Singlet Oxygen as a Mediator in the Hematoporphyrin-catalyzed Photooxidation of NADPH to NADP\(^+\) in Deuterium Oxide*

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The oxygen-dependent photooxidation of NADPH in the presence of hematoporphyrin in \(\text{D}_2\text{O}\) results in the production of enzymatically active NADP\(^+\). The reaction is not inhibited by benzoate, mannitol, superoxide dismutase, or catalase. Moreover, addition of either potassium superoxide or \(\text{H}_2\text{O}_2\) does not potentiate the reaction. This suggests \(\text{OH}^-, \text{H}_2\text{O}_2, \text{and O}_2^\cdot\) are not likely to be the reactive oxygen species in this system. The oxidation is inhibited by various singlet oxygen quenchers and inhibitors such as 1,4-diazabicyclo[2.2.2]octane, 2,5-dimethylfuran plus methanol, histidine, and methionine. In addition, the rate of oxidation in \(\text{H}_2\text{O}\) is less than one-fifth of that in \(\text{D}_2\text{O}\). The results suggest a singlet oxygen-mediated process. During the oxidation, no superoxide radical production could be detected with either ferricytochrome c or nitroblue tetrazolium. However, \(\text{H}_2\text{O}_2\) has been found as one of the products. These observations are consistent with an oxidation-reduction reaction between singlet oxygen and NADPH to form \(\text{H}_2\text{O}_2\) and NADP\(^+\), catalyzed by the light-activated photosensitizer hematoporphyrin.

Photochemical oxidation is a subject of considerable and continued interest (1, 2). A number of photosensitizing molecules, including porphyrins, have been used to catalyze the oxidation of various biological components such as proteins (3, 4), amino acids (4), nucleotides (5), and lipids (6, 7). The elucidation of the mechanisms of these oxidations is of importance for an understanding of various reactive oxygen species in this system. The oxidation is inhibited by various photosensitizers, including porphyrins, and to exclude some of the other reactive oxygen species are rapidly interconvertible under suitable conditions, it is usually difficult to ascertain the identity of the actual distal species directly involved in a specific reaction.

Reactive oxygen species are known to have various deleterious effects. For example, they have been implicated in the development of pulmonary oxygen toxicity (11), the amplification of effects of a chemical carcinogen in the development of abnormal morphology of cells in culture (12), toxic effects on proteins, bacteria, and bacteriophage (13), lipid peroxidation in biological membranes (14, 15), peroxidation of unsaturated fatty acids (16), damage to adrenal mitochondrial membranes (17), the depolymerization of hyaluronic acid (18), and damage to DNA (19).

In this study, attempts have been made to show that a specific reactive oxygen species, singlet oxygen, is the mediator in the hematoporphyrin-catalyzed photooxidation of a biological component, NADPH, to enzymatically active NADP\(^+\), and to exclude some of the other reactive oxygen species by studying the effects of various inhibitors and activators.

MATERIALS AND METHODS

Deuterium oxide (99.9%) was purchased from Sigma Chemical Co. and from Research Organic Inorganic Chemical Co., NADPH and NAD\(^+\) were purchased from P-L Biochemicals. Hematoporphyrin dihydrochloride was purchased from Sigma Chemical Co.,...
Photooxidation of NADPH by $^{1}O_{2}$ in $D_{2}O$

1,4-diazabicyclo[2.2.2]octane was obtained from Matheson, Coleman and Bell, and 2,5-dimethylfuran from the Aldrich Chemical Co. Xanthine oxidase (milk), catalase (beef liver), and isocitrate dehydrogenase (pig heart), were purchased from Boehringer Mannheim Biochemicals. Prior to use, the catalase was diluted with 10 mM potassium phosphate, pH 7.4, to 2.0 mg/ml and then dialyzed for 2 h against 500 volumes of the same buffer. Superoxide dismutase was purified from bovine erythrocytes according to the procedure of McCord and Fridovich (20). Horseradish peroxidase was purchased from Nutritional Biochemicals Co. Purified N$_{2}$ was obtained from Matheson Co.

Photooxidation was performed in an apparatus containing two 15-watt Sylvania 15T8-BL black light fluorescent bulbs which emit maximally at 375 nm (21). The apparatus was designed to protect personnel from exposure to ultraviolet radiation in order to avoid possible ocular cataract formation (22). Light intensity was determined with a Blak-Ray long wave UV meter (Ultraviolet Products Inc).

A typical reaction mixture contained 8 $\mu$m hematoporphyrin dihydrochloride, 0.4 mM NADPH, 10 mM potassium phosphate, pH 7.4, and additions as indicated. Unless otherwise indicated, all solutions were prepared with 99.8% $D_{2}O$. The final volume was 3.0 ml and photooxidation was carried out in a quartz cuvette. Oxidation of NADPH was determined from the change in absorbance at 340 nm. The light intensity used for all photooxidations was 600 micro-watts/cm$^2$. Preliminary experiments indicated no significant changes in the rate of photooxidation from pH 6.9 to 7.9.

RESULTS

Oxygen Requirement and Effect of $D_{2}O$ and $H_{2}O$—The data in Fig. 1 compare the hematoporphyrin-catalyzed photooxidation of NADPH in aerobic (air-saturated) and anaerobic (N$_{2}$-saturated) conditions, in both $D_{2}O$ and $H_{2}O$ media. Under anaerobic conditions, only a very slow rate of NADPH oxidation (as indicated by the decrease in absorbance at 340 nm) was detected and there was no significant difference between the $H_{2}O$ and $D_{2}O$ systems. On the other hand, under aerobic conditions, the rate of oxidation in $D_{2}O$ was increased to about 5 times that in the aerobic $H_{2}O$ system.

A $D_{2}O$ enhancement effect is generally observed in singlet oxygen reactions (23, 24). The lifetime of $^{1}O_{2}$ has been determined to be 2 $\mu$s in $H_{2}O$ and 20 $\mu$s in $D_{2}O$ (23–25). As would be expected if $^{1}O_{2}$ is the mediator, an increase of its lifetime in $D_{2}O$ would increase the steady state concentration of $^{1}O_{2}$ available to react with NADPH during the UV irradiation and thus potentiate the rate of photooxidation. The shortened lifetime in $H_{2}O$ as compared with $D_{2}O$ is thought to occur via the transfer of electronic energy from $^{1}O_{2}$ to the vibrational energy of $H_{2}O$ (24).

The absence of a $D_{2}O$ activation effect in the anaerobic system indicates that the oxidation in the anaerobic system was not due to contamination by traces of oxygen. Moreover, it also suggests different mechanisms of oxidation in the aerobic and the anaerobic systems. In the aerobic system in $D_{2}O$, at least 80% of the NADPH oxidation is oxygen-dependent. Thus the results in Fig. 1 support the hypothesis that singlet oxygen is the mediator in the major portion of the oxygen-dependent hematoporphyrin-catalyzed NADPH photooxidation.

Reaction Not Affected by Potentiators and Inhibitors of $O_{2}^{*}$, $OH^{*}$, and $H_{2}O_{2}$—Inhibitors of various reactive oxygen species related to singlet oxygen were tested in the photooxidation system. Table I shows that addition of superoxide dismutase did not show any significant effect in NADPH photooxidation. The dismutase activity was not lost during the irradiation as it was detected subsequently in a xanthine oxidase-cytochrome c system (20). As an additional support to exclude $O_{2}^{*}$ as a mediator of NADPH photooxidation, when KO$_{2}$ in dimethyl sulfoxide was added to the standard reaction solution (without irradiation), there was no detectable oxidation of NADPH. This is in agreement with earlier reports that radiation- and enzyme-generated O$_{2}$ is not reactive toward NADH and NADPH (26, 27).

The hydroxyl radical, $OH^{*}$, is a highly reactive oxidative species. Its second order rate constant with benzoate has been determined to be $3.3 \times 10^{9}$ M$^{-1}$ s$^{-1}$ (28), and, therefore, has frequently been used as a scavenger of $OH^{*}$. Mannitol is also widely used as a scavenger of $OH^{*}$. Experiments 4 and 5 in Table I show that both benzoate and mannitol at 10 mM (25 times more concentrated than NADPH), had no inhibitory effect.

Table I

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Additions</th>
<th>NADPH oxidized as percentage of control After 2 min irradiation</th>
<th>After 7 min irradiation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. None (control)</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>2. Superoxide dismutase, 0.06 mg</td>
<td>98</td>
<td>108</td>
<td></td>
</tr>
<tr>
<td>3. Bovine serum albumin, 0.08 mg</td>
<td>98</td>
<td>108</td>
<td></td>
</tr>
<tr>
<td>4. Benzylate, 10 mM</td>
<td>97</td>
<td>97</td>
<td></td>
</tr>
<tr>
<td>5. Mannitol, 10 mM</td>
<td>119</td>
<td>111</td>
<td></td>
</tr>
<tr>
<td>6. Catalase, 2500 units</td>
<td>91</td>
<td>104</td>
<td></td>
</tr>
<tr>
<td>7. $H_{2}O_{2}$, 0.62 mM</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>8. EDTA, 0.1 mM</td>
<td>114</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>9. $H_{2}O$ in place of $D_{2}O$</td>
<td>18</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>10. Dimethylfuran, 2.35 mM, and methanol, 125 mM</td>
<td>13</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>11. Methanol, 125 mM</td>
<td>142</td>
<td>107</td>
<td></td>
</tr>
<tr>
<td>12. Methionine, 10 mM</td>
<td>32</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>13. Histidine, 10 mM</td>
<td>18</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>14. 1,4-Diazabicyclo[2.2.2]octane, 10 mM</td>
<td>67</td>
<td>70</td>
<td></td>
</tr>
</tbody>
</table>
effect on the photooxidation of NADPH. This suggests that the reaction was not mediated by OH·.

Experiment 6 shows that the addition of catalase did not inhibit the photooxidation of NADPH, and addition of H2O2 in Experiment 7 did not potentiate the reaction. Thus H2O2 may also be excluded as the mediator in the photooxidation.

In Experiment 8, in order to test possible influence of trace metal ions in the system, EDTA was added and it was also found to be without any significant effect.

Effect of Singlet Oxygen Quenchers and Reactants—The lower half of Table I provides evidence supporting the involvement of singlet oxygen in photooxidation of NADPH in D2O. Experiment 9 shows results similar to those in Fig. 1. When H2O was substituted for D2O, only 18 and 19% of the oxidation was observed after 2 and 7 min of UV irradiation, respectively.

2,5-Dimethylfuran reacts with O2 in a Diels-Alder type reaction to form an unstable cyclic peroxide which reacts with methanol to form a comparatively more stable product (29, 30). The bimolecular rate constant for the reaction of dimethylfuran with O2 in methanol is 1.4 × 106 M⁻¹ s⁻¹ (31). Experiment 10 shows an 80% inhibition of NADPH photooxidation by the addition of dimethylfuran and methanol. Experiment 11 excludes methanol as the inhibitory factor.

The rate constants for the reactions of O2 with methionine and histidine are 3 × 105 M⁻¹ s⁻¹ and 5 × 107 M⁻¹ s⁻¹, respectively (4). Experiments 12 and 13 show that both of these amino acids are also effective inhibitors of NADPH oxidation. 1,4-Diazabicyclo[2.2.2]octane is a known quencher of O2, possibly via a charge-transfer mechanism (32, 33). In experiment 14, this compound was also shown to be an inhibitor of the photooxidation.

NADP⁺ As a Product—The nucleotide product of hemoporphyrin-catalyzed photooxidation of NADPH was analyzed enzymatically. Isocitrate and NADP-dependent isocitrate dehydrogenase were added to the irradiated reaction mixture to convert NADP⁺ back to NADPH. Table II shows that about 80% of the nucleotide product under aerobic conditions was in the form of enzymatically active NADP⁺. Table II also shows that under anaerobic conditions the rate of NADPH photooxidation was much slower, and only 40% of the nucleotide product was enzymatically active.

H2O2 Generation—In the photooxidation, the reactive oxygen species is potentially capable of accepting 1, 2, or 4 electrons to form O2, H2O2, or H2O, respectively, as the reduced product of the reaction. An attempt was made to detect O2⁻ as a transient product in the photooxidation by determining the superoxide dismutase inhibitable reduction of either ferricytochrome c or nitroblue tetrazolium. No significant reduction of either of these compounds was detected during the photooxidation of NADPH. This indicates that O2⁻ was not likely to be one of the reaction products.

In the presence of NADPH, it is difficult to measure H2O2 directly; therefore, possible formation of H2O2 during the photooxidation was determined with the aid of horseradish peroxidase. Yokota and Yamazaki (34) have shown that horseradish peroxidase can catalyze a chain oxidation of either NADH or NADPH with H2O2. We applied this system to detect H2O2 generation during the photooxidation of NADPH. Fig. 2 shows that when horseradish peroxidase was present in the cuvette during the irradiation, oxidation of NADPH continued after the end of the irradiation as a result of the peroxidatic activity of the peroxidase. When catalase was present in addition to horseradish peroxidase, this postirradiation oxidation of NADPH was eliminated. In a standard reaction mixture without irradiation, horseradish peroxidase did not cause any oxidation of NADPH. These data indicate that H2O2 was generated in the photooxidation system.

The experiment plotted in Fig. 3 was designed to further
Photooxidation of NADPH by $'O_2$ in $D_2O$

**Figure 3.** Effect of additions of $H_2O_2$ and horseradish peroxidase in the reaction mixtures after completion of UV irradiation. Photooxidation was performed as described under "Materials and Methods." ○, 2000 units of catalase added prior to UV exposure; ●, control, without catalase. At Arrow 1, 50 μl of 37 mM $H_2O_2$ was added to each cuvette. At Arrow 2, 600 units of horseradish peroxidase in 0.2 ml was added to each cuvette.

demonstrate that the postirradiation oxidation of NADPH was dependent on the presence of $H_2O_2$ and to show that catalase retained its activity after irradiation in the presence of hematoporphyrin and NADPH.

In the experiment, no detectable difference was observed in the $A_{340}$ change during the 12 min immediately after irradiation in the presence and absence of catalase. This suggests that $H_2O_2$ generated during photooxidation and still present in the sample without catalase during this period (see Fig. 2) did not contribute to NADPH oxidation in the absence of horseradish peroxidase.

When $H_2O_2$ was added at Arrow 1, no detectable oxidation was observed in either sample. When horseradish peroxidase was added at Arrow 2, however, there was a sharp reduction in $A_{340}$ in the sample without catalase, but no appreciable change in the sample with catalase.

These results indicate that catalase was not destroyed by UV irradiation and that $H_2O_2$ was required for the horseradish peroxidase-catalyzed oxidation of NADPH. Furthermore, it can be inferred that $H_2O_2$ was a product of the hematoporphyrin-catalyzed photooxidation of NADPH.

Another experiment was designed to analyze the production of $H_2O_2$ quantitatively. The concentration of $O_2$ in air-saturated aqueous solution is 0.25 mM. If $'O_2$ reacts with NADPHII as shown in Reaction 1:

$$H^+ + 'O_2 + NADPHII \rightarrow NADP^+ + H_2O_2$$  (1)

the oxygen-dependent oxidation of NADPH in a closed system would stop when oxygen is depleted, i.e. when 0.25 mM NADPH is oxidized. Thus the ratio of NADPH oxidized per molecule of oxygen will be 1. If catalase is also present in the system, however, Reaction 2 catalyzed by catalase would generate 0.5 eq of $O_2$ from $H_2O_2$:

$$2H_2O_2 \rightarrow 2H_2O + O_2$$  (2)

Therefore, in a similar system plus catalase, the ratio of NADPH oxidized per molecule of oxygen originally present in the cuvette will be 2.

Fig. 4 shows that the air-saturated cuvette without catalase began to level off after about 0.25 mM NADPH was oxidized. The slower rate of decrease thereafter could be due to anaerobic oxidation as discussed above with Fig. 1. As seen in Fig. 4, the air-saturated cuvette with catalase and the oxygen-saturated cuvette began to level off only when NADPH concentration became a rate-limiting factor, indicating that catalase had relieved the limitation of oxygen in the air-saturated system. Unfortunately, higher initial concentrations of NADPII could not be used for this time course study, because Beer’s Law is not applicable beyond 0.45 mM NADPH.

Table III shows an experiment with 0.84 mM initial concentration of NADPH. Only the total change in the concentration of NADPH after 21 min UV irradiation was determined with diluted samples. In Cuvette 1, the total amount of NADPH oxidized was about 10% greater than estimated by the concentration of available oxygen according to Reaction 1 probably contributed by anaerobic oxidation. In Cuvette 2 with catalase added, the total amount of NADPH oxidized in 21 min was 0.417 mM. The ratio of NADPH oxidized to the concentration of $O_2$ originally present in the air-saturated solution in Cuvette 2 is 1.67, which is 84% of the theoretically expected value of 2. In Cuvette 3 with oxygen-saturated solution, since oxygen was not a limiting factor, over 80% of NADPH was oxidized. Taken collectively, these results indicate that a close to stoichiometric amount of $H_2O_2$ was produced during the photooxidation of NADPII.

**DISCUSSION**

In various biological reactions involving reactive oxygen species, it has frequently been difficult to unequivocally identify the specific reactants. For example, singlet oxygen (17,
Oxygen as a limiting factor in photooxidation of NADPH in a closed system and effect of catalase

Photooxidation was carried out as described under "Materials and Methods." Stopped cuvettes were used and care was taken to avoid trapping any gas bubbles in the cell. The \( A_{\lambda 0} \) of the initial solution was 5.225, determined by measuring the absorbance of samples diluted with 2 volumes of \( H_2O \). The absorbance of the irradiated samples was also determined in diluted solutions.

\[
\frac{A_{\lambda}}{A_{\lambda 0}} = \frac{A_{\lambda \text{irradiated}}}{A_{\lambda \text{unirradiated}}} = \frac{A_{\lambda \text{unirradiated}}}{A_{\lambda \text{unirradiated}}}
\]

\( \text{Cuvette} \) | \( A_{\lambda} \text{ after 21 min ir-}\) | \( A_{\lambda} \text{ radiation} \) | \( \Delta A_{\lambda} \text{ oxidized} \) | \( \text{mM} \) | \( \text{NADPH} \)
---|---|---|---|---|---
1 | Air-saturated | 0.608 | -1.246 | 0.396 | 0.279
2 | Air-saturated plus 2000 units of catalase | 2.624 | -2.596 | 0.417 | 0.218
3 | O_2-saturated | 0.559 | -4.464 | 0.746 | 0.279

36), hydroxyl radicals (14, 36), metal ion-oxygen complexes (58, 39), and superoxide radicals (36, 39) all have been implicated in lipid peroxidation. In addition, the various reactive oxygen species mentioned above have also been implicated as the bactericidal agents accompanying phagocytosis by polymorphonuclear leukocytes (40). In some cases, possibly more than one species is involved. The main difficulty, however, is to decide that many of these species (OH-, 'O_2, and O_3) are very short lived, and all of them are rapidly interconvertible. For example, it has been suggested that O_2 spontaneously dismutates (41, 42) to 'O_2 as seen in Reaction 3. This, however, has been disputed by others (43, 44).

\[
\text{O}_2^- + \text{O}_2^- + 2\text{H}^+ \rightarrow \text{'}\text{O}_2 + \text{H}_2\text{O}_2
\]

The enzyme superoxide dismutase (20) catalyzes the dismutation of O_2 leading to the formation of ground state oxygen (42) (Reaction 4) rather than 'O_2. In either case, the spontaneous or enzyme-catalyzed dismutation of O_2 leads to H_2O_2.

\[
\text{O}_2^- + \text{O}_2^- + 2\text{H}^+ \rightarrow \text{'O}_2 + \text{H}_2\text{O}_2
\]

It has been postulated that O_2 may react with H_2O_2 to produce OH- via the Haber-Weiss reaction (45) (Reaction 5). There is, however, controversy concerning the possibility of the Haber-Weiss reaction under physiologic conditions (46-48).

\[
\text{O}_2^- + \text{H}_2\text{O}_2 \rightarrow \text{OH}^- + \text{OH}^- + \text{O}_2
\]

Direct evidence for OH- generated from Reaction 5 has not been demonstrated. Frequently its postulation is based on the inhibiting effect of both superoxide dismutase (to destroy \( \text{'O}_2 \)) and catalase (to destroy \( \text{H}_2\text{O}_2 \)) on a specific reaction. It has been suggested (16) that under certain conditions, O_2 may react with H_2O_2 to form 'O_2 and OH-. It is conceivable that Reaction 5 represents the net of a far more complex reaction that exists in biological systems.

Arneson (49) has proposed that \( \text{'O}_2 \) may be generated from a reaction between O_2 and the hydroxyl radical as seen in Reaction 6. This reaction has not been demonstrated under biological conditions.

\[
\text{O}_2^- + \text{OH}^- \rightarrow \text{'}\text{O}_2 + \text{OH}^- \]

In 1963, Kahn and Kasha (50) demonstrated that the reaction of H_2O_2 with hypochlorite results in singlet oxygen formation. It has been proposed (61) that this reaction may possibly play a role in the phagocytic killing of bacteria by the myeloperoxidase system. Overall, the \( \text{'O}_2 \) generating reaction is thought to be as outlined in Reaction 7 (52). The actual species which generates \( \text{'O}_2 \) is thought to be the chloroperoxyl ion \( \text{OOC}^- \). (52, 53).

\[
\text{H}_2\text{O}_2 + \text{OCl}^- \rightarrow \text{O}_2 + \text{H}_2\text{O} + \text{Cl}^- \]

It has recently been proposed (54) that \( \text{'O}_2 \) may arise from the base-catalyzed disproportionation of H_2O_2 as seen in Reaction 8. This is not likely to occur within the physiological pH range.

\[
\text{H}_2\text{O}_2 + 2\text{OH}^- \rightarrow \text{H}_2\text{O} + \text{OH}^- + \text{'O}_2
\]

It is clear that all of these reactive oxygen species may be closely interrelated. Consequently, although photoactivation of hematoporphyrin has been reported to produce singlet oxygen (7, 55), it is still desirable to examine possible participation of other oxygen species in the hematoporphyrin-catalyzed photooxidation of NADPH.

The results mitigate against mediation of the photooxidation by superoxide radicals because the photooxidation was not affected by superoxide dismutase and because addition of K_2O to a solution of NADPH did not result in oxidation of the nucleotide. In a similar manner, H_2O_2 was excluded as a mediator of the photooxidation; catalase had no effect on the oxidation and exogenously added H_2O_2 did not potentiate the reaction. The possible role of the hydroxyl radical as the mediator of the oxidation was explored via the use of mannitol and hemin. Since neither of these OH- scavengers inhibited the photooxidation of NADPH, OH- is not a likely candidate as a mediator of the reaction. The lack of an inhibitory effect of EDTA on the reaction suggests that metal ion-oxygen complexes are probably not involved.

On the other hand, when several compounds (methionine, histidine, dimethylfuran plus methanol, and diazabicyclooctane) which are known to react with \( \text{'O}_2 \) were tested in this system, they were all found to be effective inhibitors for the hematoporphyrin-catalyzed photooxidation of NADPH (Table I). These results, together with the differential effect of H_2O and D_2O (Fig. 1) are consistent with a singlet oxygen-mediated process.

Based on the reported rate constants for the reactions between \( \text{'O}_2 \) and various inhibitors and their effects on photooxidation of NADPH, the reaction rate constant between \( \text{'O}_2 \) and NADPH in D_2O can be estimated. Table IV shows that this constant calculated from inhibitions by dimethylfuran, methionine, and histidine varies from 2.2 to 3.1 \times 10^7 \text{M}^{-1} \text{s}^{-1}. \) Diazabicyclooctane inhibition was not included in the estimation because the experiment was carried out at an alkaline pH, while all the other experiments were at neutral pH range.
About 80% of the nucleotide product from the hematoporphyrin-catalyzed photooxidation of NADPH was identified as enzymatically active NADP+. This was assayed with an of these side reactions is still not understood. cant reduction of either ferricytochrome c or nitroblue tetrazolium during the course of photooxidation. However, generation of H$_2$O$_2$ was detected by horseradish peroxidase-catalyzed oxidation of NADPH and by the effect of catalase to provide additional oxygen for the photooxidation in a closed system. These results do not preclude the remote possibility that O$_2$ can participate in an oxidation by hydrogen or electron abstraction. This occurs in the photooxidation by phenols, the only known examples to date of this class of reactions in which O$_2$ participates. Four of these classes deal with oxidation by hydrogen or electron abstraction. The fifth class involves oxidation by hydrogen or electron abstraction without net oxygen incorporation. Specifically, this reaction involves a 2-electron transfer resulting in the oxidation of NADPH to produce enzymatically active NADP$^+$ with concomitant formation of H$_2$O$_2$.

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Singlet oxygen as a mediator in the hematoporphyrin-catalyzed photooxidation of NADPH to NADP+ in deuterium oxide.
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