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Air Oxidation of HS^- Catalyzed by An Mixed-Valence Diruthenium Complex, an Near-IR Probe for HS^- Detection

Yi-Ling Quek,[†] Choon-Hong Tan,[†] Jinsong Bian,[‡] and Dejian Huang*[,†]

[†]Department of Chemistry, National University of Singapore, 3 Science Drive 3, Singapore 117543, Singapore

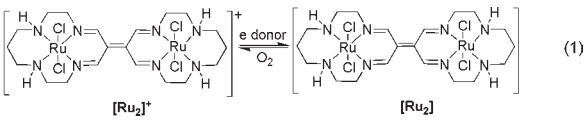
[‡]Department of Pharmacology, National University of Singapore, 10 Medical Drive, Singapore 117597, Singapore

Supporting Information

ABSTRACT: The strongly near-IR-active mixed-valence diruthenium complex $[Ru_2TIEDCl_4]Cl$, where TIED = tetraiminoethylenedimacrocycle, was found to be a highly active catalyst for air oxidation of HS^- forming polysulfide species and H_2O_2. The reaction provides a convenient method for the detection of the HS^- generation rate of a H_2S donor of medical importance.

Compounds with strong near-infrared (NIR, 650−900 nm) absorbance and/or luminescent properties are highly sought after optical materials for a number of applications. These include photovoltaic devices,[1] spectrophotometric analysis,[2] and biomedical application by taking advantage of the deep-tissue penetration of NIR waves with low background interference (either absorbance or autofluorescence from the biological matrix).[3] Mixed-valence transition-metal complexes may give rise to a strong NIR absorption band from intervalence charge-transfer (IVCT) transition.[4] Classical examples of these NIR dyes include Prussian Blue (Fe) and the Creutz−Taube ion (Ru).[5] Fundamental chemistry questions such as the synthesis, electronic spectra, and redox chemistry have been extensively studied on mixed-valence complexes,[6] yet the application of these intensively NIR-active complexes is less studied and has great potential as molecular probes, NIR light harvest devices, and/or medical applications.[7]

We were inspired by the mixed-valence diruthenium complex $[Ru_2TIEDCl_4]Cl$ (TIED = tetraiminoethylenedimacrocycle, denoted as $[\mathbf{Ru_2}]^+$, eq 1), first reported by Spreer and co-workers because of its narrow and intense band at ∼800 nm with a very high molar absorption coefficient of 68 000 M^{-1} cm^{-1}.[8] The unpaired electron in the complex is completely delocalized (class III complex), as suggested from the low dipole moment changes upon excitation, and the NIR band is the result of the delocalized intervalence transition.[9]



(1)

The valence-averaged $[\mathbf{Ru_2}]^+$ could be reduced electrochemically at 0.0 V to obtain neutral $[\mathbf{Ru_2}]$, which could be easily oxidized by oxygen to regenerate $[\mathbf{Ru_2}]^+$ (eq 1).[8] One can take advantage of the reversible redox reaction accompanied with strong NIR absorbance signal changes for the reversible sensing of redox-active molecules. Indeed, documented herein are our results on using $[\mathbf{Ru_2}]^+$ as a selective and sensitive NIR probe for the reversible sensing and quantification of hydrogen sulfide (H_2S). H_2S is a colorless and toxic gas well-known for its pungent odor of rotten eggs yet lesser known for its important biological role as a gaseous mediator for cardiovascular and neuronal health.[10] In the human body, H_2S is formed endogenously from cysteine and homocysteine by two pyridoxal-5′-phosphate-dependent enzymes, cystathionine-γ-lyase and cystathionine-β-synthetase.[11] HS^- (the pK_a of H_2S is 6.76[12]) has been detected at micromolar concentration in the lung, heart, brain, and blood.[13]

The addition of different concentrations of NaHS in a Tris-HCl buffer solution (50 mM, pH 7.40) to a $[\mathbf{Ru_2}]^+$ (16 μM) solution led to a quick (∼1 min) decay of the IVCT band at 789 nm and the concurrent appearance of a new peak at 895 nm due to $[\mathbf{Ru_2}]$, as shown in Figure 1. The spectra also feature an isosbestic point at 840 nm, indicating a quantitative reaction. The plot of the ratio of absorbance at 895 nm and that at 789 nm versus the concentration of HS^- gives a good linear response up to 16.0 μM (inset of Figure 1), but it takes over 32 μM HS^- to completely reduce $[\mathbf{Ru_2}]^+$. With this linear relationship, the quantification of HS^- is realized using $[\mathbf{Ru_2}]^+$ with a good limit of detection at 1.35 μM and the limit of quantification at 2.32 μM.

When the reaction solution was kept at room temperature for an extended period (30 min), we observed clean re-formation of $[\mathbf{Ru_2}]^+$. To further verify that the reversibility of the reaction is indeed caused by oxygen dissolved in the solution, we conducted the equimolar reaction of $[\mathbf{Ru_2}]^+$ (16 μM) with NaHS under a N_2 atmosphere. The NIR spectra show that $[\mathbf{Ru_2}]^+$ is reduced to $[\mathbf{Ru_2}]$ and remains reduced (Figure 2, dotted lines) for an extended period (42 min), indicating that the reaction between $[\mathbf{Ru_2}]^+$ and HS^- is not reversible per se. Upon exposure to air, $[\mathbf{Ru_2}]$ is oxidized back to $[\mathbf{Ru_2}]^+$ quantitatively (Figure 2, red and black solid lines). Therefore, $[\mathbf{Ru_2}]^+$ may be utilized as a reusable molecular probe for HS^- with the help of oxygen in the system.

The reusability of $[\mathbf{Ru_2}]^+$ for HS^- quantification was examined. When a limiting amount of HS^- (8.0 μM) was added to a buffered solution of $[\mathbf{Ru_2}]^+$ (16 μM), about 50% of $[\mathbf{Ru_2}]^+$ was quickly reduced by HS^- within 1 min and slowly oxidized, as demonstrated by a A_{895}/A_{789} versus time plot (Figure 3). To our surprise, a second addition of 8.0 μM HS^- only reduces about one-eighth of the total $[\mathbf{Ru_2}]^+$ revealed from the A_{895}/A_{789} ratio. The third and fourth additions of 0.5 equiv of HS^- led to results

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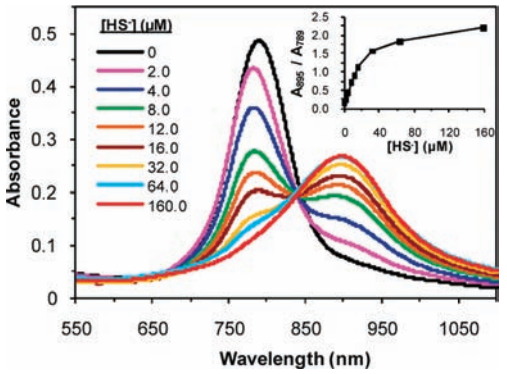

Figure 1. Absorption spectra of $[\mathbf{Ru_2}]^+$ (16 μM) in a 50 mM Tris-HCl buffer (pH 7.40) recorded 1 min after reactions with various concentrations of HS^- (2, 4, 8, 12, 16, 32, 64, and 160 μM). Inset: Plot of the absorbance ratio A_{895}/A_{789} vs $[HS^-]$. There is a linear response in the range below 16 μM HS^- with the regression line, $A_{895}/A_{789} = 0.0729\text{-}[HS^-] + 0.1353$. $R^2 = 0.9996$.

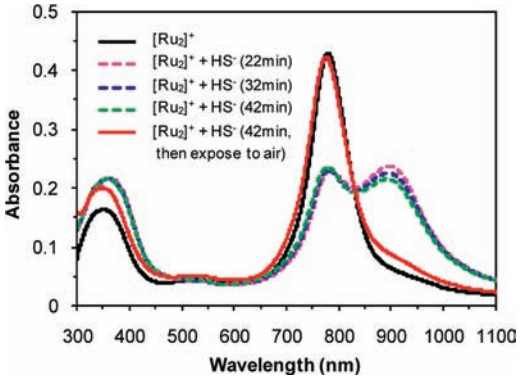

Figure 2. Absorption spectra of $[\mathbf{Ru_2}]^+$ (16 μM) in a 50 mM Tris-HCl buffer (pH 7.40) before and after the addition of HS^- (16 μM) under a N_2 atmosphere with time and upon exposure to air after 42 min.

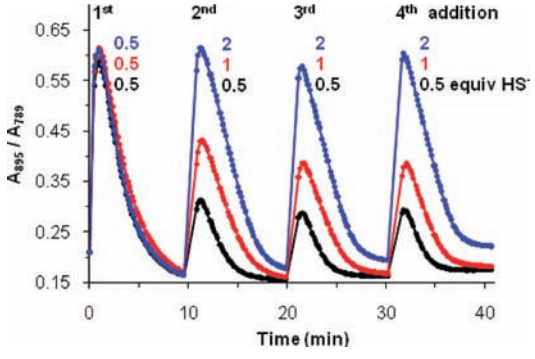

Figure 3. Catalytic circles of $[\mathbf{Ru_2}]^+$ in the HS^-/O_2 system observed by the absorbance ratio changes recorded in real time of $[\mathbf{Ru_2}]^+$ (16 μM) in a 50 mM Tris-HCl buffer (pH 7.40) after the first addition of HS^- (8 μM) and the second, third, and fourth additions of HS^- (8, 16, and 32 μM).

similar to those of the second addition (Figure 3, black line). When 16 μM HS^- were added for the second, third, and fourth round, 50% of the signal (A_{895}/A_{789}) was regained (Figure 3, red line). Full recovery of A_{895}/A_{789} is achieved after the addition of 32 μM HS^- each time (Figure 3, blue line).

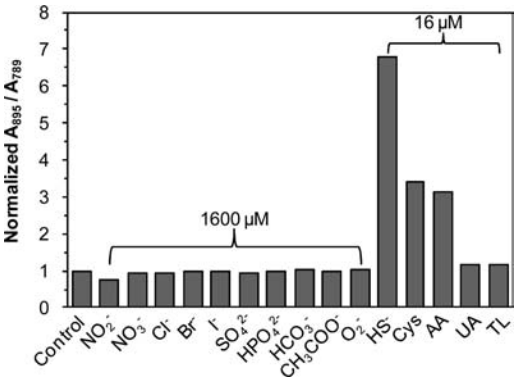

Figure 4. Absorbance ratio changes of $[\mathbf{Ru_2}]^+$ (16 μM) in a 50 mM Tris-HCl buffer (pH 7.40) recorded 1 min after the addition of various anions (1600 μM) and reductants (16 μM). Cys, AA, UA, and TL denote cysteine, ascorbic acid, uric acid, and Trolox, the water-soluble vitamin E analogue, respectively. All of the data are normalized with respect to the absorbance ratio of $[\mathbf{Ru_2}]^+$ before the addition.

To gain more insight into the reaction, we analyzed the reaction products. Under 5% $[\mathbf{Ru_2}]^+$ loading, HS^- oxidation generates about 0.5 equiv of H_2O_2 per HS^- oxidized, as quantified using a ferrous ion oxidation−xylenol orange (FOX) assay.[14] HS^- was converted to H_2S_2 as indicated by the similarity of the UV−vis spectra of the reaction products and that of authentic $NaHS_2$ prepared independently by following a literature method (Figure S3 in the Supporting Information).[15] On the basis of these data, we proposed the following catalytic reaction mechanism:

$$HS^- + [\mathbf{Ru_2}]^+ \rightarrow [\mathbf{Ru_2}] + S^{\bullet -} + H^+ \quad (2)$$

$$[\mathbf{Ru_2}] + O_2 \rightarrow [\mathbf{Ru_2}]^+ + O_2^{\bullet -} \quad (3)$$

$$S^{\bullet -} + HS^- \rightarrow [HSS^{\bullet 2-}] \quad (4)$$

$$HSS^{\bullet 2-} + O_2 \rightarrow O_2^{\bullet -} + HSS^- \quad (5)$$

$$2O_2^{\bullet -} + 2H^+ \rightarrow H_2O_2 + O_2 \quad (6)$$

Net reaction:

$$2HS^- + H^+ + O_2 \rightarrow HSS^- + H_2O_2 \quad (7)$$

First, a rapid single-electron-transfer reaction resulted in the formation of $HS^{\bullet}$, which has a low pK_a value and will dissociate to give $S^{\bullet -}$ under the reaction conditions (eq 2).[16] In the presence of oxygen, $[\mathbf{Ru_2}]$ transfers an electron to molecular oxygen in the solution at a slower rate and generates superoxide and $[\mathbf{Ru_2}]^+$ to complete the catalytic cycle (eq 3). The formed $S^{\bullet -}$ would react with HS^- to give $HSS^{\bullet 2-}$, which may lose an electron to oxygen and form HSS^-. Finally, the formed superoxide anion dismutates to oxygen and H_2O_2 (eq 6). The net reaction is a catalyzed oxidation of HS^- to HS_2^- and H_2O_2 (eq 7). Upon an extended reaction time (1 day), elemental sulfur was formed, as detected by electron ionization mass spectrometry of the reaction mixture. It takes a little over 2 equiv of HS^- to fully reduce $[\mathbf{Ru_2}]^+$, as shown in Figure 1, indicating that HS^- is consumed by other pathways (eq 4) in addition to reacting with $[\mathbf{Ru_2}]^+$. It was proposed that $S^{\bullet -}$ could react with O_2 to give a sulfur peroxyl anion radical, $SOO^{\bullet -}$, which rearranges to a sulfur dioxide anion

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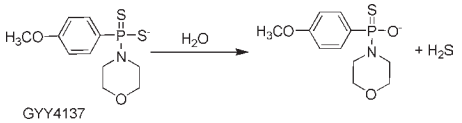
radical, $SO_2^{\bullet-}$. $SO_2^{\bullet-}$ could reduce the oxygen to give a superoxide anion and SO_2.[17] Under pH 7.4 conditions, we would expect the formation of SO_3^{2-} because the pK_{a2} of HSO_3^- is 7.20.[18] However, the UV−vis spectra of the reaction products bear little similarity to that of SO_3^{2-} (Figure S3 in the Supporting Information). In addition, if this mechanism were operating, we would expect one HS^- to generate one H_2O_2, which is not supported by our experimental data. The partial reduction of $[\mathbf{Ru_2}]^+$ in the subsequent addition of HS^- shown in Figure 3 is likely due to the reaction of formed H_2O_2 with added HS^-.[19] Consequently, more than 1 equiv of HS^- has to be added in order to reach the same degree of reduction as that in the first addition. It is foreseeable that removal of the formed H_2O_2 from the system would lead to full response in the subsequent addition of HS^-.

For a good molecular probe, high selectivity is an essential feature and $[\mathbf{Ru_2}]^+$ exhibits good selectivity for HS^- (1 equiv) compared to other anions of biological relevance. These include NO_2^-, NO_3^-, Cl^-, Br^-, I^-, SO_4^{2-}, HPO_4^{2-}, HCO_3^-, CH_3COO^-, and $O_2^{\bullet-}$ even though they are in large excess (100 equiv; Figure 4). Not all anions tested herein possess reducing activity, but ligand substitution (replacement of the Cl^-) may occur to cause a shift of the IVCT band. This is not the case here because we did not observe much change of the NIR spectra caused by these anions under the assay conditions.

The reactivity of $[\mathbf{Ru_2}]^+$ was examined with common biological reductants such as cysteine, ascorbic acid (vitamin C), uric acid, and vitamin E (in the form of Trolox, a water-soluble α-tocopherol analogue). Equimolar amounts of cysteine and ascorbic acid were able to reduce $[\mathbf{Ru_2}]^+$ to about half its amount compared to that by HS^- (Figure 4). The negative charge on HS^- may help its interaction with the cationic $[\mathbf{Ru_2}]^+$ and facilitate the reaction. Uric acid and Trolox failed to react. Nonetheless, for biological fluids containing a mixture of thiols, vitamin C, and HS^-, quantification of HS^- is only accurate after these interfering compounds are selectively removed either chemically or enzymatically.

A potential application of $[\mathbf{Ru_2}]^+$ is in quantifying H_2S by NIR absorbance. To illustrate such an application, we measured the H_2S releasing rate from a H_2S donor agent, morpholin-4-ium 4-methoxyphenyl(morpholino)phosphinodithioate, GYY4137.[20,21] Using $[\mathbf{Ru_2}]^+$ as the probe, the GYY4137 hydrolysis rate constant, k, is determined to be $(3.33 \pm 0.25) \times 10^{-7}$ s^{-1} (Figure S9 in the Supporting Information). The accuracy of our method is determined to be excellent at 98 ± 4% recovery of the control sample of HS^-.



GYY4137
cation is omitted for clarity

In conclusion, we have demonstrated that NIR-active $[\mathbf{Ru_2}]^+$ can catalyze the air oxidation of HS^- through a single-electron-transfer reaction to generate hydrogen peroxide, disulfane, and elemental sulfur. The NIR absorbance ratio of the two resting states of the catalyst, $[\mathbf{Ru_2}]^+$ and $[\mathbf{Ru_2}]$, allows for the convenient determination of HS^-.

■ ASSOCIATED CONTENT

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

■ AUTHOR INFORMATION

Corresponding Author
*E-mail: chmhdj@nus.edu.sg.

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