Controlling the Energy and Electron Transfer in a Novel Ruthenium Bipyridyl Complex: An ESR Study

Eylon Yavin,[†] Lev Weiner,[‡] Rina Arad-Yellin,[†] and Abraham Shanzer*,[†]

Department of Organic Chemistry, The Weizmann Institute of Science, Rehovot, Israel, 76100, and Department of Chemical Services, The Weizmann Institute of Science, Rehovot, Israel, 76100

Received: March 26, 2001; In Final Form: June 11, 2001

A novel ruthenium complex has been synthesized. It is composed of three bipyridyl ligands, one of which is modified and has two hydroxamate groups. Photoexcitation of the complex with blue light ($\lambda_{max} = 477$ nm) leads to the formation of a long-lived nitroxyl radical on hydroxamate as was detected and characterized by ESR. In anaerobic conditions, the radical was not formed, suggesting that a reactive oxygen species is required for generating the nitroxyl radical. The quenching of the excited state of ruthenium bipyridyl complexes by molecular oxygen can generate either singlet oxygen via energy transfer or superoxide radical via electron transfer. In this latter case the superoxide radical is confined in a cage complex (vide infra). Singlet oxygen, generated via energy transfer from Ru(II) in its excited state, is the reactive species that is responsible for the oxidation of the hydroxamate group to its corresponding nitroxyl radical. This was confirmed by using a specific quencher (sodium azide) and by following the kinetics of the nitroxyl radical formation in deuterated solvents. Moreover, we can turn on the electron-transfer pathway by liberating superoxide radicals and producing a strong oxidant, Ru(III), from the collision "cage" complex proposed earlier (Zhang, X.; Rodgers, M. A. J. *J. Phys. Chem*. **1995**, *99*, 12797−12803.) This was achieved using compounds with either chemical (spin traps) or enzymatic (superoxide dismutase) affinity to superoxide radicals. Thus, the rate and yield of the nitroxyl radical formation in the novel ruthenium complex can be increased by almost thirty times.

Introduction

In the past two decades a tremendous effort has been made to utilize the powerful oxidation potential of photogenerated ruthenium(III) polypyridyl complexes to catalyze the oxidation of water[1−4] and to photooxidize DNA and RNA.[5−10] Furthermore, ruthenium complexes incorporated into specific sites of proteins and nucleic acids were used to study photoinduced electron-transfer processes in these biopolymers.[5,11−19]

In the presence of molecular oxygen, the quenching of excited states of ruthenium tris(bipyridyl) may lead to one of two major processes. One is the generation of singlet oxygen and ground-state ruthenium via energy transfer (eq 1); and the other is the generation of superoxide radical and Ru(III) via electron transfer (eq 2). On the basis of transient absorption spectroscopy of Ru(II), Zhang and Rodgers[20] have suggested that in the latter case, a "cage" complex consisting of Ru(III) and superoxide radical could be formed. In neutral pH the cage complex is not liberated, thereby providing a zero quantum yield for the electron-transfer process. However, in acidic conditions (3 N D_2SO_4), the proton can release the cage complex (eq 3) elevating the quantum yield to 0.55.[20]

Hydroxamates are bi-dentate ligands that are found in many of the natural ion-binding molecules, especially the iron binding (siderophores) complexes. Desferrioxamine is one of such molecules. Due to its high affinity to Fe(III), it generates an octahedral complex with a stability constant of 10^{31} M^{-1}.[21] In

$$\overset{*}{Ru}L_3^{2+} + {}^3O_2 \xrightarrow{ET} RuL_3^{2+} + {}^1O_2 \quad (1)$$

$$\overset{*}{Ru}L_3^{2+} + {}^3O_2 \xrightarrow{e^-T} RuL_3^{3+} + O_2^{\bullet-} \quad (2)$$

$$\underset{\text{cage}}{\left[RuL_3^{3+}\text{---}O_2^{\bullet-}\right]} + H^+ \rightleftharpoons RuL_3^{3+} + HO_2^{\bullet} \quad (3)$$

neutral solutions, in the presence of hydroxyl and superoxide radicals, hydroxamates of desferrioxamine are oxidized to relatively stable nitroxyl radicals.[22,23] Hydroxamates with bulky groups form nitroxyl radicals in acidic solutions even at ambient light.[24] This effect has been attributed to the stabilization of the nitroxyl radical in acidic medium.

We proposed that combining a ruthenium complex with hydroxamate groups in a single molecule should provide a useful tool to monitor photoinduced reactions between Ru(II) and molecular oxygen. We assumed that Ru(III), an electron-transfer product with a high redox potential, E_0 ($Ru^{2+/3+}$) = 1.29V,[25] could oxidize the hydroxamate to its corresponding nitroxyl radical. Such a process may be monitored by following the formation of the nitroxyl radicals by ESR

With this in mind, we synthesized a ruthenium tris-bipyridine complex (**Ru-2**), in which one of the bipyridyls was substituted with two strands, each terminated by an hydroxamate group.

Excitation of **Ru-2** with blue light generates the nitroxyl radical. In this case singlet oxygen, produced by energy transfer from excited **Ru-2**, is the reactive oxygen species responsible for producing the nitroxyl radical.

Using compounds with a high affinity to superoxide radicals such as a spin trap *N-tert*-butyl-α-phenylnitrone (PBN), or an

* Author to whom correspondence should be addressed. Fax: 972-8-9342917. E-mail: abraham.shanzer@weizmann.ac.il.
[†] Department of Organic Chemistry.
[‡] Department of Chemical Services.

10.1021/jp0111113 CCC: $20.00
Published on Web 08/07/2001

enzyme−superoxide dismutase (SOD), the energy transfer pathway was switched to the electron transfer pathway providing effective generation of Ru(III). Ru(III) dramatically enhanced (thirty times) the generation of the nitroxyl radicals. This result supports the hypothesis of the "cage complex" formed in our Ru (II) complex.

Experimental Section

Instrumentation. ^{1}H NMR spectra were measured on an Avance DPX-400 MHz or DPX-250 MHz spectrometers (Bruker) using the solvent deuterium signal as an internal reference. All *J* values are given in Hertz. IR spectra were recorded on a Protégé 460 FTIR spectrometer. UV/Vis spectra were measured with a Hewlett-Packard model 8450A diode array spectrophotometer. LCQ mass spectra were taken at the Protein Center of the Technion, Haifa. Flash chromatography was performed using Merck 230-400 mesh silica gel. Thin-layer chromatography (TLC) on 60 F-254 silica gel was visualized by UV light and by one or more of the following reagents: ninhydrine, basic $KMnO_4$ solution, iodine, or by $FeCl_3$ in MeOH. X-band ESR spectra were recorded on an electron spin resonance ER 200D-SRC spectrometer (Bruker) at room temperature using a flat quartz cell (70 μL). For computer simulation of ESR spectra the public ESR software distribution developed by D. R. Duling (NIEHS) was used.

Solvent and Reagent Pretreatment. Dichloromethane (DCM) was dried by passing the solvent through a basic alumina column. Tetrahydrofuran (THF) was distilled from Na under argon. *cis*-Dichloro bis(2,2′-bipyridine) ruthenium(II) dihydrate was purchased from STREM chemicals. All enzymes (superoxide dismutase, catalase, cytochrome C, and xanthine oxidase), chemicals, and reagents were purchased from Sigma. Double distilled water and spectroscopic grade CH_3CN were used for ESR experiments. Where necessary, reactions were carried out in argon atmosphere.

Catalytic Activity of Enzymes. Enzymatic activity of catalase was assessed by adding the solution of enzyme in phosphate buffer (PBS) to solution of hydrogen peroxide in the same buffer. The time-dependent decrease of absorption of hydrogen peroxide at 240 nm ($\epsilon = 43\ M^{-1}\ cm^{-1}$) provided the value of the enzymatic activity. Activity of SOD was measured in a xanthine/xanthine oxidase and cytochrome C system according to McCord and Fridovich.[26]

ESR Experiments. The ESR experiments were carried out in CH_3CN/H_2O (93:7), PBS or DDW. A flat cell of 70 μL was used in all experiments when the recording of ESR spectra were accompanied by illumination of the cell. A 150 W lamp (Schott model KL 1500 LCD) adjusted with different filters was used as a light source. Ruthenium complexes were illuminated using a blue filter (380−500 nm). In the experiments with Rose Bengal, a yellow filter ($\lambda > 520$ nm) was used.

Synthesis. Abbreviations for the NMR spectra are as follows: s-bipy means substituted bipyridine, bipy means unsubstituted bipyridines, and ov refers to overlapping proton peaks. Proton correlation for the ruthenium complexes (**Ru-1** and **Ru-2**) was established by COESY ^{1}H NMR.

Compound **5** was synthesized according to Szemes et al.[27] with slight modifications: Bipy-4,4′-dicarboxylic acid (328 mg, 1.34 mmol) was suspended in $SOCl_2$ and was refluxed under argon for 5 h. Excess $SOCl_2$ was evaporated, the diacyl chloride was dissolved in dry THF and triethylamine (415 μL, 3 mmol) was added. Compound **4** (590 mg, 2.75 mmol) dissolved in THF was added to the reaction mixture. The pH of the reaction was adjusted to eight by adding triethylamine, and the reaction mixture was left to stir overnight at room temperature. After removal of the solvents, the residue was purified by column chromatography, using mixtures of MeOH/$CHCl_3$ (0−5%) as eluent. A 410 mg quantity of compound **5** (50% yield) as obtained.

^{1}H NMR (250 MHz, $CDCl_3$) $\delta = 8.80$ (d, $J = 5$ Hz, 2H, bipy 6,6′), 8.72 (m, 2H, bipy 5,5′), 7.76 (m, 2H, bipy 3,3′), 7.42 (m, 2H, CON**H**), 5.00−5.15 (ov, 2H of THP and 2H of NHC**H**), 4.05, 3.65 (m, 4H of THP), 3.43, 3.36 (s, 6H, NC**H**$_3$), 1.66 (br, 12H, THP), 1.48 (m, 6H, CHC**H**$_3$). **IR** ($CHCl_3$): $\nu = 1640\ cm^{-1}$ (**CO**NO).

Ru-1 was prepared by refluxing compound **5** (130 mg, 0.078 mmol) and $Ru(bipy)_2Cl_2 \cdot 6H_2O$ (109 mg, 0.078 mmol) in an 80% ethanolic solution for 4 h under argon. The solvent was removed under vacuum and the compound was purified by column chromatography eluting with CH_3CN: BuOH: 0.2 M KNO_3 (8:0.5:1.5). A 178 mg quantity of the Ru-complex **Ru-1** (83% yield) was obtained.

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.87 (m, 2H, s-bipy 3,3′), 9.2 (br, 2H, CON**H**), 8.70 (m, 4H, bipy 3,3′), 8.05 (m, 4H, bipy 4,4′), 7.90 (m, 2H, s-bipy 5,5′), 7.76 (m, 4H, bipy 6,6′), 7.64 (m, 2H, s-bipy 6,6′), 7.45 (m, 4H, bipy 5,5′), 5.31 (br, 2H, THP), 4.85, 5.00 (br, 2H, NHC**H**), 4.00 and 3.65 (m, 4H of THP), 3.30−3.36 (ov, 6H, NC**H**$_3$), 1.56−1.66 (ov, 12H, THP and 6H, CHC**H**$_3$). **IR** (KBr): ν 1640 cm^{-1} (**CO**NO).

Ru-2 was prepared by dissolving 78 mg of **Ru-1** (0.076 mmol) in acetic acid: water (1:1) and heating to 60 °C for 1 h. The solvent was evaporated and the compound was dissolved in minimum amount of MeOH and added into a cold diethyl ether solution. The flask was left overnight at 4 °C and the compound was filtered and washed with diethyl ether. A 57 mg quantity of **Ru 2** (97% yield) was obtained.

^{1}H NMR (400 MHz, CD_3CN + 10% MeOH-d_4): $\delta = 9.34$ (s, 2H, s-bipy 3,3′), 8.53 (d, $J = 7$ Hz, 4H, bipy 3,3′), 8.03 (m, 4H, bipy 4,4′), 7.86 (d, $J = 5.7$ Hz, 2H, s-bipy 5,5′), 7.72 (ov, 4H of bipy 6,6′ and 2H of s-bipy 6,6′), 7.39 (m, 4H, bipy 5,5′), 5.17 (m, 2**H**, NHCH), 3.18 (s, 6H, NC**H**$_3$), 1.36 (m, 6H, CHC**H**$_3$). **IR** (KBr): $\nu = 1635\ cm^{-1}$ (**CO**NO).

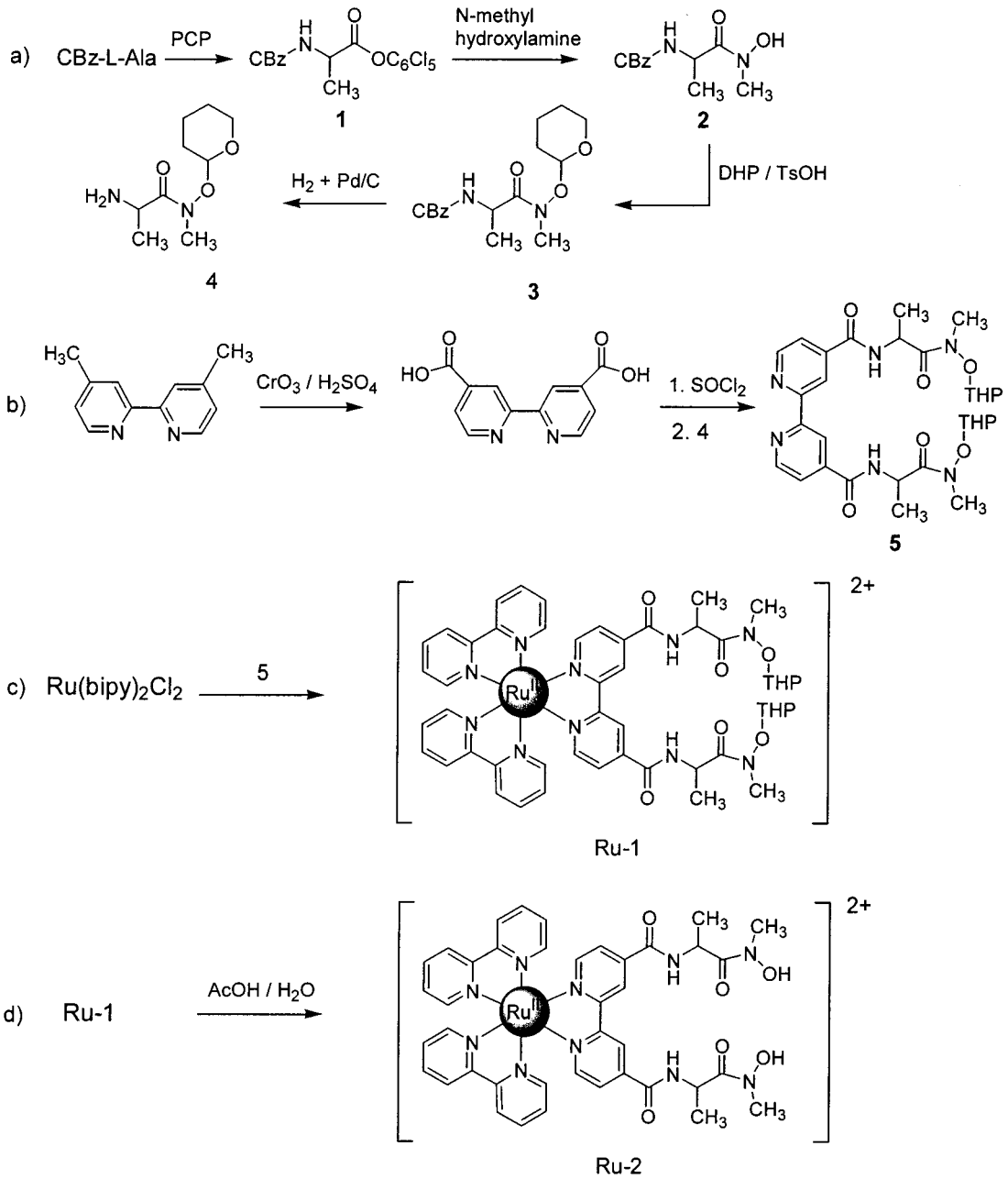
UV/Vis: λ_{max} (ϵ) = 289 (47200) and 477 (8800) nm. **MS-ES:** $m/z = 858\ [M-H]^{1+}$.

Results and Discussion

Preparation of Ruthenium Complex (Ru-2). The strategy used for the synthesis of the complex is illustrated in Scheme 1. The synthetic approach was to initially prepare the substituted bipyridine (**5**) derivative and then to react it with *cis*-$Ru(bipy)_2Cl_2 \cdot 2H_2O$. This was done by coupling the active ester of L-Cbz-Ala (**1**) with *N*-methyl hydroxylamine, which yielded the hydroxamate (**2**).[28] Protection of the OH group of the hydroxamate with THP[29] (**3**) and removal of the benzyl carbamate protection group by hydrogenation[30] yielded compound **4**, which had a free amine group. This compound was then coupled to the 4,4′-dicarboxy-2,2′-bipyridine, which was previously synthesized from 4,4′-dimethyl-2,2′-bipyridine,[31] and yielded compound **5**. This derivative and *cis*-$Ru(bipy)_2Cl_2 \cdot 2H_2O$ were refluxed in 80% ethanol yielding **Ru-1**, which was further heated in $AcOH/H_2O$ to give the nitrate salt of **Ru-2**.

*1. Energy Transfer in Ruthenium Complex (**Ru-2**).* Photoexcitation of **Ru-2** in a CH_3CN/H_2O solution by visible light led to the formation of a radical with a *g*-factor of 2.0061 (Figure 1a). We assume that the unpaired electron of the radical is split by the nitrogen ($I = 1$) of the hydroxamate and by three

SCHEME 1



protons ($3 * I = 1/2$) of the neighboring methyl group generating a 12-line ESR signal.

A computer-simulated spectrum of such a *N*-methyl hydroxamate radical is very similar to the experimental spectrum (Figure 1b). The calculated hyperfine coupling constants are $a_N = 6.83$ G and $a_H = 7.87$ G. These values are in accordance with those observed for the radical of *N*-methyl-*N*-acetylhydroxylamine.[32] Scheme 2 presents the ruthenium complex (**Ru-2-radical**) in which the nitroxyl radical is formed on either one of the two strands of the molecule.

To evaluate the role of molecular oxygen on the formation of the nitroxyl radical, we prepared a sample of **Ru-2** in anaerobic conditions. No ESR signal was observed upon irradiation, an observation that has emphasized the role of both molecular oxygen and light for the formation of the nitroxyl radical.

Irradiation of ruthenium bipyridyl complexes in neutral pH solutions generates reactive oxygen species.[20] These species are singlet oxygen molecules that are formed via energy transfer from the excited ruthenium bipyridyl complex to molecular oxygen. To prove that singlet oxygen leads to the generation of the nitroxyl radical in our complex, the following experiments have been conducted:

1. The rate of appearance of the nitroxyl radical in ESR during irradiation of **Ru-2** in the presence of sodium azide (NaN_3) was monitored. A very small ESR signal (less than 1% the intensity) was generated (Figure 2). This observation is explained by the fact that NaN_3 quenches singlet oxygen.[33−35] In a control experiment it was found that NaN_3 has a minor effect on the fluorescence spectrum of ruthenium(II) (data not shown), which suggests that the observed effect on the nitroxyl radical formation is due to quenching of singlet oxygen by NaN_3 rather than quenching of the MLCT states of the complex.

2. It is well documented that the steady-state concentration of singlet oxygen is higher in deuterated solvents, due to the increase of its lifetime.[36−39] Therefore we anticipated that in

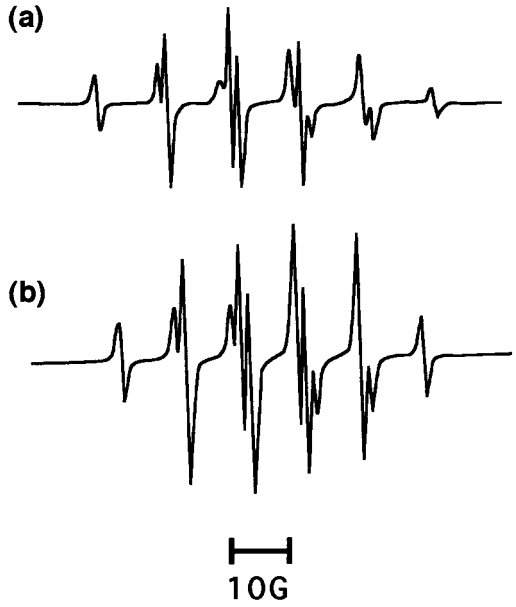


Figure 1. ESR spectrum of the nitroxyl radical; (a) experimental and (b) computer Simulation. $a_N = 6.83$ G, $a_{(3H)} = 7.87$ G.

deuterated solvents the process of the nitroxyl radical formation would be amplified. Monitoring the formation of the nitroxyl radical shows that in *d*-solvent mixture, (D_2O/CD_3CN), the formation of the radical is very fast and after ca. twenty minutes of irradiation the concentration of the nitroxyl radical has not yet reached a steady-state level. For the nondeuterated solvent mixture, a significantly lower steady-state concentration of the nitroxyl radical was obtained (Figure 2).

3. Tetramethyl piperidone (TEMP) is a spin trap commonly used for the detection of singlet oxygen in chemical and biological systems.[40,41] It forms a spin adduct, which consists of a stable nitroxyl radical (TEMPO), producing a triplet ESR spectrum.[40] Irradiation of **Ru-2** (1.8 mM) in the presence of TEMP (100 mM) has yielded an ESR signal, which is the superposition of the ESR spectra of TEMPO ($a_N = 14.8$ G) and our nitroxyl radical (Figure 3). The integral intensities of the observed radicals were comparable; however, [TEMP]/[Ru-2] ~ 50. Hence, the hydroxamate moiety and TEMP are competing for singlet oxygen produced under irradiation of our complex, and the reaction of **Ru-2** with singlet oxygen is much more efficient than that of TEMP.

Formation of nitroxyl radical in hydroxamic acids by the attack of singlet oxygen was not reported before. Therefore we decided to use a known source of singlet oxygen to examine the formation of the nitroxyl radical. This was done by studying a model compound containing a bis-hydroxamate ligand without the ruthenium tris-bipyridine core. The model compound (**m-2**), which was synthesized in our laboratory,[42] is depicted in Scheme 3. As a control experiment, a solution of Rose Bengal[43] was irradiated using a yellow filter ($\lambda > 520$ nm) in the presence of TEMP. The typical triplet ($a_N = 14.9$ G), characteristic of the paramagnetic TEMP adduct, TEMPO, indicated the production of singlet oxygen. Photoexcitation of the Rose Bengal solution in the presence of **m-2** produced the nitroxyl radical ESR spectrum, which increased with irradiation time.

The signal intensity of the nitroxyl radical formed under irradiation of **Ru-2** solution was 6-fold higher that that for equimolar solution of $Ru(bipy)_3$ and **m-2**. This may be attributed to the close proximity of the hydroxamate moiety to the ruthenium(II) ion in the bipyridyl complex, i.e., the pathway of singlet oxygen should be very short. This adds additional evidence that the oxidation process is more effective for **Ru-2** than for TEMP (see Figure 3).

Thus, singlet oxygen is the reactive oxygen species that once generated in the system, leads to the formation of the nitroxyl radical. The mechanism, which is presented in eqs 4−6, is based solely on the energy-transfer process from the excited ruthenium complex to molecular oxygen.

$$\text{Ru-2(II)} \xrightarrow{h\nu} {}^{*}\text{Ru-2(II)} \quad (4)$$

$${}^{*}\text{Ru-2(II)} + {}^{3}O_2 \xrightarrow{ET} \text{Ru-2(II)} + {}^{1}O_2 \quad (5)$$

$$\text{Ru-2(II)} + {}^{1}O_2 \longrightarrow \text{Ru-2(II)-radical} \quad (6)$$

Irradiation of **Ru-2** in a neutral pH buffer solution induces the formation of nitroxyl radicals, although the effect is less pronounced than that obtained for CH_3CN/H_2O solution. It can be attributed to the shorter lifetime of singlet oxygen in water (4.2 μs) than in acetonitrile (58 μs).[39]

Ruthenium poly-pyridyl complexes in their excited state are efficient in reducing metal ions such as copper[44−50] and iron[51−55] and can reduce molecular oxygen to superoxide radicals.[20] In our case, this reduction (via electron transfer) is assumed to be inefficient, since the cage complex that may be formed (eq 7) decays rapidly to the ruthenium complex in its ground state and molecular oxygen.[20]

$$\text{Ru-2(II)} \xrightarrow{h\nu} {}^{*}\text{Ru-2(II)} + {}^{3}O_2 \xrightarrow{e^{-}T} [\text{Ru-2(III)}\text{---}O_2^{\bullet -}] \quad (7)$$

Back $e^{-}T$

*2. Electron Transfer in Ruthenium Complex (**Ru-2**). 2.a. Electron Transfer in **Ru-2** by Chemical Recognition.* The possibility of Ru(III) generation by oxidation of Ru(II) by a sacrificial electron acceptor or by molecular oxygen was explored. In the latter case the idea was to liberate the superoxide radical from its cage complex, thereby producing Ru(III). Ru(III) has a high oxidation potential[25,56] which may lead to the formation of our nitroxyl radical and Ru(II).

Several experiments were performed:

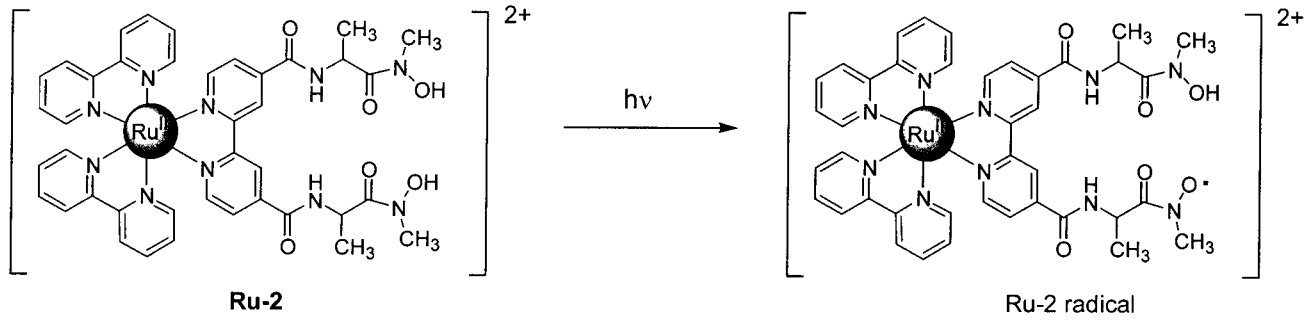
1. Solutions of **Ru-2** (1 mM) and ammonium persulfate (10 mM), as a sacrificial electron acceptor, were prepared either in CH_3CN/H_2O or in H_2O and the spontaneous formation of the nitroxyl radical via oxidation by Ru(III)[57] was observed in ambient light. More than a 10-fold increase of the nitroxyl radical formation was observed upon light excitation (data not shown).

2. As an additional electron acceptor, we used Co(EDTA) that can oxidize Ru(II)* to Ru(III).[58] Stimulation of the nitroxyl radical was observed in a mixture of **Ru-2** and Co(EDTA) under continuous irradiation.

These two experiments show that the direct oxidation of Ru(II)* to Ru(III) can stimulate the generation of the nitroxyl radical in our system.

3. To liberate superoxide radicals from our cage complex and thereby to produce Ru(III), we used a traditional spin trap, N-*tert*-butyl-α-phenylnitrone (PBN). PBN reacts with reactive oxygen species, such as hydroxyl and superoxide radicals.[59,60] The addition of PBN to both CH_3CN/H_2O and H_2O solutions of **Ru-2** has yielded a rapid enhancement of the formation of the 12 line ESR spectra of our nitroxyl radical on the hydroxamate group under irradiation. Monitoring the changes in intensity of the peak of the radical (a doublet at 3273.7 G) with time in the presence/absence of PBN has revealed a totally different behavior for the two cases (Figure 4). Addition of PBN

SCHEME 2



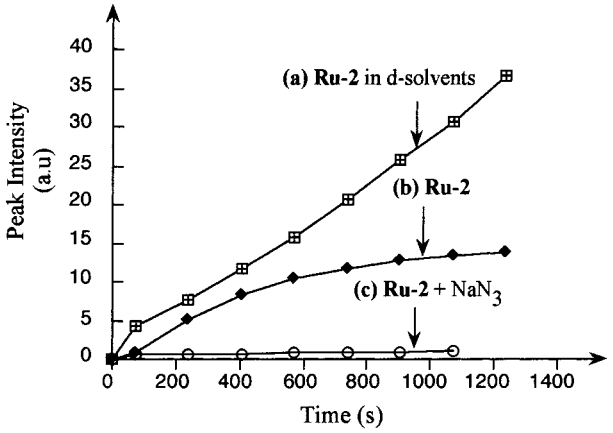


Figure 2. The kinetic profile of the radical formation of the nitroxyl radical for irradiated solution of **Ru-2** (1 mM) in (a) deuterated, (b) nondeuterated solvents, and (C) the presence of 1 mM NaN_3.

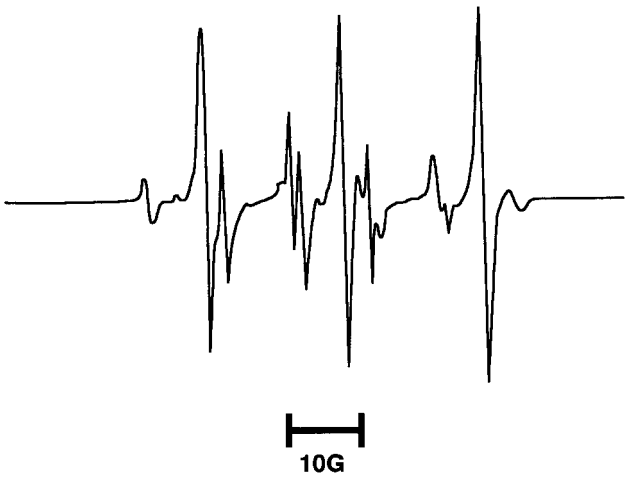


Figure 3. ESR spectrum of the nitroxyl radical superimposed on the TEMPO radical for irradiated solution of **Ru-2** (1.8 mM) and TEMP (0.1 M). Microwave power 8 mV, modulation amplitude = 0.5 G.

was found to increase dramatically the nitroxyl radical formation reaching a short steady-state level where after a rapid decrease in the nitroxyl radical ESR signal is observed. This decrease may be attributed to the further oxidation of the nitroxyl radical to its corresponding diamagnetic N-oxo-ammonium ion[61] or to the loss of radicals by termination of two adjacent nitroxyl radicals. In the case of irradiation of **Ru-2** without PBN, a monotonic linear increase of the nitroxyl radical is due to the oxidation of the hydroxamate by singlet oxygen (vide supra).

Apparently, PBN can release superoxide from the cage complex by forming the superoxide-adduct thereby liberating **Ru-2**(III), which, in turn, oxidizes the hydroxamate to its corresponding nitroxyl radical. Appearance of nitroxyl radicals

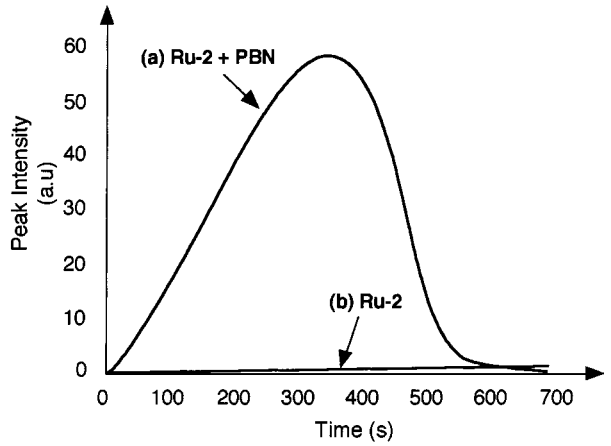
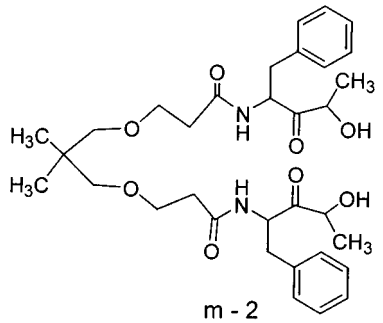


Figure 4. The kinetic profile of the nitroxyl radical formation for irradiated solution of **Ru-2** (1 mM) in the presence (a) and absence (b) of PBN (0.05 M). The ESR signal was fixed at 3273.7 G (doublet) and recorded with a receiver gain of 5×10^4 and modulation amplitude of 2 G.

SCHEME 3



of the hydroxamate was stimulated significantly by another popular spin trap DMPO (data not shown).

To support this assumption, a sample of **Ru-2** and PBN in CH_3CN/H_2O was prepared and irradiated under argon. No radical formation was observed, indicating that the PBN molecule reacts with the reactive oxygen species rather than with the ruthenium complex itself. When the sample was exposed to air and irradiated, the formation of the radical was observed.

To obtain a clearer view of the reactive oxygen species that are involved in our system, we irradiated the hydroxamate-protected ruthenium complex (**Ru-1**) in the presence of PBN and DMPO. The values $a_N = 14.15$ G and $a_H = 2.12$ G for the spin adduct of PBN (Figure 5b) are nearly the same as the hyperfine constants of PBN with superoxide and hydroxyl radicals.[62] In the case of DMPO, a quartet with an intensity ratio of 1:2:2:1 and hyperfine constants $a_N = a_H = 14.8$ G was

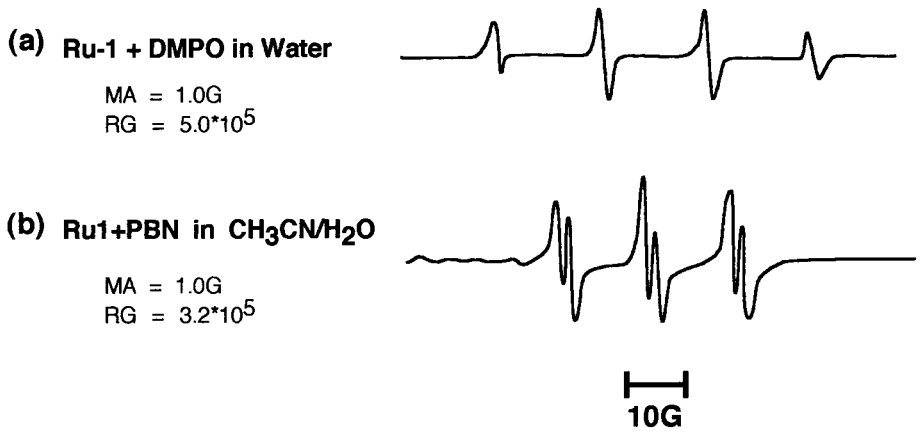


Figure 5. ESR Spectra of 1 mM **Ru-1** irradiated with two different spin traps: (a) 50 mM DMPO, and (b) 50 mM PBN. Microwave power = 20 mV.

observed (Figure 5a). Such an ESR spectrum is characteristic of the spin adduct of DMPO with OH radical. However, it may also result from a spontaneous transition of the DMPO−OOH adduct.[63]

ESR signals of the adducts of PBN and DMPO with oxygen radicals were registered upon irradiation of the complex. These spectra support the assumption that the superoxide radicals are liberated from the cage by interaction of the oxygen radicals with the spin traps. Based on these findings, the following mechanism (eqs 7−9) is proposed:

$$\overset{*}{Ru}\text{-2(II)} + {}^3O_2 \xrightarrow{e^-T} [\text{Ru-2(III)}\cdots O_2^{\bullet -}] \xrightarrow{\text{Back } e^-T} \text{Ru-2(II)} + {}^3O_2 \quad (7)$$

$$[\text{Ru-2(III)}\cdots O_2^{\bullet -}] + \text{PBN} \longrightarrow \text{Ru-2(III)} + \text{PBN-adduct} \quad (8)$$

$$\text{Ru-2(III)} \xrightarrow{\text{Ox.}} \text{Ru-2(II)-radical} \quad (9)$$

*2.b. Electron Transfer in **Ru-2** by Enzymatic Recognition.* As was observed, a relatively small molecule, such as PBN, chemically reacts with oxygen radicals in the cage complexes, thereby producing the powerful oxidant, Ru(III). The question was whether it would be possible for large molecules, such as enzymes, to recognize the superoxide radical in its cage complex.

Superoxide dismutase (SOD) and catalase catalyze the following reactions (eqs 10 and 11):

$$2O_2^{\bullet -} + 2H^+ \xrightarrow{\text{SOD}} H_2O_2 + O_2 \quad (10)$$

$$H_2O_2 \xrightarrow{\text{Catalase}} H_2O + 1/2O_2 \quad (11)$$

In fact, irradiation of buffer solutions of **Ru-2** in the presence of SOD enhanced significantly (ca. 10-fold) the formation of nitroxyl radicals. Addition of catalase to the solution had a minor effect on the radical formation, excluding hydrogen peroxide and its products (namely, hydroxyl radicals) as contributors to the nitroxyl radical formation. A kinetic profile showing the enzymatic effect on the nitroxyl radical formation is presented in Figure 6. Obviously, the affinity of SOD to superoxide radicals is sufficient to liberate the cage complex and produce Ru(III).

Ru(III) is widely used to study long-range electron transfer in biopolymers.[14,16,17] Our finding that hydroxamic acid is extremely sensitive to oxidation by Ru(III) opens up new possibilities for studying such processes. Incorporation of both ruthenium complexes and hydroxamates into a designated location on DNA or proteins may provide a powerful tool for studying electron-transfer processes.

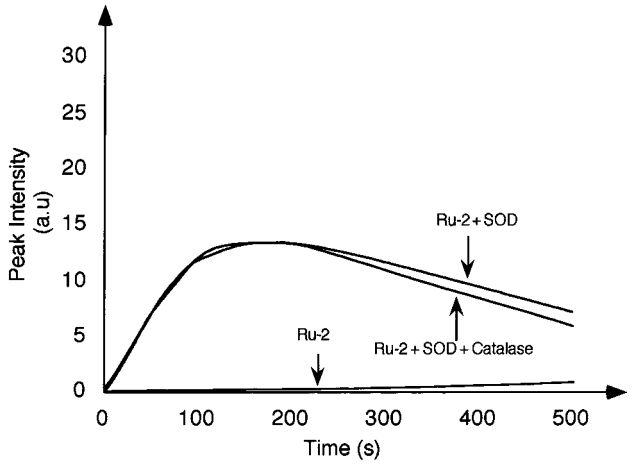


Figure 6. The effect of enzymes on the nitroxyl radical formation while irradiating a solution of **Ru-2** (1.8 mM). The ESR signal was fixed at 3625 G (singlet) and recorded with a receiver gain of 1.25×10^5 and modulation amplitude of 1 G.

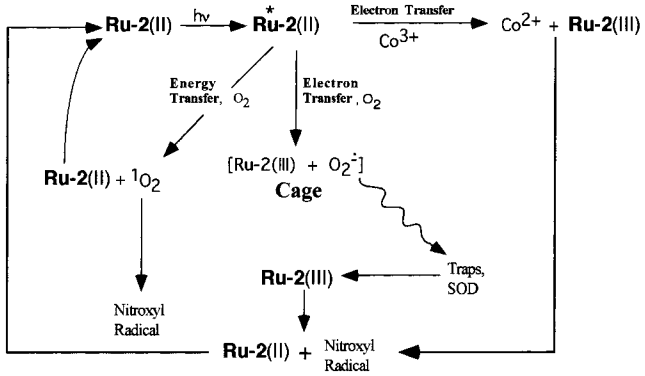


Figure 7. The miscellaneous photoinduced processes observed for the novel ruthenium complex **Ru-2**.

Summary

We have shown that in the novel ruthenium complex **Ru-2** there are two different pathways for generating nitroxyl radicals: energy- or electron-transfer pathway (Figure 7).

Photoexcitation of **Ru-2** produces a nitroxyl radical on the hydroxamate group due to singlet oxygen formed via *energy transfer* from the excited ruthenium complex to molecular oxygen. The efficiency of this process is low. By supplying an

external trigger (a spin trap or superoxide dismutase) that has a chemical/enzymatic affinity to superoxide radicals, we release the cage complex formed between Ru(III) and superoxide radical, thereby practically switching the energy-transfer pathway to that of an *electron transfer*. This, in turn, enhances dramatically the nitroxyl radical formation by ca. 30 times.

It is known that in the presence of hydroxyl radicals, hydroxamates are oxidized to their corresponding nitroxyl radicals.[64] As shown in this study, hydroxamates are also sensitive to singlet oxygen. Hence, such compounds may be used as nonselective but highly sensitive probes for sensing reactive oxygen species, which is relevant for oxidative stress in biological systems.

Finally, according to our findings, both ruthenium complexes and hydroxamates seem to be an advantageous couple for studying electron transfer stimulated by Ru(III) in biopolymers.

Acknowledgment. We thank Rachel Lazar for her dedicated work in synthesizing the model compound. This work was supported in part by the Israel Academy of Sciences. A.S. holds the Siegfried and Irma Ullman professorial chair.

References and Notes

(1) Ramaraj, R.; Kira, A.; Kaneko, M. *J. Chem. Soc., Faraday Trans. 1* **1986**, *82*, 3515−3524.
(2) Gilbert, J. A.; Eggleston, D. S.; Murphy, W. R. J.; Geselowitz, D. A.; Gersten, S. W.; Hodgson, D. J.; Meyer, T. J. *J. Am. Chem. Soc.* **1985**, *107*, 3855−3864.
(3) Ghosh, P. K.; Brunschwig, B. S.; Chou, M.; Creutz, C.; Sutin, N. *J. Am. Chem. Soc.* **1984**, *106*, 4772−4783.
(4) Kaneko, M.; Awaya, N.; Yamada, A. *Chem. Lett.* **1982**, *5*, 619−622.
(5) Wagenknecht, H. A.; Stemp, E. D. A.; Barton, J. K. *J. Am. Chem. Soc.* **2000**, *122*, 1−7.
(6) Ortmans, I.; Content, S.; Buotonnet, N.; Mesmaeker, A. K. D.; Bannawarth, W.; Constant, J.-F.; Defrancq, E.; Lhomme, J. *Chem. Eur. J.* **1999**, *5*, 2712−2721.
(7) Carter, P. J.; Cheng, C. C.; Thorp, H. H. *J. Am. Chem. Soc.* **1998**, *120*, 632−642.
(8) O'Reilly, F.; Kelly, J.; Mesmaeker, A. K. *Chem. Commun.* **1996**, 1013−1014.
(9) Cheng, C.-C.; Goll, J. G.; Neyhart, G. A.; Welch, W.; Singh, P.; Thorp, H. H. *J. Am. Chem. Soc.* **1995**, *117*, 2970−2980.
(10) Tossi, A. B.; Kelly, J. M. *Photochem. Photobiol.* **1989**, *49*, 545−556.
(11) Hamachi, I.; Tsukiji, S.; Shinkai, S.; Oishi, S. *J. Am. Chem. Soc.* **1999**, *121*, 5500−5506.
(12) Wilker, J. J.; Dmochowski, I. J.; Dawson, J. H.; Winkler, J. R.; Gray, H. B. *Angew. Chem., Int. Ed. Engl.* **1999**, *38*, 90−92.
(13) Di-Bilio, A. J.; Dennison, C.; Gray, H. B.; Ramirez, B. E.; Sykes, A. G.; Winkler, J. R. *J. Am. Chem. Soc.* **1998**, *120*, 7551−7556.
(14) Arkin, M. R.; Stemp, E. D. A.; Holmlin, R. E.; Barton, J. K.; Hormann, A.; Olson, E. J. C.; Barbara, P. F. *Science* **1996**, *273*, 475−480.
(15) Liu, R. Q.; Hahm, S.; Miller, M.; Durham, B.; Millett, F. *Biochem.* **1995**, *34*, 973−983.
(16) Murphy, C. J.; Arkin, M. R.; Ghatlia, N. D.; Bossmann, S.; Turro, N. J.; Barton, J. K. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 5315−5319.
(17) Murphy, C. J.; Arkin, M. K.; Jenkins, Y.; Ghatlia, N. D.; Bossmann, S. H.; Turro, N. J.; Barton, J. K. *Science* **1993**, *262*, 1025−1028.
(18) Winkler, J. R.; Gray, H. B. *Chem. Rev.* **1992**, *92*, 369−379.
(19) Margalit, R.; Kostic, N. M.; Che, C. M.; Blair, D. F.; Chiang, H. J.; Pecht, I.; Shelton, J. B.; Shelton, J. R.; Schroeder, W. A.; Gray, H. B. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 6554−6558.
(20) Zhang, X.; Rodgers, M. A. J. *J. Phys. Chem.* **1995**, *99*, 12797−12803.
(21) Halliwell, B.; Gutteridge, J. M. C. *Mol. Aspects Med.* **1985**, *8*, 89−193.
(22) Davies, M. J.; Donkor, R.; Dunster, C. A.; Gee, C. A.; Jonas, S.; Willson, R. L. *Biochem. J.* **1987**, *246*, 725−729.
(23) Morehouse, K. M.; Flitter, W. D.; Mason, R. P. *FEBS Lett.* **1987**, *222*, 246−250.
(24) Hinojosa, O.; Jacks, T. J.; Calamari, T. A.; Thibodeaux, D. P. *Photochem. Photobiol.* **1989**, *49*, 1−5.
(25) Kalyanasundaram, K. *Coord. Chem. Rev.* **1982**, *46*, 159−244.
(26) McCord, J. M.; Fridovich, I. *J. Biol. Chem.* **1969**, *244*, 6049−6055.
(27) Szemes, F.; Hesek, D.; Chen, Z.; Dent, S. W.; Drew, M. G. B.; Goulden, A. J.; Graydon, A. R.; Grieve, A.; Mortimer, R. J.; Wear, T.; Weightman, J. S.; Beer, P. D. *Inorg. Chem.* **1996**, *35*, 5868−5879.
(28) Dayan, I.; Libman, J.; Agi, Y.; Shanzer, A. *Inorg. Chem.* **1993**, *32*, 1467−1475.
(29) Bernady, K. F.; Floyd, M. B.; Poletto, J. F.; Weiss, M. J. *J. Org. Chem.* **1979**, *44*, 1438−1447.
(30) Bergmann, M.; Zervas, L. *Ber.* **1932**, *65*, 1192−1201.
(31) Garelli, N.; Vierling, P. *J. Org. Chem.* **1992**, *57*, 3046−3051.
(32) Green, E. S. R.; Evans, H.; Rice-Evans, P.; Davies, M. J.; Salah, N.; Rice-Evans, C. *Biochem. Pharmacol.* **1993**, *45*, 357−366.
(33) Zang, L.; Misra, B. R.; Van-Kuijk, F. J. M. G.; Misra, H. P. *Biochem. Mol. Biol. Int.* **1995**, *37*, 1187−1195.
(34) Hasty, N.; Merkel, P. B.; Radlick, P.; Kearns, D. R. *Tetrahedron Lett.* **1972**, *1*, 49−52.
(35) Hall, R. D.; Chingell, C. F. *Photochem. Photobiol.* **1987**, *45*, 459−464.
(36) Merkel, P. B.; Nilsson, R.; Kearns, D. R. *J. Am. Chem. Soc.* **1972**, *94*, 1030−1031.
(37) Foote, C. S. *Acc. Chem. Res.* **1968**, *1*, 104−110.
(38) Young, R. H.; Wehrly, K.; Martin, R. L. *J. Am. Chem. Soc.* **1971**, *93*, 5774−5779.
(39) Rodgers, M. A. J. *J. Am. Chem. Soc.* **1983**, *105*, 6201−6205.
(40) Moan, J.; Wold, E. *Nature* **1979**, *279*, 450−451.
(41) Hadjur, C.; Jeunet, A.; Jardon, P. *J. Photochem. Photobiol. B−Biology* **1994**, *26*, 67−74.
(42) Kofman, V.; Dikanov, S. A.; Haran, A.; Libman, J.; Shanzer, A.; Goldfarb, D. *J. Am. Chem. Soc.* **1995**, *117*, 383−391.
(43) Lambert, C. R.; Kochevar, I. E. *J. Am. Chem. Soc.* **1996**, *118*, 3297−3298.
(44) Langen, R.; Chang, I. J.; Germanas, J. P.; Richards, J. H.; Winkler, J. R.; Gray, H. B. *Science (Washington, DC)* **1995**, *268*, 1733−1735.
(45) Nilsson, T. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 6497−6501.
(46) Jackman, M. P.; McGinnis, J.; Powls, R.; Salmon, G. A.; Sykes, A. G. *J. Am. Chem. Soc.* **1988**, *110*, 5880−5887.
(47) Kostic, N. M.; Margalit, R.; Che, C. M.; Gray, H. B. *J. Am. Chem. Soc.* **1983**, *105*, 7765−7767.
(48) English, A. M.; DeLaive, P. J.; Gray, H. B.; Lum, V. R. *J. Am. Chem. Soc.* **1982**, *104*, 870−871.
(49) Baggott, J. E.; Pilling, M. J. *J. Phys. Chem.* **1980**, *84*, 3012−3019.
(50) Hoselton, M. A.; Lin, C. T.; Schwarz, H. A.; Sutin, N. *J. Am. Chem. Soc.* **1978**, *100*, 2383−2388.
(51) Scott, J. R.; Fairris, J. L.; McLean, M.; Wang, K.; Sligar, S. G.; Durham, B.; Millett, F. *Inorg. Chim. Acta* **1996**, *243*, 193−200.
(52) Scott, J. R.; Willie, A.; McLean, M.; Stayton, P. S.; Sligar, S. G.; Durham, B.; Millett, F. *J. Am. Chem. Soc.* **1993**, *115*, 6820−6824.
(53) Osvath, P.; Salmon, G. A.; Sykes, A. G. *J. Am. Chem. Soc.* **1988**, *110*, 7114−7118.
(54) Isied, S. S.; Worosila, G.; Atherton, S. J. *J. Am. Chem. Soc.* **1982**, *104*, 7659−7661.
(55) Lin, C. T.; Boettcher, W.; Chou, M.; Creutz, C.; Sutin, N. *J. Am. Chem. Soc.* **1976**, *98*, 6536−6544.
(56) Erkkila, K. E.; Odom, D. T.; Barton, J. K. *Chem. Rev.* **1999**, *99*, 2777−2795.
(57) Nickel, U.; Chen, Y. H.; Schneider, S.; Silva, M. I.; Burrows, H. D.; Forosinho, S. J. *J. Phys. Chem.* **1994**, *9*, 2883−2888.
(58) Ohkubo, K.; Arikawa, Y. *J. Mol. Catal.* **1985**, *33*, 65−70.
(59) Starke-Reed, P. E.; Oliver, C. N. *Archiv. Biochem. Biophys.* **1989**, *275*, 559−567.
(60) Janzen, E. G.; Haire, D. L. *Two decades of spin trapping*; JPI Press: Greenwich, CT, 1990; Vol. 1.
(61) Ford, W. E.; Rodgers, M. A. J. *J. Phys. Chem. B* **1997**, *101*, 930−936.
(62) Buettner, G. R. *Free Radical Biol. Med.* **1987**, *3*, 259−303.
(63) Pou, S.; Hassett, D. J.; Britigan, B. E.; Cohen, M. G.; Rosen, G. M. *Anal. Biochem.* **1989**, *177*, 1−6.
(64) Halliwell, B. *Biochem. Pharmacol.* **1985**, *34*, 229−233.