Luminescent Metalloreceptor with a Neutral Bis(Acylaminoimidazoline) Binding Site: Optical Sensing of Anionic and Neural Phosphodiesters

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Received September 17, 1997
Revised Manuscript Received November 17, 1997

Phosphates play a crucial role in a wide range of biological processes. Numerous efforts have been devoted to molecular recognition, [1−4] transport, [5] and catalytic hydrolysis[6] of phosphates. Further understanding of the structure and dynamics of phosphates in biological systems requires the development of nondestructive and real-time optical sensing techniques.[7] A number of excellent phosphate receptors are now known. However, few of these receptors are capable of optically detecting phosphodiesters.[8] Recently, biomimetic bis(guanidinium) residues have proven significantly useful for binding phosphodiesteres.[2] Incorporating such a binding site into a photoactive $Ru(bpy)_3^{2+}$ (bpy = 2,2′-bipyridine) should provide a new class of optical signal transduction systems for phosphodiesters. We report herein the first metalloreceptor with a neutral bis(acylaminoimidazoline) binding site, which recognizes anionic and neutral phosphodiesters with luminescent signal transduction.

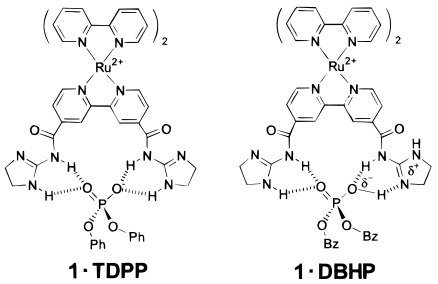
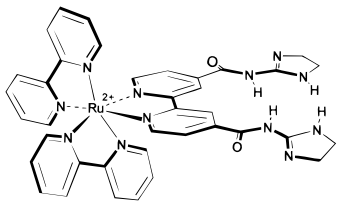


Figure 1. Proposed structures for a 1:1 complex between **1** and tetraethylammonium diphenyl phosphate (TDPP) or dibenzyl hydrogen phosphate (DBHP).



1

4,4′-Dicarboxy-2,2′-bipyridine[9] was treated with $SOCl_2$ and then condensed with 2-amino-2-imidazoline[2b] to give *N*,*N*′-bis-(2-imidazolin-2-yl)-2,2′-bipyridine-4,4′-dicarboxyamide (bimbpy) in 48% yield. Reaction of bimbpy with $Ru(bpy)_2Cl_2$[10] in refluxed 50% EtOH for 40 min gave metalloreceptor **1** in 35% yield. The pK_a values of the acylaminoimidazolines were spectrophotometrically determined to be ∼2.0 and 3.97. Two acylaminoimidazolines of **1** behaved independently and had unusually poor basicities as compared with alkyl-, aryl-, and even acylguanidines (pK_as = 7−13).[11] The reduced basicities probably reflect an electrostatic repulsion between a cationic metal center and protonated acylaminoimidazolines. In solution, the phosphate-binding site of **1** predominately existed in the unprotonated form.

The binding nature of metalloreceptor **1** was primarily studied by ^{1}H NMR spectroscopy. Titration of anionic tetraethylammonium diphenyl phosphate (TDPP) into a solution of **1** in acetone-d_6 or acetonitrile-d_3 gave downfield shifts of N*H* and 3,3′-C*H* resonances of bimbpy. These changes are consistent with the formation of a complex **1·TDPP** (Figure 1).[12] However, no further quantitative analyses were carried out because of immediate precipitation of the complex **1·TDPP** during ^{1}H NMR titrations.

Metalloreceptor **1** also formed a complex with neutral phosphodiesters, and the complex formation was significantly dependent on guest acidity and solvents. A dilution method was employed to explore complexation of **1** with neutral diphenyl hydrogen phosphate (DPHP) or dibenzyl hydrogen phosphate (DBHP), which is useful for discriminating between proton transfer and binding phenomina.[13] Upon dilution of a 1:1 mixture of **1** and DPHP in acetone-d_6 and in acetonitrile-d_3, observed

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(12) In a majority of similar phosphate receptors, a complex structure forming four hydrogen bonds to complement three oxygens of a bound phosphate have been proposed.[2e,f] However, an examination of CPK models indicated that the binding cavity of **1** is not large enough to form such hydrogen bonds.

S0002-7863(97)03263-0 CCC: $15.00
Published on Web 01/14/1998

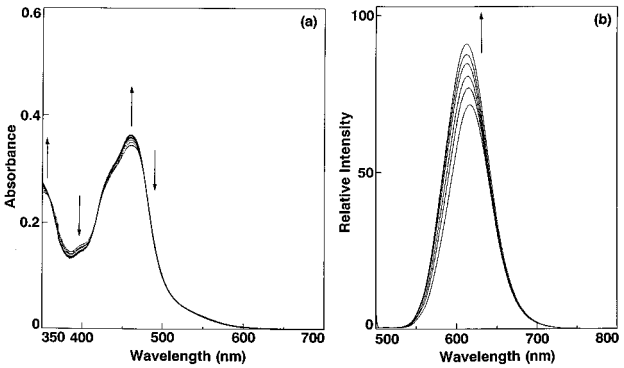

Figure 2. Change in the UV−visible absorption (a) and luminescence spectra (b) of **1** upon addition of tetraethylammonium diphenyl phosphate (TDPP) in acetone: **[1]** = 0.02 mM, **[TDPP]** = 0−6.0 mM. Excitation was at 477 nm.

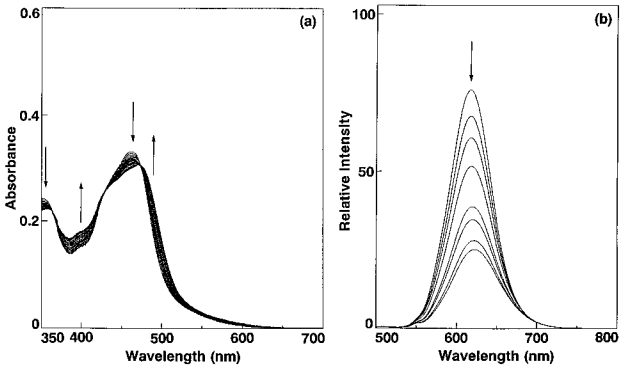

Figure 3. Change in the UV−visible absorption (a) and luminescence spectra (b) of **1** upon addition of dibenzyl hydrogen phosphate (DBHP) in acetone: **[1]** = 0.02 mM, **[DBHP]**= 0−6.0 mM. Excitation was at 474 nm.

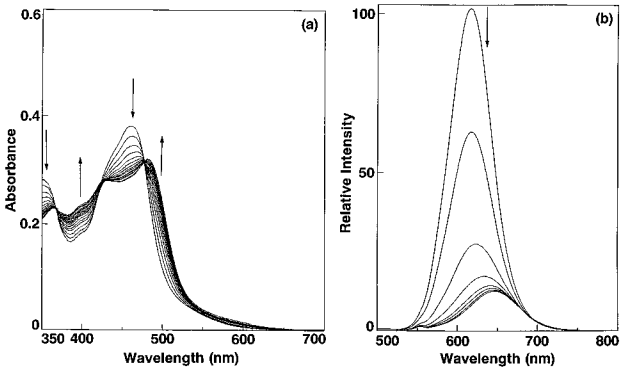

Figure 4. Change in the UV−visible absorption (a) and luminescence spectra (b) of **1** upon addition of diphenyl hydrogen phosphate (DHPP) in acetone: **[1]** = 0.02 mM, **[DHPP]**= 0−6.0 mM. Excitation was at 476 nm.

chemical shifts of **1** remained unaltered over a concentration range of 0.5−10.0 mM, implying proton transfer from DPHP to **1**. Dilution with a 1:1 mixture of **1** and DBHP in acetonitrile-d_3 also showed no changes in the ^{1}H NMR spectrum of **1**. In contrast, the same experiment in acetone-d_6 revealed distinct shifts of the N*H* and 3,3′-C*H* resonances of bimbpy, indicating the formation of a complex **1·DBHP** as shown in Figure 1. The titration curves were analyzed by nonlinear regression analysis[13] to give K_a = 4600 M^{-1}.[14] A 1:1 stoichiometry for the binding was confirmed by Job plots.[15] These results show that the neutral bis(acylaminoimidazoline) binding site of **1**, which unlike the similar bis(guanidinium) receptors,[2] can act as both hydrogen-bond donors and acceptors.

The absorption spectrum of **1** was affected by the addition of phosphodiesters. Control experiments using Ru(bpy)$_3^{2+}$ showed no UV−visible spectral changes. Therefore, UV−visible responses of **1** are due to specific association and neither are due to counter anion exchanges nor solvent polarity changes. Anionic TDPP caused a slight blue shift and an increase of the MLCT band in the visible region (Figure 2a). The presence of isosbestic points at 477, 419, and 358 nm suggests that only one complex was being formed. Neutral DBHP induced a red shift and a decrease of the MLCT band with isosbestic points at 474, 422, and 366 nm (Figure 3a). Neutral DPHP, which only caused proton transfer, provided UV−visible spectral changes similar to those observed for neutral DBHP but much more pronounced (Figure 4a). From these UV−visible absorption changes, K_as of 33 000 and 4800 M^{-1} were calculated for TDPP and DBHP, respectively. Metalloreceptor **1** showed 6.9 times higher affinity for anionic TDPP over neutral DBHP. The less preference of **1** for DBHP may be due to electrostatic repulsion between the cationic metal center and the positive charge produced on the neutral binding site of **1** upon complexation.

Optical sensing of phosphodiesters was more drastically demonstrated by virtue of luminescent response of **1** to the complex formation. Figures 2b and 3b show luminescence spectra excited at the isosbestic wavelength when varying the concentration of TDPP and DBHP, respectively. Addition of 10 equiv of DBHP to a solution of **1** in acetone induced a 32% reduction in luminescence intensity. In contrast, metalloreceptor **1** gave a 27% luminescence enhancement when 10 equiv of TDPP was added to a solution of **1** in acetone. Complexation rigidifying a luminescent receptor has been reported to result in a large increase in emission intensity because of inhibiting vibrational and rotational relaxation modes of nonradiative decay.[8c] Luminescence enhancement for anionic phosphodiesters may be due to this rigidity effect. On the other hand, neutral phosphodiesters upon complexation form different hydrogen-bonding structures from anionic phosphodiesters. In the complex **1·DBHP**, intracomplex proton transfer of the phosphoric acid hydrogen from DBHP to **1** probably causes a reduction in luminescence intensity. This is consistent with the protonation-induced luminescence profile in which DPHP caused a significant quenching (Figure 4b). Luminescent signal transduction of **1** is strikingly sensitive to hydrogen-bond interaction.

Acknowledgment. We thank the Ministry of Education, Science, Sports and Culture in Japan for financial support by a Grant-in-Aid for Scientific Research (B).

Supporting Information Available: Synthetic experimental and details of spectrophotometric pK_a measurements and binding studies (6 pages). See any current masthead page for ordering and Internet access instructions.

JA973263A

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