regardless of its bulkiness or whether the group is aromatic or aliphatic.

It has been believed that amides usually exist in trans structures, as evidenced crystallographically in many proteins and peptides, although some peptides containing proline or N-methylated amino acid residues have cis structures, especially in cyclic peptides.[6] The change of conformational preference as a consequence of N-methylation is more distinct in anilide structures than in ordinary amides with an aliphatic group at the imino end. In order to investigate the cis preference of *N*-methylamides theoretically, extensive studies based on molecular orbital calculations are in progress.[10] Further generalization or extension of the applicability of this rule is also under investigation.

The amide group is very important in drug structures, not only from a synthetic viewpoint but also because of its chemical and physical properties. This paper raises the possibility that the *N*-methylamide moiety in anilides may be bioisostere of the cis carbon-carbon double bond.

Supplementary Material Available: Listing of crystal data, atom positioning and thermal parameters, and bond lengths and angles for compounds **2**-**5** (16 pages). Ordering information is given on any current masthead page.

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Reduced Derivatives of the Manganese Cluster in the Photosynthetic Oxygen-Evolving Complex

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Photosynthetic oxygen evolution requires Mn, Cl, and Ca and is believed to take place at a multinuclear Mn cluster (the oxygen-evolving complex, OEC).[1] Substantial effort has been devoted to elucidation of the cluster's structure using a variety of physical and chemical methods. We describe herein evidence from X-ray absorption spectroscopy that treatment in the dark with either hydroxylamine or hydroquinone results in substantial reduction of the Mn.

Treatment of photosystem II with micromolar concentrations of NH_2OH results in a two-flash delay in oxygen evolution.[2,3] Longer exposure time or millimolar concentrations of NH_2OH result in an eventual inhibitory loss of Mn(II).[4,5] The two-flash delay has been interpreted as arising from rapid reduction of Mn in the dark to a state formulated as S_{-1}.[6-9] This interpretation has been questioned most recently by Guiles et al.[10] They report, on the basis of X-ray absorption near edge structure (XANES), that NH_2OH does not cause reduction in the dark, but that reduction to a species formulated as S_0* *does* occur following illumination. Since it has recently been demonstrated[11] that millimolar concentrations of Ca^{2+} stabilize the OEC reaction center complex with respect to NH_2OH- and hydroquinone-induced loss of activity, we have used this stabilization technique to permit further study of reduced OEC derivatives using XANES.

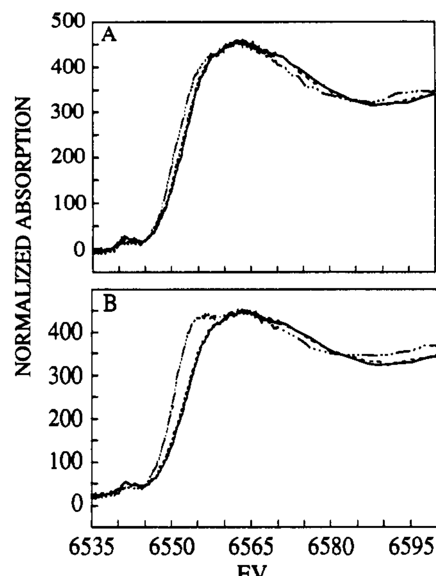

Figure 1. Normalized OEC XANES spectra: (—) S_1 control; (-··-) treated sample; (- - -) treated sample after illumination and dark adaptation. (A) NH_2OH-treated sample. (B) Hydroquinone-treated sample.

Table I. Manganese Oxidation State Composition for OEC[a]

sample	Mn(II)	Mn(III)	Mn(IV)
control (S_1)		49 (20)	51 (20)
hydroquinone	23 (7)	77 (7)	
	41 (5)		59 (5)
NH_2OH		86 (12)	14 (12)
	4 (5)	96 (5)	
	25 (7)		75 (7)

[a] Percent composition of different Mn oxidation states. Standard deviations (in parentheses) are for all combinations of models with the indicated oxidation states. For the reduced samples, several different oxidation-state models can be used to fit the data (see text).

Highly purified reaction center complex samples (specific activity = 1350 μmol of O_2 (mg of chlorophyll)$^{-1}$ h^{-1}) were prepared as previously described,[12] suspended at 0.67 mg of chlorophyll/mL, and dark adapted (>30 min at 4 °C) to prepare the OEC in the S_1 state.[13] This material was then either used directly (control) or treated with reductants (100 μM NH_2OH for 3 min or 200 μM hydroquinone for 30 min). Excess hydroxylamine was removed by 40-fold dilution. Ferricyanide was used to oxidize excess hydroquinone. A portion of each sample was diluted to 0.05 mg/mL chlorophyll and illuminated under saturated conditions for 3 min at 4 °C. As these illumination conditions allow multiple turnovers, the samples were again dark adapted for comparison with the S_1 control. Pellets of all samples were then packed into

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Lucite cuvettes in the dark and stored at 77 K. All subsequent sample handling was done in the dark. Biological activity was measured using a Clark-type O_2 electrode in an assay mixture containing 10 mM $CaCl_2$, 50 mM MES (pH 6), and 0.31 mM 2,6-dichloro-*p*-benzoquinone as an acceptor.

X-ray absorption data were measured at the Stanford Synchrotron Radiation Laboratory (SSRL) wiggler beam line 7-3 under dedicated conditions (3.0 GeV, 40 mA) using a Si(220) double-crystal monochromator detuned 50% for harmonic rejection. Samples were maintained at 10 K using a liquid He flow cryostat, and data were measured as fluorescence excitation spectra using a 13-element Ge detector array. Spectra were calibrated by simultaneously recording the absorption spectrum of $KMnO_4$, with the $KMnO_4$ preedge peak defined as 6543.3 eV. Data were reduced and normalized as previously described.[14] Normalized XANES spectra were fit to linear combinations of spectra drawn from a library of Mn(II), Mn(III), and Mn(IV) reference compounds for quantitative analysis of Mn(II) content.[15]

The OEC XANES spectra are shown in Figure 1. Dark treatment with either reductant generates a new species whose XANES spectrum is consistent with reduction of Mn. Since the changes in the XANES are completely reversed by illumination, they do not result from irreversible damage. This is consistent with there being only small activity losses (0-20%) following the treatments.

We have previously noted[15] the difficulty in determining the relative amounts of Mn(III) and Mn(IV) due to the similarity of their XANES spectra. In contrast, the unique XANES spectra observed for Mn(II) make it relatively easy to quantitate.[16] The results of quantitative fits to the XANES spectra are summarized in Table I. We find no evidence for Mn(II) in the control (S_1) sample. Our previous suggestion[14] that S_1 contains ca. 25% Mn(II) was due to Mn(II) contamination present in the Mylar windows covering those samples.[18] Quantitative comparisons of the fluorescence intensities for Mylar alone and for Mylar-covered OEC samples show that Mn contamination accounted for ca. 25% of the fluorescence intensity, thus confirming the accuracy of our quantitation method. The present samples, measured using Mn-free polypropylene windows, show <5% Mn(II), consistent with the results of Klein, Sauer, and co-workers.[10]

In contrast to the spectra for S_1, the XANES spectra for hydroquinone-treated samples can be fit only by including ca. 30% of a Mn(II) component. This is consistent with the recent observation[19] that a photoreversible Mn(II) six-line EPR signal is produced by hydroquinone treatment. The apparent amount of Mn(II) depends on the oxidation state that is assumed for the remainder of the Mn. The XANES spectra for NH_2OH-treated samples also show reduction relative to S_1. In this case, however, the data can be fit equally well by Mn(III) plus small amounts of either Mn(II) or Mn(IV) or by a ca. 1:3 mixture of Mn(II) and Mn(IV). Regardless of which model is assumed, the edge fitting confirms the reduction of Mn,[16] as expected for the observed shift in edge energy.

The reduction of Mn by hydroxylamine treatment in the dark conflicts with the conclusions of Guiles et al.[10] The difference may be due to the use of different sample preparations (the reaction center complex lacks extrinsic polypeptides and is hence more susceptible to reduction). Alternatively, the difference may lie in the interpretation of the data. The edge shift that we observe is similar to that reported by Guiles et al.[10] In the earlier experiments, this shift was attributed to the formation of inactive centers based on the observation of a Mn(II) EPR signal. As noted above, however, a Mn(II) EPR signal is not necessarily associated with inactive centers.[19] Since we observe little loss of activity and since the treatments are reversible, the reduction observed in our samples cannot be due to inactive centers (the 0-20% centers that are inactivated lose Mn(II) into the supernatant, where it does not contaminate the XANES spectrum). Experiments to resolve the origin of the difference between our conclusions and those of Guiles et al. are in progress.

Figure 1 and Table I show that significantly less Mn(II) is produced by NH_2OH than by hydroquinone. Perhaps this is not surprising considering the higher concentration and longer incubation time used for hydroquinone. However, these reaction conditions were chosen to maximize reductant concentration and exposure time without compromising activity. It is possible to generate a NH_2OH-reduced species with a XANES spectrum identical to that of hydroquinone (not shown); however, such a sample is inactive. This shows that the NH_2OH sample presented here is not simply a mixture of ca. 50% S_1 and 50% of the more reduced derivative formed by hydroquinone.

In a series of reactivity studies,[11,19] Mei and Yocum have shown that hydroxylamine and hydroquinone attack different sites in the OEC. Hydroxylamine-treated samples are EDTA sensitive,[11] but hydroquinone-treated samples are not.[19] There is a strong synergism in the ability of NH_2OH and hydroquinone to inactivate the OEC.[19] It is intriguing that the hydroquinone sensitive site gives more Mn(II) but is not susceptible to EDTA while the NH_2OH sensitive site gives less Mn(II) but is EDTA susceptible. This suggests that NH_2OH may be attacking a water-accessible Mn while hydroquinone reduces Mn that remains sequestered within the protein.

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(16) This quantitation procedure assigns all of the XANES changes to oxidation-state changes. XANES *energies* depend somewhat on ligation type;[17] however, in 50 models examined to date, the XANES *shape* for Mn(II) appears to be a unique oxidation-state marker. It should be noted, however, that a hypothetical Mn(III) complex having a Mn(II)-like structure (e.g., Mn-O distances of ca. 2.2 Å) might give changes similar to those in Figure 1.

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Long-Range Heteronuclear Spin Locking (HSL) 2D-NMR Spectroscopy and Its Application to the Resonance Assignments of Poly(*p*-*tert*-butylstyrene)

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In this paper, it is demonstrated that heteronuclear spin locking (HSL) 2D-NMR spectroscopy can be used to obtain correlations due to long-range heteronuclear interactions in organic structures, by unequivocally assigning the aromatic ^1H and ^{13}C resonances of isotactic poly(*p*-*tert*-butylstyrene), **1**.

The NMR resonances from the aromatic protons of styrene units in polymers can provide information about stereosequence and monomer unit distributions, but their chemical shift behavior is imperfectly understood. A better understanding of the aromatic resonance patterns of polymers develops concurrently with the understanding of other spectroscopic characteristics of the polymers. Isotactic **1** is a particularly good material to investigate from this standpoint because of its unusually high solubility in common solvents at ambient temperatures, despite its high

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