phosphine, cis chloride configuration (Figure 2), as proposed for **2** and suggested earlier for the analogous bis-ethylene complex, $W(CH_2{=}CH_2)_2Cl_2(PMePh_2)_2$.[2] The C–O bond distances of 1.390 (8) Å and 1.379 (8) Å are considerably longer than in free acetone (1.210 (4) Å[9]) and approach the C–O single bond length of 1.41 Å.[10] The long C–O bond distances, the large deviation from planarity of the acetone ligands (average angles O–C–C = 113.6 (6)°, C–C–C = 111.4 (6)°), and the ^{13}C chemical shift of the carbonyl carbons (96.7 ppm vs 203.5 ppm for free acetone) all suggest a large contribution from a metallaoxirane resonance form and substantial oxidation of the tungsten(II) center.[11,12] To our knowledge, complexes **2** and **4–7** are the first bis-η^2-ketone complexes, and they are rare examples of η^2-binding of a ketone ligand without electron-withdrawing substituents.[11]

Complex **4** decomposes at ambient temperatures to nonstoichiometric amounts of acetone, $W(O)Cl_2(PMePh_2)_3$,[13] and paramagnetic tungsten complex(es); an oxo–alkylidene complex has not been observed. The decomposition of complexes **5–7** and the reasons for the difference in reactivity between **2** and **4** are currently under investigation. Complex **1** does not react with diethyl ketone, probably because its steric bulk, or with γ-butyrolactone or ethyl acetate, presumably for electronic reasons. The addition of aldehydes (RC(O)H, R = Me, Et, *t*-Bu) to **1** gives a number of products.

The oxidative addition of cyclopentanone is a remarkable reaction because of the strength of the C=O bond (~160 kcal/mol[14]): it is the strongest bond that has been simply cleaved to two fragments that remain on a single metal center.[15] This is a four-electron oxidative addition reaction that occurs under very mild conditions, presumably favored by the formation of a strong[16] tungsten–oxygen multiple bond. The reaction contrasts with the typical reduction of ketones by metals leading to pinacolates and olefins via C–C coupling.[17] Cleavage of cyclopentanone may occur directly from an η^2-ketone adduct or possibly via a metallacycle formed by the head-to-tail coupling of two cyclopentanones.[18] Mechanistic studies are in progress.

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Supplementary Material Available: Spectroscopic and analytical data for **2–7** and tables of atomic coordinates, bond distances and angles, anisotropic temperature factors, and hydrogen atom coordinates for **3** and **4** (14 pages); tables of observed and calculated structure factors for **3** and **4** (35 pages). Ordering information is given on any current masthead page.

The Mechanism of Microbial Denitrification

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Denitrification,[1] the reduction of NO_3^- to N_2O and N_2 by soil bacteria, is a key process in the nitrogen cycle that controls the amount of fixed nitrogen available for plant growth; estimates indicate that 25–30% of fertilizer nitrogen is lost via denitrification.[2] Nitrite and nitrous oxide are known to be free intermediates in the pathway[1] (eq 1)

$$NO_3^- \rightarrow NO_2^- \rightarrow ? \rightarrow N_2O \rightarrow N_2 \qquad (1)$$

Efforts to design specific inhibitors of denitrification suitable for agricultural use require a knowledge of the mechanism of the first enzyme unique to denitrification, nitrite reductase. The mechanism of this key step in denitrification, reduction of NO_2^- to N_2O, has been controversial,[3] with evidence presented for NO as an obligatory free intermediate,[3-5] and for formation of N_2O either by dimerization of free nitroxyl (HNO)[6,7] (eq 2) or by nucleophilic attack of a second NO_2^- on an enzyme-bound nitrosyl intermediate[8,9] (eq 3). The existence of the nitrosyl intermediate common

$$NO_2^- + E \rightleftarrows E{\cdot}NO_2^- \underset{H_2O}{\overset{H_2O}{\rightleftharpoons}} E{\cdot}NO^+ \rightarrow E + HNO \rightarrow (1/2)N_2O \qquad (2)$$

$$NO_2^- + E \rightleftarrows E{\cdot}NO_2^- \underset{H_2O}{\overset{H_2O}{\rightleftharpoons}} E{\cdot}NO^+ \xrightarrow{NO_2^-} E{\cdot}N_2O_3 \rightarrow N_2O \qquad (3)$$

to eq 2 and 3 has been conclusively demonstrated[6,10] by $H_2{}^{18}O$ exchange and by trapping experiments with N_3^- and NH_2OH. We report herein the results of isotope exchange and trapping studies which resolve this controversy by demonstrating that $H_2{}^{18}O$, $^{14}N_3^-$, and $^{15}NO_2^-$ compete for the same enzyme-bound nitrosyl intermediate, as required by eq 3.

Cell-free extracts of *Pseudomonas stutzeri*, a typical denitrifier known to contain a heme *cd*-nitrite reductase,[11] were used, because we have found that whole cells are relatively impermeable to azide. Table I[12] shows the relative isotopic composition of N_2O produced

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0002-7863/87/1509-7214$01.50/0

Table I. Ratio of N_2O-45 (Obtained from Nitrosation with $^{14}N_3^-$) and N_2O-46 (from Denitrification) as a Function of $^{15}NO_2^-$ Concentration

$[^{15}NO_2^-]$, mM	N_2O-45/ N_2O-46	$[^{15}NO_2^-]$, mM	N_2O-45/ N_2O-46
0.05[a]	0.79 ± 0.07	0.1[b]	0.24 ± 0.01
0.10[a]	0.44 ± 0.01	0.2[b]	0.22 ± 0.02
1.0[a]	0.17 ± 0.03	0.5[b]	0.086 ± 0.001
		1.0[b]	0.052 ± 0.005

[a] 50 mM succinate as reductant. [b] Luria broth medium as reductant.

by incubating cell-free extracts with varying amounts of $^{15}NO_2^-$ in the presence of 50 mM $^{14}N_3^-$. Trapping the $E{\cdot}^{15}NO^+$ intermediate with $^{14}N_3^-$ (nitrosation) produces $^{14}N^{15}NO$ (mass 45), while denitrification produces $^{15}N_2O$ (mass 46). The results clearly show that NO_2^- competes with N_3^- for the $E{\cdot}NO^+$ intermediate.

Previous work in our laboratories[9] with whole cells of *P. stutzeri* has shown that the extent of $H_2{}^{18}O$ exchange with the $E{\cdot}NO^+$ intermediate, as determined by the ^{18}O content of product N_2O, decreases with increasing nitrite concentration, which indicates that $H_2{}^{18}O$ and NO_2^- compete for the same intermediate, $E{\cdot}NO^+$. We have observed similar behavior for cell-free extracts (data not shown). In order to examine this point more carefully, we have performed an isotope dilution experiment, in which cell-free extracts are incubated with 1 mM $^{15}NO_2^-$, 50 mM $^{14}N_3^-$, and 9% $H_2{}^{18}O$, and the ^{18}O content of N_2O originating from denitrification (N_2O-48/(N_2O-46 + N_2O-48)) is compared to that of N_2O originating from nitrosation (N_2O-47/(N_2O-45 + N_2O-47)). Nitrite is a "sticky" substrate for *P. stutzeri* nitrite reductase,[13,14] and the free NO_2^- pool does not equilibrate rapidly with $H_2{}^{18}O$. Consequently, the ^{18}O content of $^{15}N_2O$ produced by denitrification should be the same as (eq 2) or approximately one-half that of (eq 3) the $E{-}^{15}NO^+$ pool, as monitored by trapping with $^{14}N_3^-$. We find experimentally that $^{15}N_2O$ from denitrification is 52.7 ± 2% equilibrated[15] with the $H_2{}^{18}O$, while $^{14}N^{15}NO$ from nitrosation is 80.5 ± 2.4% equilibrated with the $H_2{}^{18}O$ solvent, consistent with eq 3. (Even at high $[NO_2^-]$ in the absence of N_3^-, we always observe ca. 8% equilibration of N_2O with $H_2{}^{18}O$, possibly indicating that some ^{18}O exchange occurs via an intermediate containing an N–N bond, as has been observed for nitrosation with NH_2OH.[9])

These results demonstrate that H_2O, N_3^-, and *nitrite* compete for a common intermediate, $E{\cdot}NO^+$. This provides the first direct evidence that unambiguously distinguishes between the mechanisms of eq 2 and 3 and indicates that denitrification occurs by *sequential* reaction of two nitrite ions with the enzyme, as first proposed by us.[8] This conclusion is supported by recent isotope effect studies.[16,17] Further mechanistic studies are in progress to define the second nitrite binding site and the factors which cause purified nitrite reductase to produce primarily NO rather than N_2O.[1,3]

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Photochemical Dehydrofragmentation Reactions: Importance of Donor and Acceptor Structure in Determination of Reactivity in Radical Ion Pairs Formed in Electron-Transfer Photoreactions

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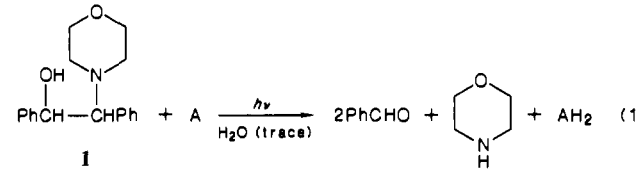
Photoinduced electron-transfer reactions are the subject of considerable interest due both to the versatility of net chemical reactions which can be achieved as well as to the variety of intermediates and mechanistic complexities encountered in their study.[1-8] For initially neutral donors and acceptors quenching of excited singlet states is frequently dominated by formation of a geminate ion pair, A^-/D^+, which can subsequently decay by back electron transfer or, in moderately polar to polar solvents, by cage escape to form free ions.[7-10] In nonpolar solvents the back electron transfer process acts as an effective clock to limit the lifetime for reaction of the geminate pair to the ns time scale; consequently only relatively rapid reactions can occur efficiently from the geminate pair and these frequently involve participation of the solvent or other reagents present in high concentration.[11-14] We have previously reported the chemically clean and moderately efficient C–C bond photofragmentation reactions of β-amino-alcohols initiated by electron transfer to excited acceptors (eq 1).[15,16] We report here a mechanistic study where this reaction



(1)

is restricted to the geminate pair and which emphasizes the complimentary roles of reduced acceptor and oxidized donor in facilitating chemical reaction in competition with fast back electron

(12) Experimental conditions: 50 mM $Na^{14}N_3$; reductant was 50 mM succinate or Luria broth medium; cell-free extracts of *P. stutzeri*[9] prepared by French press (12000 psi, 2 passages); all reagents in 25 mM HEPES buffer, pH 7.3; samples incubated at 25 °C overnight, 2–3 replicates per $[^{15}NO_2^-]$, 2 injections per sample, 2 integrations per peak. Sterile controls (autoclaved for 20 min at 200 °C) gave no detectable N_2O. Gas samples were analyzed with an HP 5985 GC/MS system equipped with a Porapak Q column. The mass spectrometer was operated in EI mode with selective ion monitoring. System temperatures were as follows: injector, 80 °C; column, 55 °C; ion source, 100 °C. The electron multiplier voltage was 1400–3000 MeV, and the autotune value was 2000 MeV. The amount of extract added was such that the extent of reaction for the incubation period and for the lowest $[NO_2^-]$ did not exceed 20%; thus, the $[NO_2^-]$ and $[N_3^-]$ did not change significantly during the course of the experiments.

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