Oxidation and Reduction of Nitrite Ion in the TiO₂ Photo-induced Catalytic Reaction

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During a photo-induced catalytic reaction under near UV irradiation to an aqueous suspension of TiO₂, about 95% of NO₂⁻ was oxidized to NO₃⁻, but NH₄⁺ was not detected. The oxidation was inhibited by the addition of mannitol or under anaerobic conditions. The nitration of HPA was observed in the presence of t-buthanol, suggesting the formation of ONOO⁻. An ESR spectrum gave a triplet signal at g = 2.041, in the presence of NO₃⁻, mannitol, FeSO₄, and MGD, indicating the reduction of NO₂⁻ to NO.

Key words: nitric oxide; nitrite; peroxynitrite; photo-induced catalysis; titanium dioxide

The initial event in the photo-induced catalytic reaction of TiO₂ in light with an energy greater than the band gap (3.2 eV for the anatase form of TiO₂, corresponding to light of about 385 nm) is well documented to promote an electron (e⁻) from the valence band to the conduction band, creating an electron vacancy or hole (h⁺) at the valence band edge (vb), according to Eq. 1.¹

\[ \text{TiO}_2 + hv \rightarrow e^- + h^+_\text{vb} \]  

We reported recently,² the generation scheme of active oxygen in the TiO₂ photo-induced catalytic reaction. Dioxygen molecule and H₂O₂, the dismutation product of O₂⁻, are reduced by e⁻ to produce O₂ and ·OH, respectively.

\[ O_2 + e^- \rightarrow O_2^- \]  
\[ 2O_2^- + 2H^+ \rightarrow H_2O_2 + O_2 \]  
\[ H_2O_2 + e^- \rightarrow ·OH + OH^- \]

There is another route of ·OH generation, the oxidation of H₂O by h⁺vb.

\[ h^+_\text{vb} + OH^- \rightarrow ·OH or h^+_\text{vb} + H_2O \rightarrow ·OH + H_2O \]

These active species of oxygen as well as e⁻ and h⁺vb would support a wide variety of TiO₂ dependent photo-induced catalytic reactions, oxidation, oxidative cleavage, reduction, geometric and valence isomerization, substitution, condensation, and polymerization.³

We showed here that NO₂⁻ is oxidized by ·OH and reduced by e⁻, producing NO and NO₂, respectively, by the photo-induced catalytic reaction of TiO₂, and that the nitration of HPA by ONOO⁻, formed from a reaction of NO with O₂.³ NO is known as one of the important intercellular messengers, as a mediator of vaso-constricting action in mammals, and as the molecule that collaborates with reactive species of oxygen to trigger transcriptional activation of defense genes and the hypersensitive response in plants.⁴,⁵ ONOO⁻ and/or HONO₂ induces lipid peroxidation, oxidizes Met and Cys residues, and causes nitration of Tyr residues in proteins, depletes antioxidants, and damages DNA.⁶

The photo-induced catalytic reaction was started by illuminating with near UV light (3.6 mW/cm²) obtained by filtering light with a heat-cut filter and a UV-D33S pass-filter (Toshiba, Japan) which passed light of 295–390 nm from a 200-W xenon lamp equipped in the UV-illuminating system L-5662-01 (Hamamatsu Photonics, Hamamatsu, Japan). The intensity of near UV light was measured with a UV-power meter (Hamamatsu Photonics, C6080-03). The reaction mixture containing TiO₂ as a powder (ϕ = 5 µm) was stirred magnetically during the reaction. Anaerobic experiments were done by introducing argon gas to the reaction mixture for at least 20 min before the start of reaction, and bubbling during the reaction at a flow rate of 5 ml/min. Upon near UV irradiation, NO₂⁻ was decreased with time, and disappeared after 90 min of irradiation.

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Abbreviations: HPA, 4-hydroxyphenyl acetic acid; nitro-HPA, 4-hydroxy-3-nitro-phenylacetic acid; NO, nitric oxide; NOR-3, (±)-(E)-ethyl-2-[(E)-hydroxyimino]-5-nitro-3-hexeneamine; ONOO⁻, peroxynitrite; MGD, methylglucaminedithiocarbamate; Fe(MGD)₃, ferrous complex of MGD
tion under the aerobic conditions. NO$_3^-$ was converted to NO$_2^-$ with about 95% yield of the decreased amount of NO$_3^-$ as shown in Fig. 1. No NH$_4^+$ was detected during the reaction. The oxidation of NO$_3^-$ to NO$_2^-$ was inhibited by the addition of 250 mM mannitol, a OH scavenger and the anaerobic condition, under which the yield of OH generation is only 18% of that observed in aerobic reaction. These results indicated the oxidation of NO$_3^-$ proceeded according to the equation [6] by OH generated by reactions [4] and [5].

\[ \text{NO}_3^- + 2 \cdot \text{OH} \rightarrow \text{NO}_2^- + \text{H}_2\text{O} \]  

[6]

On the other hand, after the reaction in the presence of an OH scavenger, the spectrum of HPA being added to the reaction mixture showed a distinct shoulder near 430 nm in an alkaline solution (Fig. 2). As an authentic nitro-HPA (Sigma, H0257) showed a distinct peak at 430 nm in an alkaline solution, the reaction product was possibly nitro-HPA. HPLC analysis using a reverse phase column (ODS 120T, Tosoh) done under the same conditions as reported to analyze nitro-HPA, a low but significant amount of nitro-HPA in the reaction mixture was detected. No nitration of HPA proceeded in anaerobic reaction, without irradiation, or in the absence of NO$_3^-$ (data not shown). The nitration of phenolic moieties proceeds by ONOO$^-$, the reaction product between O$_2^-$ and NO. This nitration is catalyzed by transition metals, superoxide dismutase, peroxidase, proteins containing copper, and catalase, but can also occur spontaneously. The nitration of HPA suggested the generation of NO from NO$_3^-$ to form ONOO$^-$ with O$_2$ which is the co-product of the aerobic TiO$_2$-photo-induced catalytic reaction [2].

\[ \text{O}_2^- + \text{NO} \rightarrow \text{ONOO}^- \quad (k = 6.7 \pm 0.9 \times 10^8 \text{ M}^{-1}\text{s}^{-1})[7] \]

ONOO$^-$ + HPA $\rightarrow$ nitro-HPA  

[8]

By the addition of Fe(MDG)$_2$ (Dojin Laboratories, Kumamoto, Japan) to the aerobic reaction mixture contained NO$_3^-$ and mannitol, an ESR spectrum showed a characteristic triplet signal at $g = 2.041$ (Fig. 3). This triplet was the same as that of NO trapped with Fe(MDG)$_2$, in which NO was formed from N0$_2^-$ and Fe(MDG)$_2$, a typical NO generating reagent. An identical triplet signal had been detected by trapping NO generated from sodium nitroprusside with Fe(MDG)$_2$. The ESR analysis indicated the generation of NO by the reduction of NO$_2^-$ in TiO$_2$ photo-induced catalytic reaction. NO$_2^-$ is reduced by a hydrated electron (e$_{h}^-$) induced by pulse-radiolysis. In our previous report, we detected the reduction of 2,6-dichlorophenolindophenol or ferricyanide by e$_{h}^-$ generated by the photo-induced reaction of TiO$_2$. As the reduction of these com-

![Fig. 1. Disappearance of NO$_3^-$ and Formation of NO$_2^-$ During Photo-induced Catalytic Reaction of TiO$_2$.](image1)

The reaction mixture contained 50 mM K-phosphate, pH 7.4, 5 mg/ml TiO$_2$ (anatase, Wako Pure Chemical Industries) and 100 mM NaNO$_3$. The reaction was started by near UV irradiation. During the photo-induced catalytic reaction, aliquots were withdrawn from the mixture to measure NO$_3^-$, NO$_2^-$ and NH$_4^+$, NO$_3^-$ (●) was measured spectrophotometrically based on the Griess reaction. NO$_3^-$ (●) and NH$_4^+$ (●) were assayed enzymatically using the F-Kit (Boehringer Mannheim).

![Fig. 2. Absorption Spectra of Nitro-HPA and the Reaction Product from HPA after the Photo-induced catalytic Reaction of TiO$_2$.](image2)

The reaction mixture (1 ml) contained 50 mM K-phosphate, pH 7.4, 5 mg TiO$_2$, 5 mM NaNO$_3$, 100 mM i-butyl alcohol, and 1 mM HPA. After near UV irradiation for 1 hr, 30 $\mu$M of 1 mM NaOH was added, then the mixture was centrifuged to remove TiO$_2$. The spectrum of the supernatant fluid was recorded (line 1). The spectra of authentic nitro-HPA (125 $\mu$M) in alkaline solution (line 2) and in 50 mM K-phosphate, pH 7.4 (line 3) are also shown.
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The reaction mixture contained 50 mM K-phosphate, pH 7.4, 5 mg/ml TiO$_2$, 5 mM NaNO$_2$, 250 mM mannitol, 10 mM MGD, and 1 mM FeSO$_4$. ESR spectra were measured at room temperature by a JEOL JES-FE1X spectrometer with 100 kHz field modulation. The modulation width was 0.25 mT and time constant was 0.1 sec. ESR spectrum of the reaction mixture after 5 min from the start of irradiation (line 1), and without irradiation (line 3). The spectrum of NO trapped with Fe(MGD)$_2$ (line 2) was also recorded in a mixture contained 2 mM NOR-1 as the NO generator, MGD and FeSO$_4$.

Fig. 3. ESR Spectra of NO Trapped with Fe(MGD)$_2$ and of Aerobic Photo-catalytic Reaction Product from NO$_2^-$. The reaction mixture contained 50 mM K-phosphate, pH 7.4, 5 mg/ml TiO$_2$, 5 mM NaNO$_2$, 250 mM mannitol, 10 mM MGD, and 1 mM FeSO$_4$. ESR spectra were measured at room temperature by a JEOL JES-FE1X spectrometer with 100 kHz field modulation. The modulation width was 0.25 mT and time constant was 0.1 sec. ESR spectrum of the reaction mixture after 5 min from the start of irradiation (line 1), and without irradiation (line 3). The spectrum of NO trapped with Fe(MGD)$_2$ (line 2) was also recorded in a mixture contained 2 mM NOR-1 as the NO generator, MGD and FeSO$_4$.

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First indication of the oxidation as well as the reduction of NO$_2^-$ by the photo-induced catalytic reaction of TiO$_2$. The reaction generating NO and ONOO$^-$ may be applicable to biological systems to elucidate the functions of active species of nitrogen.

References