), 3.62−3.55 (m, 2 H, NHC*H*$_2$(CH$_2$)$_2$CH$_3$), 1.72 (pent, 2 H, J = 7.3 Hz, NHCH$_2$C*H*$_2$CH$_2$CH$_3$), 1.48 (sext, 2 H, J = 7.3 Hz, NHCH$_2$CH$_2$C*H*$_2$CH$_3$), 0.99 (t, 3 H, J = 7.3 Hz, NH(CH$_2$)$_3$C*H*$_3$). IR (KBr) ν/cm^{-1}: 3395 (br, NH), 1656 (m, C═O). Positive-ion ESI-MS ion cluster at m/z 332 {M + H$^+$}$^+$.

Re(CO)$_3$(dpqa)Br. A mixture of Re(CO)$_5$Br (162 mg, 0.40 mmol) and dpqa (132 mg, 0.40 mmol) in 20 mL of benzene was refluxed under nitrogen for 4 h. The mixture was evaporated to dryness to obtain a yellow solid. Recrystallization of the crude product from CH$_2$Cl$_2$/diethyl ether afforded Re(CO)$_3$(dpqa)Br as yellow crystals. Yield: 256 mg (95%). ^{1}H NMR (300 MHz, acetone-d_6, 298 K, TMS): δ 10.06 (d, 1 H, J = 8.2 Hz, H4′ of pyrido ring of dpqa), 9.88−9.85 (m, 2 H, H4 of pyrido ring of dpqa and H3 of quinoxaline ring of dpqa), 9.68−9.62 (m, 2 H, H6 and H6′of pyrido rings of dpqa), 9.16 (s, 1 H, CON*H* of dpqa), 8.38−8.28 (m, 2 H, H5 and H5′ of pyrido rings of dpqa), 3.61−3.54 (m, 2 H, NHC*H*$_2$(CH$_2$)$_2$CH$_3$), 1.74−1.69 (m, 2 H, NHCH$_2$-(C*H*$_2$)CH$_2$CH$_3$), 1.51−1.43 (m, 2 H, NH(CH$_2$)$_2$C*H*$_2$CH$_3$), 0.98 (t, 3 H, J = 7.3 Hz, NH(CH$_2$)$_3$C*H*$_3$). IR (KBr) ν/cm^{-1}: 3416 (br, NH), 2960 (w, CH), 2023 (s, C≡O), 1915 (s, C≡O), 1885 (s, C≡O), 1664 (m, C═O). Positive-ion ESI-MS ion cluster m/z at 682 {M + H}$^+$, 602 {M − Br$^-$}$^+$.

[Re(CO)$_3$(dpqa)(CH$_3$CN)](CF$_3$SO$_3$). To a suspension of Re(CO)$_3$(dpqa)Br (269 mg, 0.40 mmol) in 200 mL of CH$_3$CN was added AgCF$_3$SO$_3$ (102 mg, 0.39 mmol). The mixture was refluxed under nitrogen for 24 h in the dark. The off-white AgBr precipitate was removed by filtration using Celite. The filtrate was evaporated to dryness to give a yellow solid. Recrystallization of the crude product from CH$_2$Cl$_2$/diethyl ether afforded [Re(CO)$_3$(dpqa)(CH$_3$CN)](CF$_3$SO$_3$) as yellow crystals. Yield: 239 mg (75%). ^{1}H NMR (300 MHz, acetone-d_6, 298 K, TMS): δ 10.26 (d, 1 H, J = 8.2 Hz, H4′ of pyrido ring of dpqa), 9.97 (d, 1 H, J = 8.5 Hz, H4 of pyrido ring of dpqa), 9.86 (s, 1 H, H3 of quinoxaline ring of dpqa), 9.79−9.74 (m, 2 H, H6 and H6′ of pyrido rings of dpqa), 9.25 (s, 1 H, CON*H* of dpqa), 8.50−8.41 (m, 2 H, H5 and H5′ of pyrido rings of dpqa), 3.60−3.54 (m, 2 H, NHC*H*$_2$(CH$_2$)$_2$CH$_3$), 1.76−1.69 (m, 2 H, NHCH$_2$C*H*$_2$CH$_2$CH$_3$), 1.50−1.42 (m, 2 H, NH-(CH$_2$)$_2$C*H*$_2$CH$_3$), 1.00−0.95 (m, 3 H, NH(CH$_2$)$_3$C*H*$_3$). IR (KBr) ν/cm^{-1}: 3457 (br, NH), 2939 (w, CH), 2038 (s, C≡O), 1910 (s, C≡O), 1669 (m, C═O), 1157 (m, CF$_3$SO$_3^-$), 1034 (m, CF$_3$SO$_3^-$). Positive-ion ESI-MS ion cluster at m/z 643 {M − CF$_3$SO$_3$}$^+$.

[Re(CO)$_3$(dpqa)(py-4-CH$_2$-NH-biotin)](PF$_6$) (1). A mixture of [Re(CO)$_3$(dpqa)(CH$_3$CN)](CF$_3$SO$_3$) (129 mg, 0.16 mmol) and py-4-CH$_2$-NH-biotin (54 mg, 0.16 mmol) in 30 mL of anhydrous THF/MeOH (3:1, v/v) was refluxed under nitrogen for 12 h. The mixture was evaporated to dryness to give a yellow solid. The complex was converted to the hexafluorophosphate salt by metathesis with KPF$_6$ and then purified by column chromatography on alumina. The desired product was eluted with CH$_3$CN/MeOH (10:1, v/v). Recrystallization of the crude product from CH$_3$CN/diethyl ether afforded complex **1** as yellow crystals. Yield: 114 mg (66%). ^{1}H NMR (300 MHz, acetone-d_6, 298 K, TMS): δ 10.20 (t, 1 H, J = 8.5 Hz, H4′ of pyrido ring of dpqa), 10.02−9.94 (m, 3 H, H4, H6 and H6′ of pyrido rings of dpqa), 9.82 (s, 1 H, H3 of quinoxaline ring of dpqa), 9.31 and 9.24 (s, 1 H, CON*H* of dpqa), 8.56−8.49 (m, 4 H, H5 and H5′ of pyrido rings of dpqa and H2 and H6 of pyridine), 7.71 (s, 1 H, py-CH$_2$-N*H*), 7.25 (d, 2 H, J = 5.0 Hz, H3 and H5 of pyridine), 5.82−5.61 (m, 2 H, NH of biotin), 4.52−4.40 (m, 1 H, NCH of biotin), 4.27−4.25 (m, 3 H, NCH of biotin and py-4-C*H*$_2$-NH), 3.58−3.51 (m, 2 H, NHC*H*$_2$(CH$_2$)$_2$CH$_3$), 3.26−3.14 (m, 1 H, SCH of biotin), 1.73−1.32 (m, 10 H, NHCH$_2$(C*H*$_2$)$_2$-CH$_3$ and COCH$_2$C$_3$*H*$_6$ of biotin), 0.96 (t, 3 H, J = 7.3 Hz, NH(CH$_2$)$_3$C*H*$_3$). IR (KBr) ν/cm^{-1}: 3442 (br, NH), 2930 (m, CH), 2028 (s, C≡O), 1916 (s, C≡O), 1680 (m, C═O), 845 (s, PF$_6^-$). Positive-ion ESI-MS ion cluster at m/z 936 {M − PF$_6$}$^+$, 602 {M − PF$_6$ − py-4-CH$_2$-NH-biotin}$^+$. Anal. Calcd for C$_{38}$H$_{39}$N$_9$O$_6$SPF$_6$-Re·H$_2$O·CH$_3$CN: C, 42.14; H, 3.89; N, 12.28. Found: C, 42.27; H, 4.08; N, 12.48.

[Re(CO)$_3$(dpqa)(py-3-CO-NH-en-NH-biotin)](PF$_6$) (2). The preparation of complex **2** was similar to that of complex **1** except that py-3-CO-NH-en-NH-biotin (63 mg, 0.16 mmol) was used instead of py-4-CH$_2$-NH-biotin. Complex **2** was isolated as yellow crystals. Yield: 131 mg (72%). ^{1}H NMR (300 MHz, acetone-d_6, 298 K, TMS): δ 10.19 (d, 1 H, J = 8.2 Hz, H4′ of pyrido ring of dpqa), 10.13−10.01 (m, 2 H, H6 and H6′ of pyrido rings of dpqa), 9.97 (d, 1 H, J = 8.2 Hz, H4 of pyrido ring of dpqa), 9.82 (s, 1 H, H3 of quinoxaline ring of dpqa), 9.23 and 9.16 (s, 1 H, CON*H* of dpqa), 8.92 (s, 1 H, H2 of pyridine), 8.85−8.80 (m, 1 H, H6 of pyridine), 8.59−8.51 (m, 2 H, H5 and H5′ of pyrido rings of dpqa), 8.35−8.34 (s, 1 H, py-3-CO-N*H*), 8.24 (d, 1 H, J = 8.2 Hz, H4 of pyridine), 7.53−7.48 (m, 2 H, H5 of pyridine and en-N*H*-biotin), 5.71−5.65 (m, 2 H, NH of biotin), 4.48−4.42 (m, 1 H, NCH of biotin), 4.26−4.21 (m, 1 H, NCH of biotin), 3.58−3.50 (m, 2 H, NHC*H*$_2$(CH$_2$)$_2$CH$_3$), 3.44−3.33 (m, 4 H, C$_2$*H*$_4$-NH-biotin), 3.11−2.99 (m, 1 H, SCH of biotin), 2.63 (d, 1 H, J_{gem} = 13.4 Hz, SCH of biotin), 2.16 (t, 2 H, J = 7.3 Hz, COC*H*$_2$C$_3$H$_6$ of biotin), 1.73−1.40 (m, 10 H, NHCH$_2$(C*H*$_2$)$_2$CH$_3$ and COCH$_2$C$_3$*H*$_6$ of biotin), 0.96 (t, 3 H, J = 7.3 Hz, NH(CH$_2$)$_3$C*H*$_3$). IR (KBr) ν/cm^{-1}: 3437 (br, NH), 2935 (m, CH), 2034 (s, C≡O), 1922 (s, C≡O), 1653 (m, C═O), 843 (s, PF$_6^-$). Positive-ion ESI-MS ion cluster at m/z 993 {M − PF$_6$}$^+$, 602 {M − PF$_6$ − py-3-CO-NH-en-NH-biotin}$^+$. Anal. Calcd for C$_{40}$H$_{42}$N$_{10}$O$_7$SPF$_6$Re·H$_2$O·CH$_3$CN: C, 42.14; H, 3.96; N, 12.87. Found: C, 42.12; H, 4.19; N, 12.76.

[Re(CO)$_3$(dpqa)(py-4-CH$_2$-NH-cap-NH-biotin)](PF$_6$) (3). The preparation of complex **3** was similar to that of complex **1** except that py-4-CH$_2$-NH-cap-NH-biotin (72 mg, 0.16 mmol) was used instead of py-4-CH$_2$-NH-biotin. Complex **3** was isolated as yellow crystals. Yield: 92 mg (48%). ^{1}H NMR (300 MHz, acetone-d_6, 298 K, TMS): δ 10.20 (d, 1 H, J = 7.3 Hz, H4′ of pyrido ring of dpqa), 10.02−9.94 (m, 3 H, H4, H6 and H6′ of pyrido rings of dpqa), 9.83 (s, 1 H, H3 of quinoxaline ring of dpqa), 9.20 (s, 1 H, CON*H* of dpqa), 8.62−8.47 (m, 4 H, H5 and H5′ of pyrido rings of dpqa and H2 and H6 of pyridine), 7.74 (s, 1 H, py-CH$_2$-N*H*), 7.23 (d, 2 H, J = 5.6 Hz, H3 and H5 of pyridine), 7.08 (s, 1 H, cap-N*H*-biotin), 6.00−5.63 (m, 2 H, NH of biotin), 4.40−4.44 (m, 1 H, NCH of biotin), 4.38−4.25 (m, 3 H, NCH of biotin and py-4-C*H*$_2$-NH), 3.58−3.52 (m, 2 H, NHC*H*$_2$(CH$_2$)$_2$CH$_3$), 3.18−3.13 (m, 1 H, SCH of biotin), 2.93−2.90 (m, 2 H, C*H*$_2$-NH-biotin), 2.15−2.08 (m, 4 H, C*H*$_2$C$_4$H$_8$-NH-biotin and COC*H*$_2$C$_3$H$_6$ of biotin), 1.84−1.09 (m, 16 H, NHCH$_2$(C*H*$_2$)$_2$CH$_3$, CH$_2$C$_3$*H*$_6$CH$_2$-NH-biotin and COCH$_2$C$_3$*H*$_6$ of biotin), 0.96 (t, 3 H, J = 7.3 Hz, NH(CH$_2$)$_3$C*H*$_3$). IR (KBr) ν/cm^{-1}: 3426 (br, NH), 2930 (m, CH), 2033 (s, C≡O), 1921 (s, C≡O), 1655 (s, C═O), 840 (s, PF$_6^-$). Positive-ion ESI-MS ion cluster m/z at 1049 {M − PF$_6$}$^+$, 602 {M − PF$_6$ − py-4-CH$_2$-NH-cap-NH-biotin}$^+$. Anal. Calcd for C$_{44}$H$_{50}$-N$_{10}$O$_7$SPF$_6$Re·2H$_2$O: C, 42.96; H, 4.42; N, 11.39. Found: C, 42.73; H, 4.70; N, 11.55.

[Re(CO)$_3$(dpqa)(py)](PF$_6$) (4). The preparation of complex **4** was similar to that of complex **1** except that pyridine (13 mL, 0.16 mmol) was used instead of py-4-CH$_2$-NH-biotin. Complex **4** was

isolated as yellow crystals. Yield: 106 mg (80%). ^{1}H NMR (300 MHz, acetone-d_6, 298 K, TMS): δ 10.23 (d, 1 H, J = 8.5 Hz, H4′ of pyrido ring of dpqa), 10.05−9.95 (m, 3 H, H4, H6 and H6′ of pyrido rings of dpqa), 9.81 (s, 1 H, H3 of quinoxaline ring of dpqa), 9.17 (s, 1 H, CON*H* of dpqa), 8.67 (m, 2 H, H5 and H5′ of pyrido rings of dpqa), 8.56−8.48 (m, 2 H, H2 and H6 of pyridine), 7.91 (t, 1 H, J = 7.6 Hz, H4 of pyridine), 7.40 (t, 2 H, J = 6.7 Hz, H3 and H5 of pyridine), 3.58−3.51 (m, 2 H, NHCH_2(CH$_2$)$_2$CH$_3$), 1.73−1.64 (m, 2 H, NHCH$_2$CH_2CH$_2$CH$_3$), 1.50−1.38 (m, 2 H, NHCH$_2$-CH$_2$CH_2CH$_3$), 0.96 (t, 3 H, J = 7.3 Hz, NH(CH$_2$)$_3$CH_3). IR (KBr) ν/cm^{-1}: 3396 (br, NH), 2035 (s, C≡O), 1918 (s, C≡O), 1679 (m, C═O), 842 (s, PF_6^-). Positive-ion ESI-MS ion cluster at m/z 681 $\{M - PF_6\}^+$, 602 $\{M - PF_6 - py\}^+$. Anal. Calcd for $C_{27}H_{22}N_6O_4PF_6Re\cdot 0.5CH_3CN$: C, 39.74; H, 2.80; N, 10.76. Found: C, 39.59; H, 3.10; N, 10.58.

Coupling of 9-Anthraldehyde to Avidin. 9-Anthraldehyde (0.62 mg, 3.49 μmol) in anhydrous DMSO (160 μL) was added to avidin (12 mg) in 1.5 mL of 50 mM carbonate buffer pH 9.4. After the mixture was stirred slowly in the dark at room temperature for 4 h, 10 μL of 5 M $NaCNBH_3$ in 1 M aqueous NaOH solution was added. The mixture was stirred for another 12 h in the dark at room temperature. After the solid residue was removed by centrifugation, the solution was loaded onto a PD-10 column that had been equilibrated with 50 mM Tris-Cl buffer pH 7.4. The first band that came out from the column with intense blue fluorescence was collected. The solution was then concentrated with a YM-30 centricon, and the conjugate An-Av was washed with Tris-Cl buffer successively, and finally purified by size-exclusion HPLC to remove any free labels.

Emission Titrations. Aliquots (5 μL) of the rhenium(I) biotin complex in DMSO (0.55 mM) were added to a solution of avidin or An-Av (protein concentration = 3.8 μM) in 2 mL of 50 mM potassium phosphate buffer pH 7.4 at 1-min intervals. The emission intensities of the solutions were measured upon excitation at 350 nm.

Homogeneous Competitive Assay for Biotin. Avidin (or An-Av) was added to a mixture of unmodified biotin analyte and the rhenium(I) biotin complex in 50 mM potassium phosphate buffer pH 7.4/DMSO (9:1, v/v). The concentrations of the rhenium(I) biotin complex and avidin (or An-Av) were 4.6 and 1.2 μM, respectively, while that of biotin varied from 1×10^{-3} to 1×10^{-9} M. The assay mixtures were incubated at room temperature for 1 h, and the emission intensities of the solutions were then measured.

Determination of k_{on}, k_{off}, and K_d. The first dissociation constants K_d of the avidin-adducts of rhenium(I) biotin complexes were defined as k_{off}/k_{on} where k_{on} was the on-rate constant for the binding of the fourth complex to Re_3-avidin; and k_{off} was the off-rate constant for the departure of the first complex from the adduct Re_4-avidin.[4b] For the determination of k_{on}, 200 μL of avidin (ca. 25 μM) in 50 mM potassium phosphate buffer pH 7.4 at 298 K was added to 2 mL of the rhenium(I) biotin complex solution (ca. 9 μM) in a mixture of potassium phosphate buffer and DMSO (49:1, v/v). The rate of increase of emission intensity was measured. For the determination of k_{off}, the dissociation of avidin-bound rhenium(I) biotin complex was induced by addition of a 100-fold excess of unmodified biotin to a solution of the adduct Re_4-avidin (ca. 2.3 μM with respect to avidin). The rate of decrease of emission intensity was measured.

HABA Assays and Competitive Association and Dissociation Assay. Details of these assays have been described previously.[13f]

Physical Measurements and Instrumentation. Equipment for characterization and photophysical and electrochemical studies has been described previously.[13e] Luminescence quantum yields of the complexes were measured by the optically dilute method[17] using an aerated aqueous solution of $[Ru(bpy)_3]Cl_2$ (Φ = 0.028)[18] as the standard solution.

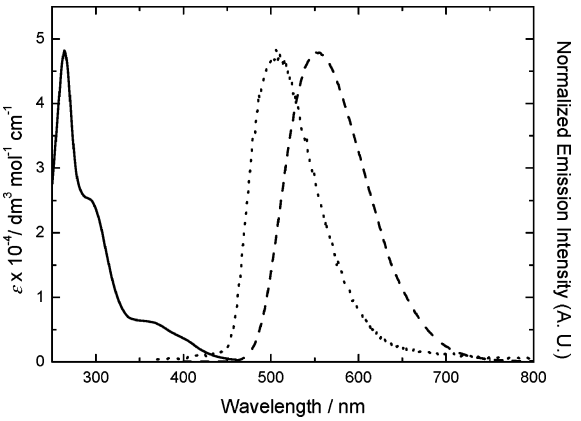

Figure 1. Electronic absorption (—) and emission spectra of complex **1** in CH_2Cl_2 at 298 K (---) and in EtOH/MeOH (4:1, v/v) at 77 K (···).

Table 1. Electronic Absorption Spectral Data for Complexes **1**−**4** at 298 K

complex	medium	λ_{abs}/nm (ϵ/dm^3 mol^{-1} cm^{-1})
1	CH_2Cl_2	264 (41685), 297 sh (21285), 367 sh (5140), 406 sh (2620)
	CH_3CN	263 (48080), 304 sh (20765), 362 sh (5935), 395 sh (2825)
2	CH_2Cl_2	263 (47655), 303 sh (22255), 367 sh (5805), 402 sh (3160)
	CH_3CN	263 (48185), 303 sh (22060), 363 sh (6065), 394 sh (3010)
3	CH_2Cl_2	265 (44295), 298 sh (22150), 368 sh (5320), 402 sh (3090)
	CH_3CN	263 (45470), 300 sh (21450), 363 sh (5610), 394 sh (2815)
4	CH_2Cl_2	264 (48195), 306 sh (20430), 371 sh (5740), 406 sh (3000)
	CH_3CN	263 (42790), 299 sh (20230), 357 sh (5675), 391 sh (2790)

Results and Discussion

Synthesis. The luminescent rhenium(I) amidodipyridoquinoxaline complexes **1**−**4** were prepared in moderate yields from the reactions of $[Re(CO)_3(dpqa)(CH_3CN)](CF_3SO_3)$ and the ligands py-4-CH$_2$-NH-biotin, py-3-CO-NH-en-NH-biotin, py-4-CH$_2$-NH-cap-NH-biotin, or pyridine in THF/MeOH, followed by metathesis with KPF_6 and purification by column chromatography on alumina. All the complexes were characterized by ^{1}H NMR, positive-ion ESI-MS, and IR and gave satisfactory elemental analyses. The complexes were soluble in common organic solvents such as alcohols, acetone, and chlorinated solvents, but sparingly soluble in water.

Electronic Absorption and Emission Properties. The electronic absorption spectral data of complexes **1**−**4** are summarized in Table 1. The electronic absorption spectrum of complex **1** in CH_2Cl_2 at 298 K is shown in Figure 1. The intense absorption bands of complex **1** in CH_2Cl_2 and CH_3CN at ca. 263−304 nm with extinction coefficients on the order of 10^4 dm^3 mol^{-1} cm^{-1} are assigned to spin-allowed intraligand (^{1}IL) ($\pi \rightarrow \pi^*$) (dpqa and pyridine ligands) transitions. Additionally, complex **1** displayed moderately intense absorption shoulders and bands at ca. 362−406 nm, which are assigned to spin-allowed metal-to-ligand charge-transfer (^{1}MLCT) (dπ(Re) $\rightarrow$ π^*(dpqa)) transitions.[5,6b,7b,8−10,12a,13,19−27] Since biotin and the spacer-arms do

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Table 2. Photophysical Data for Complexes **1**−**4** and **1a**−**4a**

complex	medium (T/K)	λ_{em}/nm	τ_o/μs	Φ
1	CH_2Cl_2 (298)	553	0.92	0.19
	CH_3CN (298)	573	0.34	0.049
	buffer[a] (298)	574	0.23	0.0021
	glass[b] (77)	506	6.06	
2	CH_2Cl_2 (298)	552	1.03	0.12
	CH_3CN (298)	568	0.47	0.038
	buffer[a] (298)	572	0.31	0.0030
	glass[b] (77)	502	5.57	
3	CH_2Cl_2 (298)	550	0.86	0.22
	CH_3CN (298)	573	0.32	0.037
	buffer[a] (298)	577	0.24	0.0023
	glass[b] (77)	505	5.90	
4	CH_2Cl_2 (298)	552	1.05	0.20
	CH_3CN (298)	573	0.37	0.065
	buffer[a] (298)	571	0.25	0.0049
	glass[b] (77)	504	5.72	
1a[c]	CH_2Cl_2 (298)	548	1.12	0.28
	CH_3CN (298)	568	0.40	0.086
	buffer[a] (298)	572	0.11	0.034
	glass[b] (77)	506	6.67	
2a[c]	CH_2Cl_2 (298)	544	1.24	0.15
	CH_3CN (298)	562	0.52	0.065
	buffer[a] (298)	566	0.21	0.033
	glass[b] (77)	504	6.18	
3a[c]	CH_2Cl_2 (298)	548	1.10	0.24
	CH_3CN (298)	568	0.40	0.054
	buffer[a] (298)	572	0.13	0.032
	glass[b] (77)	504	6.45	
4a	CH_2Cl_2 (298)	549	1.00	0.34
	CH_3CN (298)	565	0.45	0.057
	buffer[a] (298)	574	0.12	0.034
	glass[b] (77)	506	6.53	

[a] 50 mM potassium phosphate pH 7.4 containing 2.5% DMSO (DMSO was used for solubility reasons). [b] EtOH/MeOH (4:1, v/v). [c] From ref 13f.

not exhibit significant absorption, the absorption characteristics of complexes **1**−**3** are very similar to those of complex **4** (Table 1).

Excitation of complexes **1**−**4** in fluid solutions at 298 K and in alcohol glass at 77 K resulted in yellow to green luminescence. The photophysical data of complexes **1**−**4** and their dpq analogues **1a**−**4a**[13f] are summarized in Table 2. The emission spectra of complex **1** in CH_2Cl_2 at 298 K and in alcohol glass at 77 K are shown in Figure 1. The emission spectra of complexes **1**−**4** exhibited a broad band at ca. 550−553 nm in CH_2Cl_2, 568−573 nm in CH_3CN, and 571−577 nm in buffer solution at 298 K. These solvent-dependent emission bands are attributed to an ^{3}MLCT (dπ(Re) → π^*-(dpqa)) excited state.[5,6,7b,8,9,12a,13a−d,f,g,19−23,24b,25−27] Again, the emission properties of complexes **1**−**3** are very similar to those of complex **4**, indicating that the biotin moiety and the spacer-arms do not have significant effects on the emission characteristics of complexes **1**−**3**. The emission of complexes **1**−**4** occurs at slightly lower energy than that of their dpq analogues **1a**−**4a**[13f] (Table 2). These observations are accounted for by the electron-withdrawing amide substituent of the dpqa ligand. This substituent stabilizes the empty π^* orbitals of the diimine ligand and thus lowers the ^{3}MLCT transition energy of the complexes.

Table 3. Electrochemical Data for Complexes **1**−**4**[a]

complex	oxidation, $E_{1/2}$ or E_a/V	reduction, $E_{1/2}$ or E_c/V
1	+1.77[b]	−0.97, −1.52, −1.78,[b] −1.96,[c] −2.21[b]
2	+1.74[c]	−0.96, −1.53,[c] −1.72,[b] −1.90,[c] −2.13[c]
3	+1.80[b]	−0.99, −1.51,[c] −1.77,[c] −1.95,[c] −2.21[b]
4	+1.76[c]	−1.00, −1.58, −1.77,[b] −1.96,[c] −2.21[b]

[a] In CH_3CN (0.1 mol dm^{-3} ^{n}Bu$_4$NPF$_6$) at 298 K, glassy carbon electrode, sweep rate 100 mV s^{-1}, all potentials versus SCE. [b] Irreversible waves. [c] Quasireversible waves.

It is important to note that while complexes **1**−**4** in relatively nonpolar CH_2Cl_2 show emission quantum yields that are comparable to those of their dpq counterparts (Table 2) and other rhenium(I) polypyridine complexes,[7b,8,9b,12a,13a,b,f,g,21b,25,26b,27] their quantum yields in more polar aqueous buffer are extremely small (ca. 10^{-3}) (Table 2). Specifically, all the complexes undergo very significant reduction in emission quantum yields upon changing the solvent from CH_2Cl_2 to aqueous buffer (Φ_{buffer}:$\Phi_{CH_2Cl_2}$ = 1.8%). The corresponding changes for complexes **1a**−**4a** are milder (Φ_{buffer}:$\Phi_{CH_2Cl_2}$ = 14.4%). We ascribe the significant decrease in emission quantum yields for complexes **1**−**4** to both the higher solvent polarity and hydrogen bond interactions between the amide group of the dpqa ligand and water.[15] Such a large difference in the emission properties of these rhenium(I) dpqa complexes in different media implies that they could serve as sensitive probes for the hydrophobicity of their local surroundings. In rigid glass at 77 K, these complexes expectedly emitted at higher energy (ca. 502−506 nm) (Table 2) due to strong rigidochromism.[19,22,23,25]

Electrochemical Properties. The electrochemical properties of complexes **1**−**4** have been studied by cyclic voltammetry. The electrochemical data are listed in Table 3. The cyclic voltammograms of all the complexes displayed a quasireversible/irreversible rhenium(II/I) oxidation wave at a potential between ca. +1.7 and +1.8 V versus SCE.[8,13d,e,g,20a,21,23,24,25a,26a,27] The first reversible reduction couples at ca. −1 V for all the complexes are assigned to reduction of the dpqa ligand. In addition, complexes **1**−**4** exhibited quasireversible/irreversible waves at more negative potentials, which are tentatively assigned to reduction of the dpqa ligand, except for those at ca. −1.5 V that could be associated with the rhenium(I/0) couple.[24a,27b]

Avidin-Binding Studies. The avidin-binding properties of complexes **1**−**3** have been investigated using the standard

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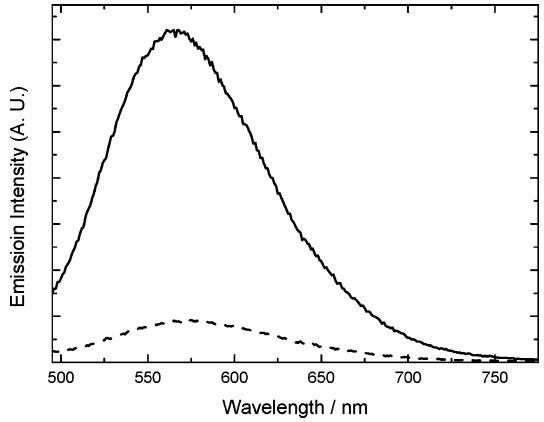

Figure 2. Emission spectra of complex **1** (17.3 μM) in the presence of 0 μM (---) and 3.8 μM (—) of avidin in 50 mM potassium phosphate buffer pH 7.4/DMSO (97:3, v/v) at 298 K.

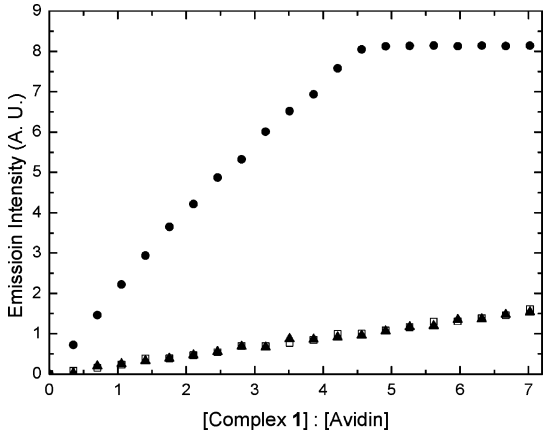

Figure 3. Luminescence titration curves for the titrations of (i) 3.8 μM avidin (●), (ii) 3.8 μM avidin and 380 μM unmodified biotin (▲), and (iii) a blank phosphate buffer solution (□) with complex **1**.

HABA assay.[2,3] Binding of HABA to avidin results in an increase in the absorbance at 500 nm, owing to the formation of the quinone tautomer of HABA at the biotin-binding sites of the protein. Additions of complexes **1**−**3**, respectively, into a mixture of HABA and avidin led to a decrease of absorbance at 500 nm, indicating that the bound HABA molecules were displaced by the complexes. In contrast, complex **4** did not give similar observations, indicating the lack of protein-binding. The plots of $-\Delta A_{500\ nm}$ versus [Re]:[avidin] for complexes **1**−**3** showed that the equivalence points occurred at [Re]:[avidin] = from ca. 4.3 to 4.7. Assuming that avidin can only specifically bind the complexes at the four biotin-binding sites, the occurrence of equivalence points at [Re]:[avidin] > 4 reveals that the binding of these rhenium(I) biotin complexes to avidin is not substantially stronger than that of HABA. To gain further insights into the avidin-binding properties of the biotin complexes **1**−**3**, luminescence titrations[4a−c] and competitive assays[28] have been performed.

Similar to other luminescent transition metal polypyridine biotin complexes,[13c,e,f,14] complexes **1**−**3** displayed enhanced emission in the presence of avidin. Figure 2 shows the emission spectra of complex **1** in the absence and presence of avidin. Emission titrations of avidin using complexes **1**−**4** have also been carried out, and the emission titration curves for complex **1** are shown in Figure 3. The equivalence points of the titrations occurred at ca. 4.6, 4.4, and 4.1 for complexes **1**, **2**, and **3**, respectively. At the equivalence points, complexes **1**−**3** revealed a ca. 8.05−3.05-fold increase in emission intensities and a ca. 1.79−1.25-fold extension in emission lifetimes (Table 4).[29] These changes are ascribed to the binding of the biotin moieties of the complexes to avidin because (i) no changes were observed when the avidin solution was presaturated with unmodified biotin (Figure 3 and Table 4) and (ii) the biotin-free complex **4** did not display similar changes. We suppose that the increase in emission intensities and emission lifetimes is a result of the enhanced hydrophobicity and rigidity of the local surroundings of the rhenium(I) biotin complexes. It is interesting to note that the emission enhancement factors are related to the chain lengths of the spacer-arms between the luminophore and biotin moieties. Specifically, complex **1** showed an 8.05-fold increment while complex **3** displayed a smaller enhancement factor of 3.05 (Table 4). We reason that the C-6 aminocaproic acid spacer-arm of complex **3** renders the luminophore to be more exposed to the polar bulk solution even after the biotin moiety binds to the protein. Also, the increase of rigidity for this complex is lower as a result of its longer and more flexible spacer-arm. Importantly, it should be emphasized that the emission enhancement factors of complexes **1**−**3** are significantly large (ca. 8.05−3.05) compared to other luminescent biotin-transition metal complexes.[13c,f,14] In particular, the rhenium(I) dpq biotin complexes **1a**−**3a** only showed enhancement factors of ca. 1.75−1.15.[13f] These findings are apparently the consequence of the very low emission intensities of the free rhenium(I) dpqa biotin complexes in aqueous solution (Table 2).

Table 4. Emission Data for Complexes **1**−**4** in Aerated 50 mM Potassium Phosphate Buffer pH 7.4 at 298 K

complex[a]	[avidin] = 0 μM [biotin] = 0 μM I (τ/μs)	[avidin] = 3.8 μM [biotin] = 0 μM I (τ/μs)	[avidin] = 3.8 μM [biotin] = 380 μM I (τ/μs)
1	1.00 (0.19)	8.05 (0.34)	0.96 (0.21)
2	1.00 (0.19)	7.86 (0.30)	1.00 (0.19)
3	1.00 (0.20)	3.05 (0.25)	1.09 (0.19)
4	1.00 (0.21)	1.00 (0.21)	1.09 (0.21)

[a] [**1**] = 17.5 μM, [**2**] = 16.7 μM, [**3**] = 15.5 μM, [**4**] = 15.2 μM.

The first dissociation constants K_d of the rhenium-avidin adducts have been estimated from the on-rates and off-rates of the rhenium-avidin adducts from kinetic experiments (Table 5).[4b] The K_d values range from ca. 1.6×10^{-9} to 6.7×10^{-8} M, which are about 6−7 orders of magnitude larger than that of the native biotin−avidin system (K_d = ca. 10^{-15}

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(29) In degassed solutions, the avidin-induced emission enhancement factors for complexes **1**−**3** were 12.21, 9.92, and 4.29, respectively, while the emission lifetimes were increased to 0.55, 0.61, and 0.27 μs, respectively. On the basis of the photophysical data in Table 2 and the enhancement factors, the radiative decay rate constants of the free complexes vary from 9.1×10^3 to 9.6×10^3 s^{-1}, while those of the avidin-bound complexes range from 3.7×10^4 to 4.9×10^4 s^{-1}. The difference of increase in emission intensities and lifetimes of the complexes upon binding to avidin is associated with the increase in the radiative decay rate constants.

Table 5. First Dissociation Constants for the Rhenium−Avidin Adducts in 50 mM Potassium Phosphate Buffer pH 7.4 at 298 K and Results of Competitive Association and Dissociation Assays

complex	K_d/M	association assay/ % avidin bound	dissociation assay/ % avidin bound
1	6.7×10^{-8}	36	45
2	1.2×10^{-9}	55	85
3	1.6×10^{-9}	68	83

M).[1−3] The K_d value for complex **1** is 1 order of magnitude larger than those for complexes **2** and **3**, revealing that a longer spacer-arm can alleviate the steric hindrance between the rhenium(I) dpqa luminophore and the protein, and thus give stronger binding.

Competitive Association and Dissociation Assays. Competitive binding of complexes **1**−**3** and native biotin to avidin has been investigated by association and dissociation assays. In the association assays, complexes **1**−**3** and native biotin competed for the binding sites of avidin. In the dissociation assays, the complexes bound to avidin were challenged by native biotin. The percentage of the rhenium-avidin adducts formation was determined by emission intensity measurements with reference to the control experiments in which native biotin was absent. The results of the assays showed that ca. 36−85% complexes remained associated with avidin molecules in the presence of native biotin (Table 5). The rhenium-bound avidin percentages of complexes **2** and **3** are higher than that of complex **1**, which is in line with the smaller K_d values and smaller off-rate constants for the avidin-adducts of complexes **2** and **3**. It should be noted that the complex-bound avidin values for each of the complexes should be similar in the association and dissociation assays. The observed differences (Table 5) are due to the fact that equilibrium conditions have not been established in the assays, and thus the results have been affected by kinetic factors. However, these data are still useful in comparing the avidin-binding properties of the three complexes.

Emission Titrations Involving Anthracene-Conjugated Avidin. We have previously studied the avidin-binding properties of other luminescent biotin-transition metal complexes in different emission quenching experiments.[13c,f,14b,c] For example, a rhenium(I) and an iridium(III) biotin complex exhibit resonance-energy transfer (RET) emission quenching when they bind to avidin molecules that have been modified by the nonfluorescent energy absorbers QSY-7[13c] and Malachite Green,[14c] respectively. In other studies, the negatively charged poly(D-glutamate:D-lysine)(6:4)-QSY-7 conjugate and electron-acceptor methyl viologen have been used to quench the emission of free rhenium(I)[13f] and ruthenium(II)[14b] polypyridine biotin complexes, respectively, to achieve higher avidin-induced emission intensity enhancement factors (I/I_0). In the current work, we employ anthracene to study the avidin-binding behavior of the rhenium(I) biotin complexes. The use of anthracene is interesting because when it is in close proximity to a luminescent rhenium(I) polypyridine complex, it quenches the emission of the complex via the exchange mechanism.[30] Meanwhile, its own fluorescence is also quenched by the rhenium(I) complex by RET.[30] Schanze and co-workers have exploited these interquenching properties and designed a series of interesting rhenium(I)-spacer-anthracene complexes as probes for double-stranded DNA molecules.[9a,c] In aqueous solution, the rhenium(I) diimine unit is close to the hydrophobic anthracene pendant, and its ^{3}MLCT emission is thus quenched. In the presence of double-stranded DNA molecules, the anthracene unit intercalates strongly into the base-pairs of the duplex and is no longer close to the rhenium(I) luminophore, leading to the appearance of typical ^{3}MLCT emission. The reason for using anthracene in the current work is that its characteristic fluorescence is very intense, which is useful for detection purposes. Other advantages are that its fluorescence properties can complement our rhenium(I) biotin probes because anthracene emits at much higher energy with a characteristic structured band, and that emission quenching of the biological host is observed instead of emission enhancement of the probes.

In the current study, we have labeled avidin with 9-anthraldehyde by reductive amination to yield a fluorescent conjugate. From the spectral data, the anthracene-to-avidin ratio of the conjugate An-Av was determined to be ca. 4.3. The absorption band of the conjugated protein at 280 nm originates from both the protein and the label, while those at 336, 351, 370, and 389 nm are the characteristic absorption features of the organic molecule. In 50 mM potassium phosphate buffer pH 7.4, the conjugate exhibited vibronically structured emission bands at 420, 440, and 477 (sh) nm, typical of the fluorescence of anthracene. The conjugate An-Av was titrated with complexes **1**−**4**, respectively, and the emission of the titration mixtures at ca. 565 nm was monitored. The titrations were compared to two control experiments in which either An-Av was absent or An-Av was blocked with excess biotin molecules. Our results showed that complexes **1**−**3** still displayed small emission enhancement and lifetime extension upon binding to the quencher-modified conjugate An-Av. However, owing to the very intense fluorescence of anthracene, the ^{3}MLCT emission intensity enhancement of the complexes at ca. 565 nm could not be determined accurately by steady-state emission studies. Emission lifetime measurements using a nanosecond Nd:YAG laser source showed that the emission lifetimes of these complexes at ca. 565 nm increased from ca. 0.19 to 0.28 μs in the presence of An-Av.[31] Similar to the case of unmodified avidin, these observations are attributable to the binding of the complexes to An-Av. However, this increase is less substantial compared to the titrations of native avidin with the complexes (Table 4), indicative of emission quenching of the complexes by the anthracene molecules.

It is interesting to note that the fluorescence of An-Av at 420, 440, and 477 (sh) nm was also quenched by the rhenium(I) biotin complexes. The emission spectra of anthracene-modified avidin in the absence and presence of complex **1** are shown in Figure 4. The results of the titrations of An-Av with the same complex in the absence and presence

(30) Schanze, K. S.; MacQueen, D. B.; Perkins, T. A.; Cabana, L. A. *Coord. Chem. Rev.* **1993**, *122*, 63.

(31) Time-resolved measurements showed that the emission intensities of the complexes at ca. 565 nm were enhanced by ca. 1.2−4.8-fold.

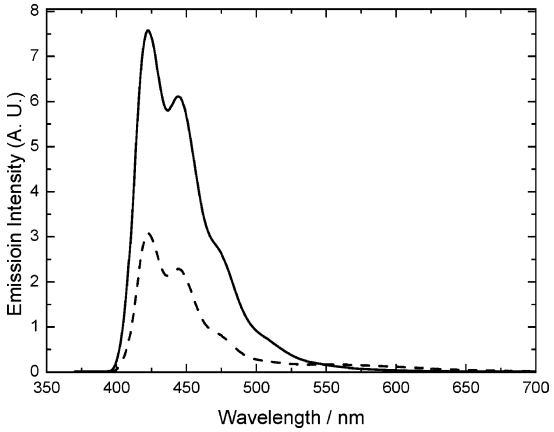

Figure 4. Emission spectra of An-Av (3.8 μM) in the presence of 0 (—) and 17.3 (---) μM of complex **1** in 50 mM potassium phosphate buffer pH 7.4/DMSO (97:3, v/v) at 298 K.

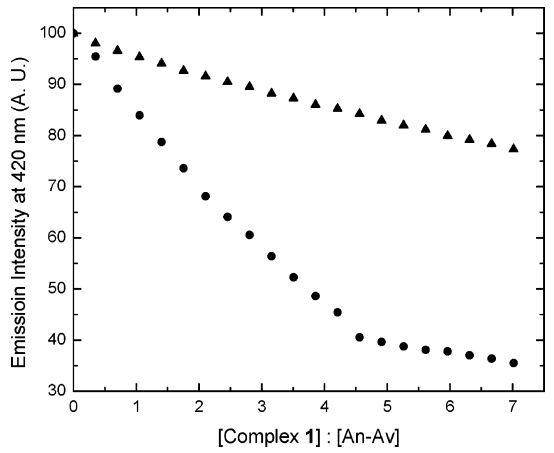

Figure 5. Luminescence titration curves for the titrations of (i) 3.8 μM An-Av (●), and (ii) 3.8 μM An-Av and 380 μM unmodified biotin (▲) with complex **1**. The emission intensities of the solution at 420 nm were monitored.

of excess biotin are displayed in Figure 5. At the equivalence points (at [Re]:[An-Av] = ca. 4.1−4.6), complexes **1**, **2**, and **3** reduced the fluorescence intensities of An-Av at 420 nm by ca. 60%, 53%, and 55%, respectively. However, we noted that An-Av also exhibited fluorescence quenching in the control experiments in which (i) the An-Av was blocked with excess biotin molecules (Figure 5), and (ii) the biotin-free complex **4** was used as the titrant (Figure S8). The reason is that the added rhenium complexes also absorb at the excitation wavelength (350 nm), thus leading to decreasing excitation power for the anthracene moiety. In these controls, no equivalence points were observed (Figures 5 and S8), and the fluorescence was lowered by only ca. 14% at [Re]:[An-Av] = 4:1. The higher extents of quenching of An-Av by complexes **1**−**3** indicate that the quenching was a consequence of the specific binding of the complexes to An-Av. In view of the overlap between the fluorescence spectrum of An-Av and the absorption spectra of rhenium(I) complexes, the fluorescence quenching of anthracene is likely to occur via the distance-dependent RET mechanism.[9a,c] It is reasonable to assume that the reduced fluorescence of An-Av in the control experiments might also be due to the nonspecific hydrophobic interactions between the complexes and An-Av.

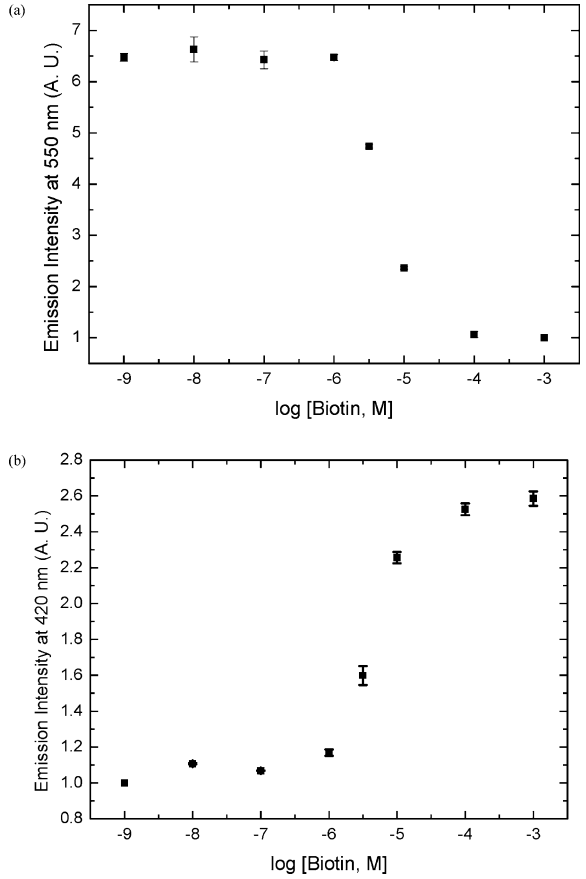

Figure 6. Results of homogeneous competitive assays for biotin using complex **2** and (a) avidin and (b) An-Av. The emission intensities of the solutions were monitored at 550 and 420 nm, respectively. The data were average of triplicate experiments ±1 standard deviation.

Homogeneous Competitive Assay for Biotin. In view of (i) the enhancement of the ^{3}MLCT emission of the rhenium(I) biotin complexes upon their binding to native avidin, and (ii) the fluorescence quenching of anthracene upon binding of these complexes to the An-Av conjugate, we have designed two new homogeneous assays for biotin. In these assays, the rhenium(I) biotin complexes were mixed with native biotin of various concentrations followed by addition of (i) avidin or (ii) An-Av. After incubation, the ^{3}MLCT emission intensities of the complexes at ca. 567 nm in the first assay or the fluorescence of anthracene of An-Av at ca. 420 nm in the second assay were measured. The concentration range of biotin analyte was varied from 1×10^{-3} to 1×10^{-9} M. The results of two selected assays using complex **2** are shown in Figure 6. A lower biotin analyte concentration led to a higher degree of binding of the complex to the avidin and anthracene-modified avidin, and thus an enhanced ^{3}MLCT emission intensity in the first assay (Figure 6a) and a reduced anthracene fluorescence intensity in the second (Figure 6b). The concentration range of biotin that can be determined by these assays was between ca. 1×10^{-6} and 1×10^{-4} M, which was comparable to related assays in which other luminescent biotin or avidin conjugates were employed.[13f,14]

Conclusions

Three luminescent rhenium(I) amidodipyridoquinoxaline biotin complexes have been synthesized and characterized, and their photophysical, electrochemical, and avidin-binding properties have been investigated. The properties of these complexes have been compared to those of their biotin-free counterpart. All the complexes exhibited ^{3}MLCT emission in fluid solutions at room temperature and in alcohol glass at 77 K. Luminescence titration experiments showed that these complexes exhibited emission enhancement and lifetime extension upon binding to avidin. On the basis of the interquenching properties of these complexes and anthracene, new homogeneous assays for biotin using these complexes, avidin, and anthracene-labeled avidin have been designed.

In conclusion, we have successfully demonstrated that, by using the interesting dpqa ligand, the emission of the rhenium(I) biotin complexes can be effectively suppressed in aqueous buffer, and significantly increased upon binding to avidin. Specifically, the emission enhancement factors have been increased to ca. 8.05−3.05 from ca. 2.98−1.15 that were observed in other rhenium(I) polypyridine biotin complexes that we reported recently.[13f] Actually, the most important remark of the current work is that the environment-sensitive rhenium-dpqa moiety is not limited to biotin−avidin recognition, but can be generally applied to other substrate−protein systems. Thus, the results of the current work are very important in the design of more efficient and sensitive biological probes and the development of novel biosensors.

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Supporting Information Available: HABA assays for complexes **1**−**3**; emission titrations of avidin and An-Av with complexes **2** and **3**; determination of k_{on}, k_{off}, and K_d; emission titration of An-Av with complexes **2**−**4**; and results of homogeneous competitive assays for biotin using complexes **1** and **3**. This material is available free of charge via the Internet at http://pubs.acs.org.

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