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Excited-State Chiral Discrimination Observed by Time-Resolved Circularly Polarized Luminescence Measurements

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In this communication we report evidence for chiral discrimination in intermolecular energy-transfer processes between chiral (but *racemic*) donor molecules and chiral (enantiomerically *resolved*) acceptor molecules in aqueous solution. The donor molecules are nine-coordinate, tris-terdentate complexes of terbium(III) with dipicolinate (dpa) ligands, and the acceptor molecules are six-coordinate, tris-bidentate complexes of ruthenium(II) with 1,10-phenanthroline (phen) ligands. The structures of both the $Tb(dpa)_3^{3-}$ and $Ru(phen)_3^{2+}$ have trigonal dihedral (D_3) symmetry, and each has either left-handed (Λ) or right-handed (Δ) configurational chirality about the metal ion. The left- or right-handedness of a given enantiomer is defined by the dispositions and relative orientations of the chelate rings about the trigonal symmetry axis of the structure. *rac*-$Ru(phen)_3^{2+}$ may be resolved by chemical means,[1] and the separated pure enantiomers are inert with respect to racemization in neutral aqueous solution at room temperature. On the other hand, $Tb(dpa)_3^{3-}$ complexes are highly labile in aqueous solution and, under equilibrium conditions, they exist as racemic mixtures of Λ and Δ enantiomers.[2]

In this study, we have examined the chiroptical absorption and luminescence properties of $Tb(dpa)_3^{3-}$ in neutral aqueous solutions also containing varying amounts of resolved $Ru(phen)_3^{2+}$. Circular dichroism (CD) measurements were used to probe the chirality of the *ground-state* population of the $Tb(dpa)_3^{3-}$ complex, while circularly polarized luminescence (CPL) measurements were used to probe the chirality of the luminescent, *excited-state* population of the $Tb(dpa)_3^{3-}$ complex.[3] No CD was observed in the transition regions unique to the $Tb(dpa)_3^{3-}$ complex, which implies that the ground-state population exists as a racemic mixture, even in the presence of the resolved $Ru(phen)_3^{2+}$ cosolute. The CPL spectrum (Figure 1), measured in the $^7F_5 \leftarrow {}^5D_4$ transition region of terbium, shows that the emitting-state population of the $Tb(dpa)_3^{3-}$ is nonracemic, with differing populations of the Λ and Δ enantiomers. This spectrum is identical with published spectra for the $Tb(dpa)_3^{3-}$ complex,[4] which indicates that this species is intact under these conditions. Figure 2 shows the optical activity observed from the excited state (expressed as the emission dissymmetry factor, $g_{em} = 2(I_l - I_r)/(I_l + I_r)$, where $I_{l(r)}$ is the intensity of left (right) circularly polarized light emitted)[3] as a function of the ratio of the concentrations of resolved Δ-(−)-$Ru(phen)_3^{2+}$ to $Tb(dpa)_3^{3-}$. The optical activity is *enhanced* as compared to that optical activity expected from a population of static, 1:1 ion pairs of $Tb(dpa)_3^{3-}$ and $Ru(phen)_3^{2+}$ complexes. For a static, ion-pairing mechanism, we would expect a linear increase in dissymmetry value as a function of the concentration of added Δ-(−)-$Ru(phen)_3^{2+}$. This is clearly not the case. The optical activity thus arises from a *dynamic* mechanism involving the excited state of $Tb(dpa)_3^{3-}$ and the resolved $Ru(phen)_3^{2+}$.

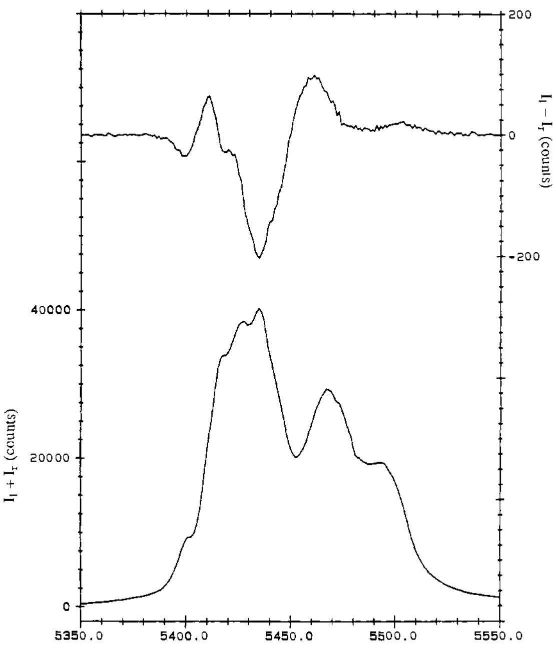

Figure 1. Circularly polarized luminescence (top, $I_l - I_r$) and total luminescence (bottom, $I_l + I_r$) for a solution 10 mM in $Tb(dpa)_3^{3-}$ and 5 μM in Λ-(+)-$Ru(phen)_3^{2+}$ at neutral pH. Excitation wavelength 325 nm (He–Cd laser). The emission corresponds to the $^7F_5 \leftarrow {}^5D_4$ transition of Tb^{3+}.

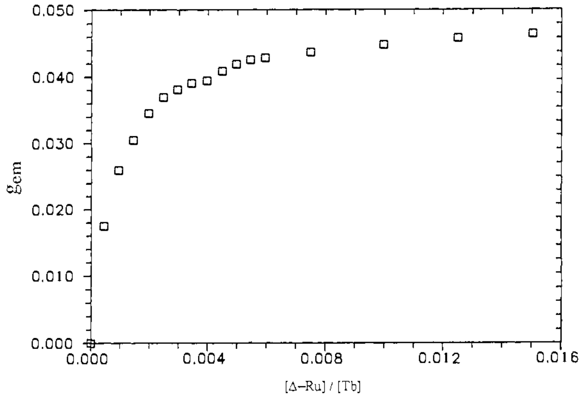

Figure 2. Dissymmetry factor, $g_{em} = 2(I_l - I_r)/(I_l + I_r)$, plotted vs the ratio of Δ-(−)-$Ru(phen)_3^{2+}$ to $Tb(dpa)_3^{3-}$ concentrations. $Tb(dpa)_3^{3-}$ concentration 15 mM, excitation wavelength 280 nm, emission at 543 nm.

Presented in Figure 3 are the time-resolved CPL and TL (total luminescence) spectra observed for a solution 10 mM in *rac*-$Tb(dpa)_3^{3-}$ and 5 μM in Λ-(+)-$Ru(phen)_3^{2+}$. The solution was excited at 325 nm by using a mechanically chopped (53 Hz) helium–cadmium laser. The time-resolved CPL and TL at 543.5 nm were recorded on a differential photon-counting CPL/TL spectrophotometer constructed at the University of Virginia.[5] Figure 3b shows the time dependence of g_{em} calculated from the data in Figure 3a. Here, we clearly see the increase in magnitude of the optical activity as the excited-state population decreases, showing that the rates of quenching of the two $Tb(dpa)_3^{3-}$ enantiomers by the resolved $Ru(phen)_3^{2+}$ are different.

This creation of optical activity as a result of "chiral quenching" of the excited state is analogous to the creation of an enantiomeric excess as the result of asymmetric photodestruction of a racemic mixture with circularly polarized light, described by Mitchell,[6]

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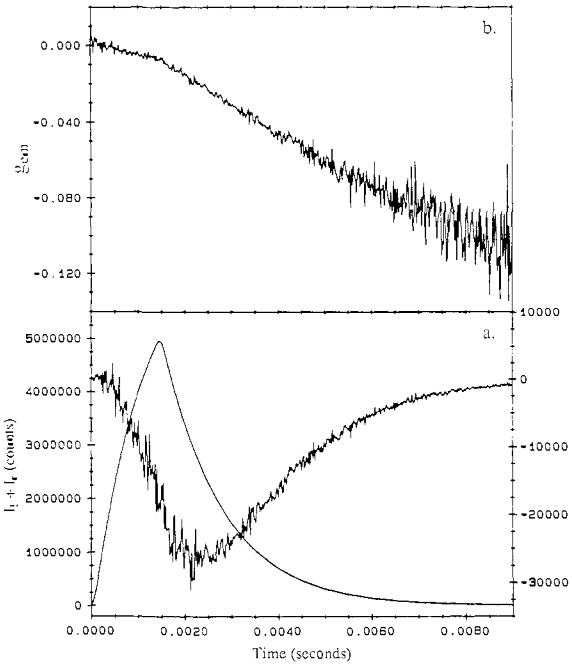

Figure 3. (a) Time-resolved circularly polarized luminescence (noisy trace, right scale) and total luminescence (smooth trace, left scale) plotted vs time for a solution 10 mM in $Tb(dpa)_3^{3-}$ and 5 μM in Λ-(+)-$Ru(phen)_3^{2+}$. Excitation wavelength 325 nm (He–Cd laser, chopped at 53 Hz), emission at 543.5 nm. The laser first excites the sample at time 0.0 and is chopped off at time 0.0016 s. The initial rise in the TL intensity reflects the kinetics of the emission. (b) Dissymmetry factor calculated from data in (a) plotted vs time.

Kagan et al.,[7] and Rau.[8] Kagan developed an equation expressing the enantiomeric excess as a function of time:

$$y = \frac{[S] - [R]}{[S] + [R]} = \tanh\,[\tfrac{1}{2}(k_S - k_R)t] \qquad (1)$$

Here, y is the enantiomeric excess, and k_S and k_R are the first-order rate constants for the photodestruction of the S and R enantiomers by the circularly polarized light. For our system, an analogous equation can be derived:

$$g_{em}(t) = g_{em}(\lim)\tanh\,[\tfrac{1}{2}(k_{\Lambda\Lambda} - k_{\Delta\Lambda})[Q]t] \qquad (2)$$

where $g_{em}(t)$ is the dissymmetry at time t, $g_{em}(\lim)$ is the limiting dissymmetry for fully resolved (Λ or Δ) $Tb(dpa)_3^{3-}$, [Q] represents the concentration of the resolved $Ru(phen)_3^{2+}$ quencher, and $k_{\Lambda\Lambda}$ and $k_{\Delta\Lambda}$ are the rate constants for quenching of the Λ-$Tb(dpa)_3^{3-}$ isomer by Λ-$Ru(phen)_3^{2+}$ and of the Δ-$Tb(dpa)_3^{3-}$ by Λ-$Ru(phen)_3^{2+}$, respectively. By fitting of the dissymmetry data in Figure 3b to eq 2, we derived approximate values for the rate constants of 1×10^8 and 2×10^8 $M^{-1}\,s^{-1}$ and a limiting dissymmetry factor magnitude for the $Tb(dpa)_3^{3-}$ complex of 1.2×10^{-1}. We are currently uncertain as to which interaction (Λ-Λ or Δ-Λ) produces the larger quenching rate. However, the difference between the two rates demonstrates that the enantioselectivity in this quenching process is very large.

This induction of optical activity into a large population of (excited) racemic terbium complexes by a small, resolved population of ruthenium complexes results in a large amplification of the optical activity of the system. This, then, is a very sensitive probe of transition metal complex enantiomeric resolution. We are continuing to develop this probe and are using it to study the enantioselective binding of transition metal complexes to DNA.

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Carbon-13 Spin System Directed Strategy for Assigning Cross Peaks in the COSY Fingerprint Region of a Protein

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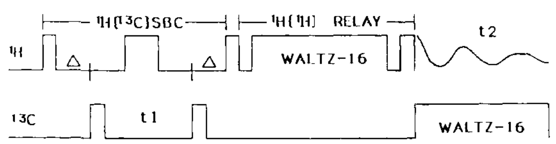
We previously demonstrated that the ^{13}C spin systems of amino acids in proteins (uniformly labeled with ^{13}C to a level of about 30%) can be traced out and classified according to 18 different amino acid types by a single $^{13}C\{^{13}C\}$ double quantum correlation ($^{13}C\{^{13}C\}$DQC) experiment.[1-3] The remaining ambiguities of Glu = Gln and Asp = Asn can be resolved by means of a $^{13}C\{^{15}N\}$ single-bond correlation ($^{13}C\{^{13}N\}$SBC) experiment.[4] ^1H spin systems then can be elucidated by using $^1H\{^{13}C\}$ single-bond correlation ($^1H\{^{13}C\}$SBC) data to translate carbon assignments into assignments of directly bonded hydrogens.[5] In principle, data from these three experiments are sufficient for extensive identification of cross peaks in the ^1H COSY fingerprint region (recorded in ^1H_2O). In practice, however, overlaps of C^α or H^α resonances from different residues lead to ambiguities in such cross assignments. These ambiguities, which appear in $^1H\{^{13}C\}$SBC or ^1H COSY spectra, can be resolved by additional information that links the ^{13}C and ^1H spin systems through other scalar coupling pathways. We show here that $^1H\{^{13}C\}$ single-bond correlation with ^1H relay ($^1H\{^{13}C\}$SBC–^{1}HR) data, along with ^1H correlated relay (^1H RELAY)[6] data, provides such pathways to extensive residue-type identifications of $[H^\alpha,H^N]$ cross peaks in the COSY[7] fingerprint region. Such identifications are a prerequisite for sequential resonance assignments based on interresidue NOESY (nuclear Overhauser effect spectroscopy)[8] connectivities.[9] The protein sample studied was the oxidized form of ferredoxin (M_r = 11 000) from *Anabaena* 7120 (a photosynthetic cyanobacterium).

In this work, homonuclear Hartmann–Hahn mixing[10] was used to provide the ^1H relay in the $^1H\{^{13}C\}$SBC experiment. This approach differs from that designed by Brühwiler and Wagner, which incorporates an additional coherence transfer step.[11] The pulse sequence (1) used is[10]



(1)

The $[H^\alpha,(C^\alpha,C^\beta)]$ connectivities from the $^1H\{^{13}C\}$SBC–^{1}HR spectrum (Figure 1B) can be correlated directly with $[(C^\alpha,C^\beta),C^{\alpha+\beta}]$ connectivities from the $^{13}C\{^{13}C\}$DQC spectrum (Figure 1A). Similarly, the $[(H^\alpha,H^\beta),C^\alpha]$ connectivities from the $^1H\{^{13}C\}$SBC-^{1}HR spectrum (Figure 2A) can be related directly

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