Communication

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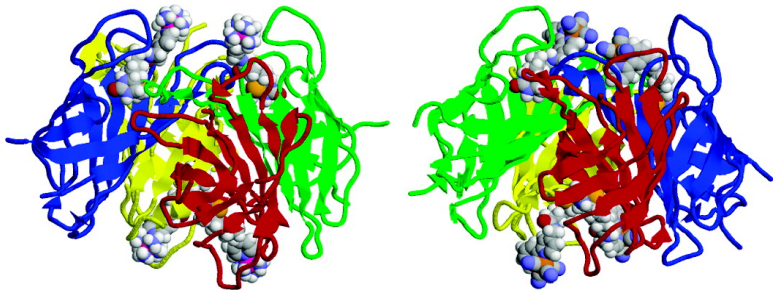

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Synthesis and Electrochemical Characterization of a Transition-Metal-Modified Ligand−Receptor Pair

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The energetics of weak interactions (van der Waals forces, hydrogen bonding) are difficult to quantify in biological ligand−receptor pairs. Insight into the biochemical role these forces play is critical to an understanding of signal transduction events and the drug discovery process. These weak interactions are essential in biological electron transfer (ET) events and are described in the semiclassical Marcus theory of donor−acceptor pairs. Modifying a native protein substrate to include a donor complex will permit an investigation of ligand−receptor binding events in solution where the acceptor is a redox mediator or an electrode. We have prepared a series of iron- and ruthenium-modified protein substrates. We report the synthesis and electrochemical characterization of these complexes in both free and protein-bound environments.

The biotin−avidin ligand−receptor pair was chosen for study primarily because of the low K_D and synthetic versatility of the ligand. Avidin binds to biotin with one of the strongest noncovalent interactions known and is highly resistant to denaturation over wide ranges of temperature and pH.[1] The solid-state structure of various forms of avidin has been determined by X-ray crystallography.[2] Several studies that incorporate metal complexes onto biotin have shown that these complexes display spectroscopic differences upon binding to avidin.[3] Further, electrochemical experiments involving both immobilized and solubilized avidin have shown that the binding affinity is not affected over the duration of the electrochemical experiments.[4]

Ligands incorporating biotin and desthiobiotin were synthesized by coupling with 4-aminomethyl pyridine according to a previously reported method,[5] and to 4-aminomethyl-4′-methyl-2,2′-bipyridine using standard peptide coupling syntheses to give 4-BMP, 4-DMP, 4-BMB, and 4-DMB.[6,7] These ligands were used to synthesize electrochemically active transition metal complexes capable of binding avidin.

A series of modified ruthenium pentaammine complexes were synthesized by reduction of $[Ru(NH_3)_5Cl]Cl_2$ according to literature methods.[8] An excess of ligand was combined with freshly generated aqueous $[(H_2O)Ru(NH_3)_5]^{2+}$ to give two linkage isomers of $[(4\text{-BMP})Ru(NH_3)_5](PF_6)_2$ ($\mathbf{1}_N$[4] and $\mathbf{1}_S$) in the case of 4-BMP or $[(4\text{-DMP})Ru(NH_3)_5](PF_6)_2$ (**2**) in the case of 4-DMP (Figure 1). These complexes were characterized by spectroscopic methods. $[(4\text{-DMP})Ru(NH_3)_5]^{2+}$ was oxidized using K[Co(edta)] and was purified using ion exchange chromatography to give $[(4\text{-DMP})Ru(NH_3)_5]Cl_3$ (**3**) (Figure 1). This complex was characterized by ESI-MS, and the UV−visible spectrum closely matched the spectrum for $[(\text{pyridine})Ru(NH_3)_5]Cl_3$.[9]

The Fe(II)−biotin complexes were prepared by modifications of literature procedures.[10] The ligand (4-BMB or 4-DMB) was heated briefly with $(NH_4)_2Fe(SO_4)_2\cdot 6H_2O$ (ligand:Fe 3:1), and 2 equiv of CN^- was added to give $[Fe(L)_2(CN)_2]$ as a crystalline solid.

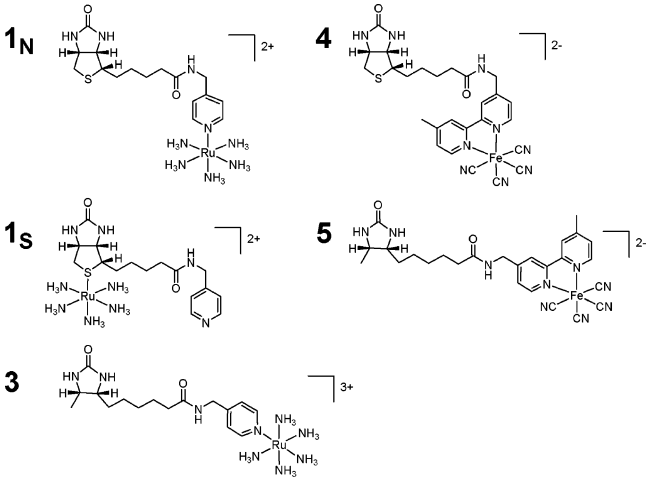

Figure 1. Structures of $\mathbf{1}_N$, $[(4\text{-BMP})_NRu(NH_3)_5]^{2+}$; $\mathbf{1}_S$, $[(4\text{-BMP})_SRu(NH_3)_5]^{2+}$; **3**, $[(4\text{-DMP})Ru(NH_3)_5]^{3+}$; **4**, $[Fe(4\text{-BMB})(CN)_4]^{2-}$; and **5**, $[Fe(4\text{-DMB})(CN)_4]^{2-}$.

This intermediate was dissolved in hot water with a large excess of CN^- and refluxed overnight to give $[Fe(4\text{-BMB})(CN)_4]^{2-}$ (**4**) or $[Fe(4\text{-DMB})(CN)_4]^{2-}$ (**5**) (Figure 1). The identity of these complexes was confirmed by spectroscopic methods.

Cyclic voltammetry (CV) of $\mathbf{1}_N$ in pH 3 phosphate buffer showed a reversible event at +0.275 V versus NHE. The potential for the N-bound isomer ($\mathbf{1}_N$) is comparable to that for $[(\text{pyridine})Ru(NH_3)_5]^{2+/3+}$ (+0.298 V), while the potential for $\mathbf{1}_S$ (+0.560 V) is comparable to that of $[(S(CH_3)_2)Ru(NH_3)_5]^{2+/3+}$ (+0.500 V).[11,12] CV of **2** and **3** in pH 6 HEPES both showed one reversible event at +0.313 V. CV of iron complexes **4** and **5** in pH 7.0 phosphate buffer solutions showed reversible redox events at +0.530 and +0.531 V respectively, which are comparable to that of $[Fe(4,4'\text{-dimethyl-}2,2'\text{-bipyridine})(CN)_4]^{2-}$ (+0.509 V).[13]

Upon addition of egg-white avidin to a CV solution of each of the compounds $\mathbf{1}_N$ and $\mathbf{1}_S$, **2**, **3**, **4**, and **5**, the current signals decrease dramatically for each of the compounds except $\mathbf{1}_S$, which is too large and too highly charged for the binding pocket (Figure S1).[2] The dramatic decrease in current signal is not surprising. The bound metal complexes are kinetically inaccessible because the protein effectively reduces the electronic coupling between these complexes and the electrode. Addition of biotin restores the signal, indicating that the binding is reversible (Figure S2).

Electrochemical mediators were employed to investigate the accessibility of the bound metal complexes, and the results are shown in Figure 2. Chosen for its reported potential of +0.322 V vs NHE, $[(4\text{-Cl-pyridine})Ru(NH_3)_5]^{3+}$ (**6**) was synthesized in a manner similar to that of **3**.[11] CVs were obtained for **3** and **6** separately ($E_{1/2}$ (**3**) = +0.281 V; (**6**) +0.317 V) and then for a

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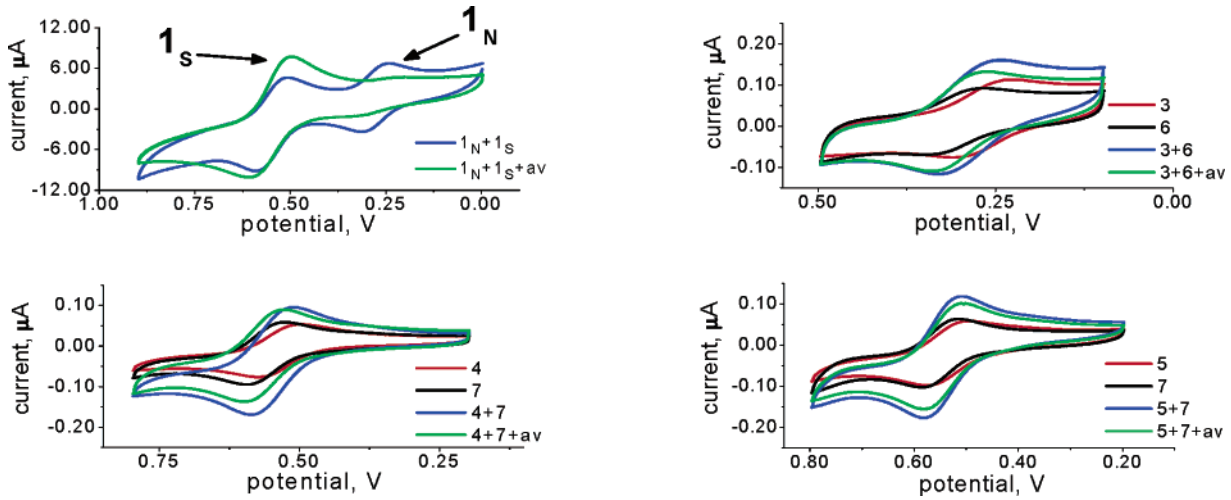

Figure 2. At a scan rate of 0.005 V/s, cyclic voltammetry in phosphate buffer shows that, upon addition of avidin (av) to a solution of avidin-binding complex ($\mathbf{1}_N$, **3**, **4**, or **5**) and mediator ($\mathbf{1}_S$, **6**, or **7**), the current for the mediator increases.

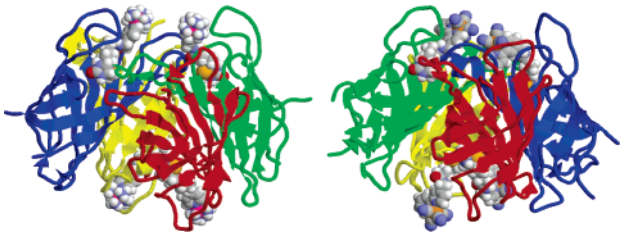

Figure 3. The final structure of MD simulation of avidin tetramer (ribbon) with modified biotin (spacefill). The picture on the left shows $\mathbf{1}_N$ bound to avidin, while the picture on the right shows **4** bound to avidin.

solution of the two complexes combined, which showed one quasi reversible couple at +0.295 V. Upon addition of avidin to the solution, the observed wave shifted back to the potential of the mediator. The current increased by 53% over that of **6** alone, which is consistent with mediator activity. The current for $\mathbf{1}_S$ increases by 75% when avidin is added to the $\mathbf{1}_N/\mathbf{1}_S$ mixture, indicating that the S-bound species $\mathbf{1}_S$ acts as a mediator. Similar results were obtained using $[Fe(2,2'\text{-bipyridine})(CN)_4]^{2-}$ (**7**, $E_{1/2} = +0.551$ V) and **4** and **5**, in which the current increased by 69 and 81%, respectively, over that of **7** alone (Figure 2). From these results, we conclude that the protein-bound metal centers are electronically accessible.

Molecular dynamics (MD) simulations of $\mathbf{1}_N$, **3**, **4**, and **5** were carried out to address the potential location of the metal while the ligand is protein-bound. The final structures from 400 ps MD simulations of $\mathbf{1}_N$ and **4** are shown in Figure 3. In all cases, the metal complex is partially shielded by the protein from solvent.

Five biotin- and desthiobiotin-containing metal complexes have been characterized. CV experiments confirm that binding of the metal complexes to avidin takes place in a manner consistent with the native ligand−receptor pair, and that the complexes, while bound, are electrochemically addressable, in agreement with previous reports.[4] Therefore, it will be possible to measure ET kinetics of the protein-bound metal center using electrochemical methods described by Chidsey[14] and Miller et al.,[15] in which self-assembled alkanethiol monolayers are employed to measure electron-transfer kinetics of tethered ferrocenes and heme species, respectively. Our future studies will use solution and surface methodologies to interrogate the ET properties of these artificial ligands.

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Supporting Information Available: Experimental procedures, spectroscopic and analytical data for the ligands and the metal complexes. This material is available free of charge via the Internet at http://pubs.acs.org.

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