

Communication

## Polarized Emission from a Rhenium Metal–Ligand Complex

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We report the first observation of polarized emission from a rhenium–phenanthroline complex,  $\text{Re}(\text{CO})_3(\text{phen})\text{Cl}$ . Highly luminescent rhenium complexes are known, with quantum yields near 0.5 and lifetimes in excess of 10  $\mu\text{s}$ . The detection of polarized emission suggests the use of rhenium complexes as probes of the hydrodynamics of large macromolecular complexes and for use in fluorescence polarization immunoassays with gated detection.

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**KEY WORDS:** Polarized emission; rhenium–phenanthroline; rhenium–phenanthroline complexes.

### INTRODUCTION

During the past 10 years there has been considerable interest in the use of luminescent metal–ligand complexes for solar energy conversion,<sup>(1–4)</sup> as luminescent probes in polymers,<sup>(5)</sup> and as oxygen sensors.<sup>(6)</sup> Recently it has been demonstrated that molecules of the type  $\text{Ru}(\text{bpy})_2(\text{dcbpy})^{2+}$  display high anisotropy values when excited in the long-wavelength band.<sup>(7)</sup> It was shown that this polarized emission was sensitive to rotational motions<sup>(7,8)</sup> and could be used to measure the rotational motions of high molecular weight macromolecular complexes.<sup>(8)</sup> The possibility of measuring such slow rotational motions is a consequence of the long luminescence decay times of these complexes, typically in the range of 500 ns.

A wealth of information is available about luminescent metal–ligand complexes.<sup>(9–11)</sup> However, the use of these complexes as biochemical probes is in its infancy and little is known about the possibility of polar-

ized emission from such complexes. In a recent report we demonstrated that an osmium metal–ligand complex also displays polarized emission<sup>(12)</sup> and could be excited with the long wavelengths currently available from laser diodes. While the long-wavelength excitation of the osmium complexes is desirable, such complexes display low quantum yields and short lifetimes as a result of the energy gap law.<sup>(13)</sup> It is known that metals other than ruthenium and osmium can yield luminescent complexes. In particular, rhenium complexes are also known to be highly luminescent.<sup>(14–16)</sup> Demas and co-workers have demonstrated that certain rhenium complexes display high quantum yields, in excess of 0.7, and lifetimes in excess of 10  $\mu\text{s}$  in fluid solutions at room temperatures.<sup>(17)</sup> Demas and others have also demonstrated that, in the presence of highly sterically hindered  $\alpha$ -diimine ligands, the rhenium complexes can exhibit lifetimes ranging from 3 to 13  $\mu\text{s}$ , with quantum yields near 30%.<sup>(18)</sup> However, to date no information is available about whether such rhenium metal–ligand complexes will display polarized emission and thus allow measurement of their hydrodynamic properties, either when free or when bound to macromolecules. In the present report we describe polarized emission from  $\text{Re}(\text{CO})_3(\text{phen})\text{Cl}$ . These preliminary data suggest that the high quantum yields and long lifetimes may render the rhenium complexes excellent probes of macromolecular dynamics.

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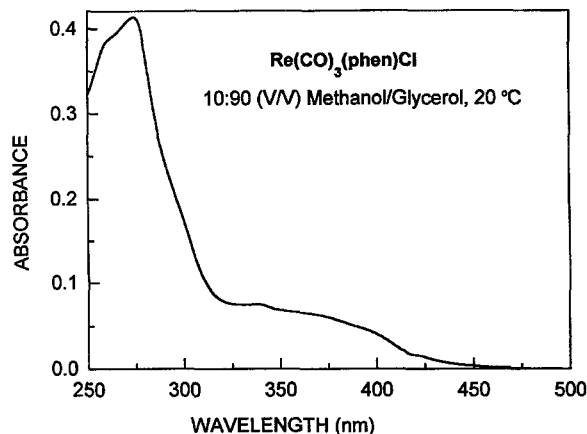


Fig. 1. Absorption spectrum of  $\text{Re}(\text{CO})_3(\text{phen})\text{Cl}$ .

## MATERIALS AND METHODS

Excitation anisotropy spectra were collected as usual, with the anisotropy defined by

$$r = \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + 2I_{\perp}} \quad (1)$$

where  $I_{\parallel}$  and  $I_{\perp}$  are the intensities measured with vertically polarized excitation and the emission polarization parallel ( $\parallel$ ) or perpendicular ( $I_{\perp}$ ) to the excitation. The values of the polarized intensities were corrected for the transmission efficiency for the polarized components by the detection optics. The reported emission spectra are corrected for variation in the detector efficiency with observation wavelength.

Fluorescence intensity and anisotropy decays were measured by time-correlated single-photon counting. The excitation wavelength was 360 nm from the frequency-doubled output of a Pyridine2 dye laser. The emission was isolated above 475 nm by use of a long-pass filter. The data were fit by the usual procedures of nonlinear least squares, using software from IBH, Inc. (Edinburgh, Scotland). Intensity decays were fit to

$$I(t) = I_0 e^{-t/\tau} \quad (2)$$

where  $\tau$  is the decay time. Anisotropy decays were fit to a double-exponential model,

$$r(t) = \sum_{i=1}^2 r_{0i} e^{-t/\theta_i} \quad (3)$$

where  $r_{0i}$  are the amplitudes and  $\theta_i$  are the correlation times. The anisotropy decays were found to be at least biexponential, and only the long correlation time is reported.

The rhenium complex  $\text{Re}(\text{CO})_3(\text{phen})\text{Cl}$  was from two sources. One sample was synthesized at Binghamton University, and the second sample was synthesized in this laboratory. *fac*- $\text{Re}(\text{CO})_3(\text{phen})\text{Cl}$  was prepared by a modification of the method reported previously.<sup>(13)</sup> In a typical preparation equimolar quantities of  $\text{Re}(\text{CO})_5\text{Cl}$  (Aldrich) and 1,10-phenanthroline (Aldrich) in THF at reflux for 2 h. The yellow product precipitated from solution and was filtered and washed with hexane and recrystallized by dissolving in  $\text{CH}_2\text{Cl}_2$  and precipitated with hexane. The spectral properties of both samples were identical.

## RESULTS

The absorption spectrum of the rhenium tricarbonyl phenanthroline in glycerol/methanol (90/10, v/v) is shown in Fig. 1. The absorption maximum is near 275 nm, with the tail of the absorption extending to about 450 nm. Because of this absorption, and therefore excitation, wavelengths are rather short, one might expect significant autofluorescence from biological samples when using the emission of this complex. However, we expect the rhenium probes to display long decay times, and they may be used with gated detection, so that the autofluorescence should cause no difficulties since it typically displays shorter decay times.

The emission spectrum of  $\text{Re}(\text{CO})_3(\text{phen})\text{Cl}$  is conveniently shifted to long wavelengths with an emission maximum near 560 nm at room temperature. The emission intensity is strongly dependent on temperature as shown by the increased intensity at  $-65^\circ\text{C}$  compared with  $12^\circ\text{C}$  (Fig. 2, bottom). There is a dramatic shift in emission maximum with temperature, suggesting that the complex can also be useful as a polarity probe when bound to macromolecules. The complex is fluorescent in fluid solvents at room temperature. In methylene chloride, in the absence of oxygen, we found a homogeneous intensity decay with a lifetime of 296 ns (Table I), as observed by time-correlated single-photon counting. In the presence of dissolved oxygen, from equilibration with air, the decay time reduced to 140 ns. We also examined the intensity decays in glycerol/methanol at various temperature. The decay was found to be a single exponential, with the decay time increasing from 243 ns at  $20^\circ\text{C}$  to 852 ns at  $-55^\circ\text{C}$ . Given the long decay times in this polar solvent, one can expect the rhenium complexes to display reasonable quantum yields and decay times in aqueous solution and when bound to macromolecules.

We questioned whether the  $\text{Re}(\text{CO})_3(\text{phen})\text{Cl}$  complex would display polarized emission in the absence of

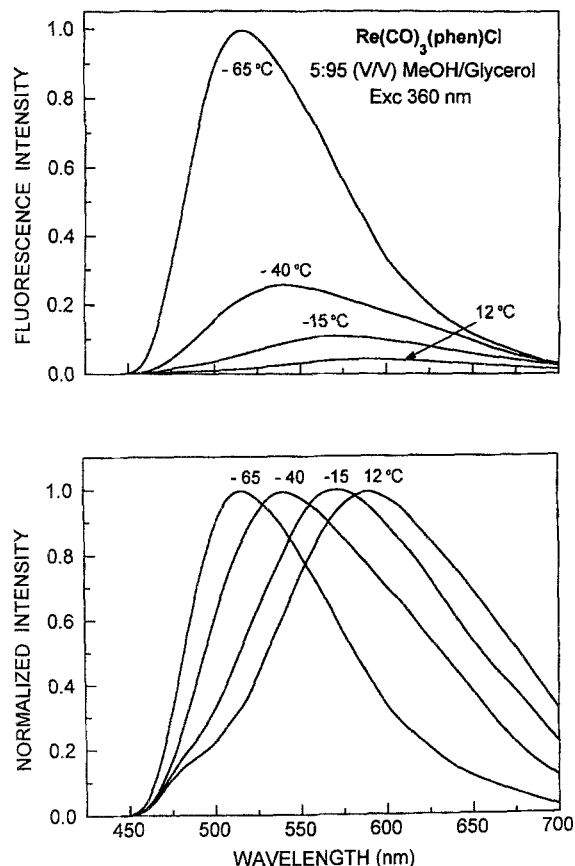


Fig. 2. Temperature-dependent emission spectra of  $\text{Re}(\text{CO})_3(\text{phen})\text{Cl}$  (top). Intensity-normalized spectra (bottom).

rotational diffusion. Hence the solution was cooled to  $-65^\circ\text{C}$ . The excitation anisotropy spectrum is shown in Fig. 3. This complex shows maximal anisotropies in excess of 0.3 and these high anisotropies are broadly available from 330 to 450 nm. Observation of significantly nonzero anisotropies from the rhenium complex indicates the possibility of long-lifetime anisotropy probes for use in biochemistry and clinical chemistry.

Rhenium complexes are known to display dual emission, which can originate from either a metal-to-ligand charge transfer state (MLCT) or a ligand-centered state (LC).<sup>(15,16)</sup> The MLCT states typically show an unstructured emission, whereas the LC states often display a structured emission which is characteristic of the ligand. The emission spectra in Fig. 2 are unstructured, suggesting that under our experimental conditions the emission is from the MLCT state. To clarify further the nature of the emission, and to characterize these complexes for use as biophysical probes, we examined the anisotropy across the emission spectrum, that is, the

Table I. Intensity Decay Times and Rotational Correlation Times of  $\text{Re}(\text{CO})_3(\text{phen})\text{Cl}$

| Solvent                          | Conditions <sup>a</sup> | °C  | $\tau$ (ns) | $\theta$         |
|----------------------------------|-------------------------|-----|-------------|------------------|
| Acetonitrile                     | Air                     | 20  | 56          | —                |
|                                  | Argon                   | 20  | 176         | —                |
| Methylene chloride               | Air                     | 20  | 140         | —                |
|                                  | Argon                   | 20  | 296         | —                |
| Glycerol methanol<br>(95/5, v/v) | Air                     | 20  | 243         | 21.4 ns          |
|                                  | Air                     | 0   | 309         | 167 ns           |
|                                  | Air                     | -19 | 337         | 362 ns           |
|                                  | Air                     | -35 | 545         | >1 $\mu\text{s}$ |
|                                  | Air                     | -55 | 852         | >1 $\mu\text{s}$ |

<sup>a</sup>The samples were either in equilibrium with air or purged with argon to remove dissolved oxygen.

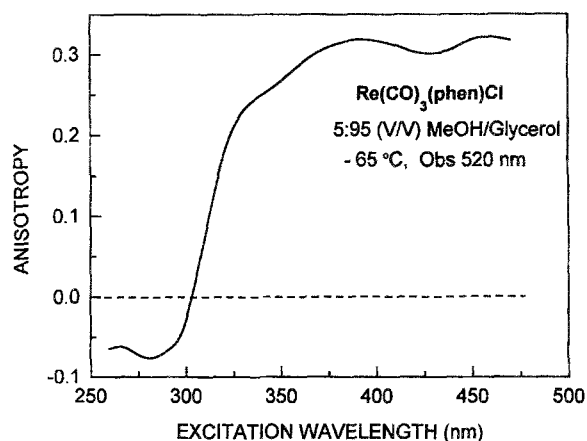


Fig. 3. Excitation anisotropy spectrum of  $\text{Re}(\text{CO})_3(\text{phen})\text{Cl}$  in methanol/glycerol (5/95, v/v),  $-65^\circ\text{C}$ . The emission was observed at 520 nm.

emission anisotropy spectrum (Fig. 4). This anisotropy is rather constant across the emission and displays a gradual decrease with increasing wavelength. This decrease is consistent with the residual effects of spectral relaxation. It is known that polar molecules often display such wavelength-dependent decreases in anisotropy due to longer mean decay times on the long-wavelength side of the emission.<sup>(19)</sup> Hence, for the purpose of using  $\text{Re}(\text{CO})_3(\text{phen})\text{Cl}$  as a biophysical probe, its emission anisotropy is consistent with emission from a single state.

And, finally, we measured the anisotropy decays of the complex glycerol/methanol at various temperatures. In these preliminary measurements the anisotropy decays were found to be somewhat complex, with ev-

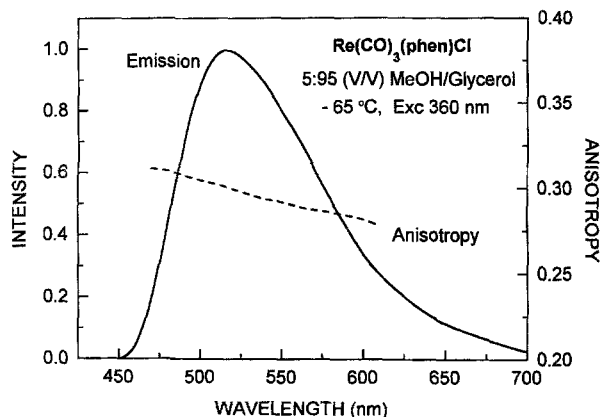


Fig. 4. Emission (—) and emission anisotropy spectrum of  $\text{Re}(\text{CO})_3(\text{phen})\text{Cl}$  in methanol/glycerol (5/95, v/v),  $-65^\circ\text{C}$  (---). The excitation wavelength was 360 nm.

idence of both a temperature-independent short correlation time (5–20 ns) and a temperature-dependent long correlation time. At present we do not understand the origin of the short correlation time. The long correlation time ranged from 21.4 ns to over 1  $\mu\text{s}$  at  $-35$  and  $-55^\circ\text{C}$ . This dependence on temperature and viscosity shows that the anisotropy decay of  $\text{Re}(\text{CO})_3(\text{phen})\text{Cl}$  is sensitive to solvent viscosity. Hence such complexes should serve as probes of the hydrodynamics of macromolecules.

## CONCLUSION

A rhenium metal–ligand complex was shown to display polarized emission. This suggests that conjugatable rhenium metal–ligand complexes, which display high quantum yields and decay times in excess of 10  $\mu\text{s}$ ,<sup>(17)</sup> can be designed for use as probes of macromolecular dynamics. The design of such complexes will undoubtedly be aided by the wealth of information available on the chemical synthesis and photophysical properties of rhenium complexes.<sup>(14–16, 20–22)</sup> Hence, we can imagine such complexes being used to replace shorter-lived fluorophores currently used in immunoassays,<sup>(23)</sup> analytical<sup>(24)</sup> and clinical<sup>(25)</sup> chemistry, cell biology,<sup>(26)</sup> and fluorescence microscopy.<sup>(27)</sup> A minor disadvantage of the Re probe is the relatively short excitation wavelength. However, it has recently been shown that pulsed light-emitting diodes (LEDs) can provide ultraviolet wavelengths down to 370 nm.<sup>(28)</sup> This mode of excitation should be particularly useful since pulsed excitation is compatible with gated detec-

tion for suppression of short-lived autofluorescence. The large Stokes' shift of the Re complexes also aids in eliminating such interference. Hence, the Re complexes should find wide use with simple light sources and instrumentation for many potential applications in the biomedical sciences.

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