Evidence against OH Radical Formation during Photocatalyzed Reduction of Manganese(III) in K₂[Mn(III)-2-α-hydroxyethyl-isochlorin e₄]acetate in Oxygen Free Aqueous Alkaline Solutions*

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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-α-hydroxyethyl-isochlorin e₄. 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 solvents[1-5]. This effect, however, has also been reported to occur in aqueous alkaline solutions in the absence of reducing agents[1, 6-8]. Photoreduction of manganese(III) in Mn(III)-chlorin e₆ in 2N NaOH was found to be accompanied by formation of H_2O_2[6]. This result was interpreted by assuming that the Mn(III)-chlorin compound photooxidized axially complexed OH⁻ ions[6-8]. Therefore, Glikman et al.[6, 8] suggested that decomposition of water in photosynthesis could be brought about through photooxidation 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 II[9, 10] and the OH radical model of photosynthetic oxygen evolution is not compatible with the observed oxygen flash yield pattern in photosynthesis[11], the photochemical reaction postulated by Glikman et al. appears to be highly improbable in the light of the results obtained by us with a very similar compound, namely Mn(III)-2-α-hydroxyethyl-isochlorin e₄.

Experimental

Preparation and purification of K₂[Mn(III)-2-α-hydroxyethyl-isochlorin e₄] acetate (3) has been described previously[12]. The absorption spectra of 3 and its corresponding Mn(IV)- and Mn(II)-compounds (4 and 2) are shown in Fig. 1.

H_2O_2 test reactions were performed in a special tube described by Engelsma et al.[5]. Quantitative determination of H_2O_2 was carried out using the phenolphthaline test reaction[13]. Phenophthaleïn – the reaction product of phenolphthaline with H_2O_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^{-5} mm) using the freeze- and thaw-technique according to Engelsma et al.[5].

The kinetics of the reduction reaction was followed by measuring the time course of the absorbance $E_\lambda(t)$ 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 $|E_\lambda(t) - E_\lambda(\infty)|$ was plotted against time. The application of this method presupposes that there are no intermediate reaction products that contribute to the absorbance.
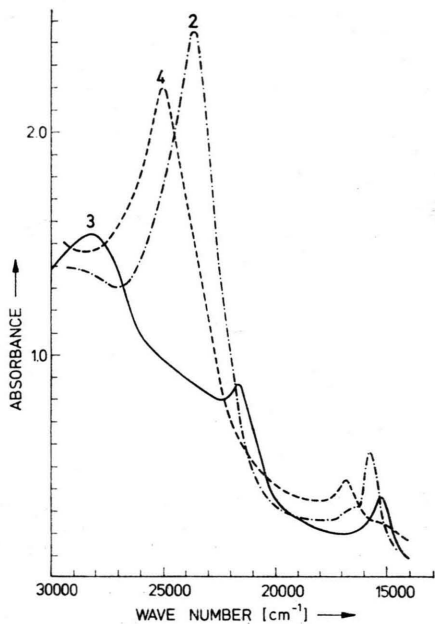

Fig. 1. Absorption spectra of K_2[Mn(III)-2-α-hydroxy-ethyl-isochlorin e_4]acetate (3) in 2 N NaOH at 20 °C and of its corresponding Mn(II) and Mn(IV)-complexes.
Concentration: 1.5×10^{-4} M/L.

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. Incompatability of OH radical formation with the kinetics of photoreduction

After complete photoreduction of Mn(III)-chlorin e_6 GLIKMAN and ZABRODA[6] reported H_2O_2 formation up to 57% of the stoichiometric amount. Contrary to this result, however, we have not been able to detect any H_2O_2 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 H_2O_2).
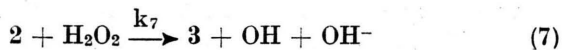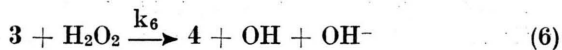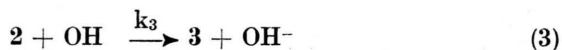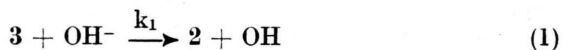
This negative result could be due to the reaction of OH radicals and H_2O_2 with 3 and 2. The chlorin ligand would be expected to be readily oxidized by these agents with the eventual formation of bile pigments[14]. No spectroscopic evidence for these reactions has been obtained upon photoreduction of 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 O_2 cannot be attributed to cleavage of the chlorin nucleus by oxidative attack of OH or H_2O_2, 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 ($E_0 \cong 400$ mV**) and OH^-/OH ($E_0 \cong 2000$ mV[15]).

But addition of oxygen free H_2O_2 dissolved in 2 N NaOH in stoichiometric amounts to a degassed solution of 3 or 2 in 2 N NaOH revealed that H_2O_2 oxidized the central metal ion instantaneously to the oxidation state 4 in both cases***. 4 was identified from its intense absorption band at 400 nm [1,12].

This compound, however, is not stable in alkaline aqueous solutions. It spontaneously reverts to 3 without adding any reducing agents[12].

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

$$3 + OH^- \xrightarrow{k_1} 2 + OH \tag{1}$$

$$3 + OH \xrightarrow{k_2} 4 + OH^- \tag{2}$$

$$2 + OH \xrightarrow{k_3} 3 + OH^- \tag{3}$$

$$4 \xrightarrow{k_4} 3 \tag{4}$$

$$2\,OH \xrightarrow{k_5} H_2O_2 \tag{5}$$

$$3 + H_2O_2 \xrightarrow{k_6} 4 + OH + OH^- \tag{6}$$

$$2 + H_2O_2 \xrightarrow{k_7} 3 + OH + OH^- \tag{7}$$

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* Band VI (28150 cm^{-1}) and band IV (15250 cm^{-1}) are assigned to $\pi \rightarrow \pi^*$-ligand transitions[1]. 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 mV[1]. This must also be true for 3/2, since H_2O_2 was found to oxidize 3 in 2 N NaOH ($E_0 = 400$ mV for HO_2^-/O_2^- when $a_{OH^-} = 1$ M/L[15]).

*** Oxidation of side chains of the porphyrin nucleus by H_2O_2 has also been reported[14,16]. We observed this reaction only in the presence of excess amounts of H_2O_2.

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 $[OH]_{ss}$ and $[H_2O_2]_{ss}$ yields the kinetic equations*

$$-\frac{dc_{II}}{dt} \cong k_1 \frac{c_{III}^2}{c_{III} + c_{II}} \Rightarrow dC_2 \cong k_1 \frac{C_3^2}{C_3 + C_2} \quad (8)$$

$$-\frac{dc_{III}}{dt} \cong 2k_1 \frac{c_{III}^2}{c_{III} + c_{II}} - k_4 c_{IV} \quad (9)$$

$$-\frac{dc_{IV}}{dt} \cong k_4 c_{IV} - k_1 \frac{c_{III}^2}{c_{III} + c_{II}} \quad (10)$$

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

* According to the theory of the rate constant of diffusion controlled reactions[17] the relations $k_2 = k_3$ and $k_6 = k_7$ hold. The contribution of the term involving $[H_2O_2]_{ss}$ is negligible in (8)–(10).
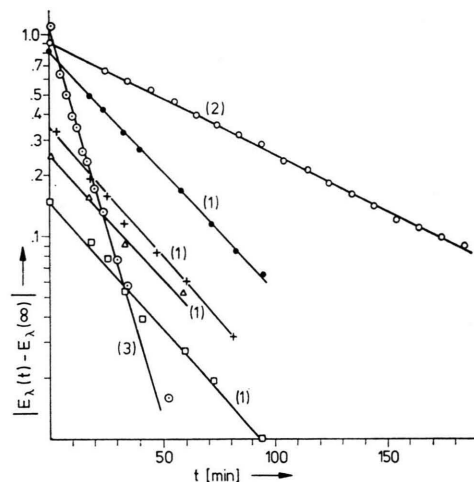

Fig. 2. Semilogarithmic plot of the kinetics of the reduction reactions in 2 N NaOH at 24 °C.
Curve 1: Photoreduction of K_2[Mn(III)-2-α-hydroxy-ethyl-isochlorin e_4]acetate.
Measuring wavelengths: △ 355 nm, + 400 nm, ● 423 nm, □ 462 nm.
Concentration: 1.31×10^{-4} M/L,
Optical path length: 0.8 cm.
Curve 2: Reduction of K_2[Mn(III)-2-α-hydroxyethyl-isochlorin e_4]acetate in the dark in the absence of reducing agents.
Concentration: 1.35×10^{-4} M/L,
Measuring wavelength: 423 nm,
Optical path length: 0.8 cm.
Curve 3: Reduction of K_2[Mn(IV)-2-α-hydroxyethyl-isochlorin e_4]acetate in the dark in the absence of reducing agents.
Concentration: 1.47×10^{-4} M/L,
Measuring wavelength: 400 nm,
Optical path length: 1 cm.

$\Delta H^{\neq} = 18$ 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

Table I. Temperature dependence of the rate constants of the reduction of K_2[Mn(III)-2-α-hydroxyethyl-isochlorin e_4]acetate in 2 N NaOH in the dark.
Concentration: 1.35×10^{-4} M/L.
Measuring wavelength: 423 nm.

T [°C]	$k \times 10^4$ sec^{-1}
24	2.1
40	8.6
55	32.0

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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