Evidence against OH Radical Formation during Photocatalyzed Reduction of Manganese(III) in $K_2[Mn(III)-2-a$-hydroxyethyl-isochlorin $e_4]$ acetate in Oxygen Free Aqueous Alkaline Solutions*

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OH Radical, Mn(III)-Chlorin, Kinetics, Photoreduction

The hypothesis of GLIKMAN and ZABRODA (Biochemistry [USSR] 34, 239 [1969]) that the primary electron donor during photoreduction of manganese(III) in Mn(III)-hydroxychlorin compounds in oxygen free aqueous alkaline solutions is the axially bound OH$^-$ ion was tested with Mn(III)-2-a-hydroxyethyl-isochlorin $e_4$. It has been shown that
1) the primary generation of OH radicals upon irradiation of the complex is highly improbable,
2) light is not essential for the reduction reaction,
3) the kinetics of photoreduction of the Mn(III)-compound in 2 N NaOH clearly is not compatible with OH radical formation.

Introduction

Photoreduction of the central metal ion in transition metal complexes of porphyrins and chlorins is commonly observed in electron donor solvents1-5. This effect, however, has also been reported to occur in aqueous alkaline solutions in the absence of reducing agents1,6-8. Photoreduction of manganese(III) in Mn(III)-chlorin $e_4$ in 2N NaOH was found to be accompanied by formation of H$_2$O$_2$. This result was interpreted by assuming that the Mn(III)-chlorin compound photooxidized axially complexed OH$^-$ ions8. Therefore, GLIKMAN et al.6,8 suggested that decomposition of water in photosynthesis could be brought about through photo-oxidation of OH$^-$ ions by an as yet unknown Mn(III)-chlorin compound. However, apart from the fact that this proposal presumably is without any photosynthetic significance, since manganese is not an integral part of the photoactive center of photosystem II9,10 and the OH radical model of photosynthetic oxygen evolution is not compatible with the observed oxygen flash yield pattern in photo-

* Measurements were taken from M. MÜLLER, Diplomarbeit Frankfurt 1974.

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Experimental

Preparation and purification of $K_2[Mn(III)-2-a$-hydroxyethyl-isochlorin $e_4]$ acetate (3) has been described previously12. The absorption spectra of 3 and its corresponding Mn(IV)- and Mn(III)-compounds (4 and 2) are shown in Fig. 1. H$_2$O$_2$ test reactions were performed in a special tube described by ENGELSLA et al.5. Quantitative determination of H$_2$O$_2$ was carried out using the phenolphthaline test reaction12. Phenolphthalein – the reaction product of phenolphthaline with H$_2$O$_2$ in the presence of CuSO$_4$ – was determined by difference absorption spectrometry.

Reduction of 3 can only be observed in oxygen free solutions. Dissolved oxygen was removed in high vacuum (10$^{-3}$ mm) using the freeze- and thaw-technique according to ENGELSLA et al.5.

The kinetics of the reduction reaction was followed by measuring the time course of the absorbance $E_i(t)$ at different wavelengths in the visible and near UV part of the spectrum with a Cary 15 spectrophotometer. In order to account for the contribution of the reaction product to the absorbance $|E_i(t)|-E_i(\infty)$ was plotted against time. The application of this method presupposes that there are no intermediate reaction products that contribute to the absorbance.

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Light experiments were done with a 900 W Xenon lamp (Osram, Germany) whose UV radiation was excluded by a 400 nm-cut-off filter (WG 400, Schott, Germany). The irradiated solutions were kept at constant temperature.

EPR experiments were carried out with a Varian E-12 spectrometer.

Results and Discussion

1. Incompatibility of OH radical formation with the kinetics of photoreduction

After complete photoreduction of Mn(III)-chlorin e₅ GLIKMAN and ZABRODA⁵ reported H₂O₂ formation up to 57% of the stoichiometric amount. Contrary to this result, however, we have not been able to detect any H₂O₂ after photoreduction of 3 (c = 1×10⁻⁴ M/L) in 2 N NaOH at 20 °C beyond the limits of experimental accuracy (4×10⁻⁶ M/L H₂O₂).

This negative result could be due to the reaction of OH radicals and H₂O₂ with 3 and 2. The chlorin ligand would be expected to be readily oxidized by these agents with the eventual formation of bile pigments¹⁴. No spectroscopic evidence for these reactions has been obtained upon photoreduction of 3. The peak positions of the absorption bands of 3 in the visible and near UV region* were found to be identical within ± 1 nm before reduction and after reoxidation of 2 with oxygen. The slight absorbance loss (7%) observed after reoxidation with O₂ cannot be attributed to cleavage of the chlorin nucleus by oxidative attack of OH or H₂O₂, since this effect was also found to occur on spontaneous reduction of 3 in the dark (see section 2) to the same extent. The formation of OH radicals in the dark, however, can be definitely ruled out on thermodynamic grounds. This is easily seen merely from comparison of the redox potentials of the systems 3/2 (E₀ ≈ 400 mV** and OH⁻/OH (E₀ ≈ 2000 mV¹⁵).

But addition of oxygen free H₂O₂ dissolved in 2 N NaOH in stoichiometric amounts to a degassed solution of 3 or 2 in 2 N NaOH revealed that H₂O₂ oxidized the central metal ion instantaneously to the oxidation state 4 in both cases***. 4 was identified from its intense absorption band at 400 nm¹,¹².

This compound, however, is not stable in alkaline aqueous solutions. It spontaneously reverts to 3 without adding any reducing agents¹².

Thus, if OH radicals were actually formed photochemically, these results would suggest the following reaction scheme:

\[
\begin{align*}
3 + OH⁻ & \rightarrow 2 + OH \\
3 + OH & \rightarrow 4 + OH⁻ \\
2 + OH & \rightarrow 3 + OH⁻ \\
4 & \rightarrow 3 \\
2 OH & \rightarrow H₂O₂ \\
3 + H₂O₂ & \rightarrow 4 + OH + OH⁻ \\
2 + H₂O₂ & \rightarrow 3 + OH + OH⁻
\end{align*}
\]

* Band VI (28150 cm⁻¹) and band IV (15250 cm⁻¹) are assigned to π→π⁎-ligand transitions¹. They should be indicative of chemical alterations of the chlorin nucleus.

** The redox potentials of Mn(III)-hydroxy-porphyrin and chlorin complexes in aqueous solution do not differ much and are well below 400 mV¹. This must also be true for 3/2, since H₂O₂ was found to oxidize 3 in 2 N NaOH (E₀ = 400 mV for HO₂⁻/O₂⁻ when aOH⁻ = 1 M/L¹⁵).

*** Oxidation of side chains of the porphyrin nucleus by H₂O₂ has also been reported¹⁴,¹⁶. We observed this reaction only in the presence of excess amounts of H₂O₂.
Apart from the slow photocatalyzed reactions (1) and (4), the scheme is made up by rapid diffusion controlled dark reactions. Application of the steady state approximation to the calculation of \([\text{OH}]_{ss}\) and \([\text{H}_2\text{O}_2]_{ss}\) yields the kinetic equations:

\[
\frac{dc_{\text{III}}}{dt} \approx k_1 \frac{c_{\text{III}}^2}{c_{\text{III}} + c_{\text{II}}} \Rightarrow \frac{dc_2}{dt} \approx k_1 \frac{C_3^2}{C_3 + C_2} \quad (8)
\]

\[
\frac{dc_{\text{III}}}{dt} \approx 2k_1 \frac{c_{\text{III}}^2}{c_{\text{III}} + c_{\text{II}}} - k_4 c_{\text{IV}} \quad (9)
\]

\[
\frac{dc_{\text{IV}}}{dt} \approx k_4 c_{\text{IV}} - k_1 \frac{c_{\text{III}}^2}{c_{\text{III}} + c_{\text{II}}} \quad (10)
\]

Thus the scheme predicts the reduction reaction to be approximately first order only at the beginning of the reaction and only if the steady state approximation can be applied to 4 as well.

This result, however, clearly is not compatible with experiment. After correction for the absorbance contribution of 2 the reduction reaction followed up to 95–98% completion at four different wavelengths is found to be exactly first order in complex concentration within experimental accuracy of 2% (Fig. 2).

This clear contradiction between theory and experiment strongly argues against OH radical formation upon photoreduction of 3.

2. The primary photochemical generation of OH radicals is highly improbable

Further support of this conclusion is obtained from two groups of experiments that render the primary photochemical generation of OH radicals unlikely.

1) We have not been able to detect any OH radicals by EPR spectroscopy at 93 K after irradiation of an oxygen free solution of 3 (5 × 10⁻⁴ M/L) in 2 N NaOH for 6.5 hours at the same temperature with visible light.

2) Reduction of 3 has also been observed in the dark though with somewhat reduced rate!

Like the photoreduction the dark reaction is also first order in pigment concentration (Fig. 2). The rate constants strongly depend on temperature (Table I). From an Arrhenius plot of the rate constants the activation energy is determined to be ΔH⁺ = 18 kcal/mole. Thus the energy content of a quantum of visible or near IR light is by far sufficient to overcome the energy barrier for reduction.

The rate of Mn(II)-formation measured at 423 nm is identical with the rate of Mn(III)-decrease measured at 355 nm and 462 nm. This is shown in Fig. 2 for the photoreduction but was also found in the dark.

Table I. Temperature dependence of the rate constants of the reduction of K₂[Mn(III)-2-α-hydroxyethylisochlorin e₄]acetate in 2 N NaOH in the dark.

<table>
<thead>
<tr>
<th>T [°C]</th>
<th>k × 10⁴ sec⁻¹</th>
</tr>
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<tbody>
<tr>
<td>24</td>
<td>2.1</td>
</tr>
<tr>
<td>40</td>
<td>8.6</td>
</tr>
<tr>
<td>55</td>
<td>32.0</td>
</tr>
</tbody>
</table>

* According to the theory of the rate constant of diffusion controlled reactions the relations \(k_2 = k_3\) and \(k_4 = k_7\) hold. The contribution of the term involving \([\text{H}_2\text{O}_2]_{ss}\) is negligible in (8)–(10).
with the dark reaction. Hence electron transfer to the central metal ion determines the rate of the reduction reaction.

Because of the existence of several charge-transfer transitions in the visible and near IR region\(^1\) the catalytic action of light indicates that electron transfer from the chlorin ligand to the metal ion occurs.

These results corroborate the hypothesis that the reduction of 3 essentially is a dark reaction which may be catalyzed by visible light.

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