Photodynamic Activity of Ascorbic Acid-modified TiO_2 Nanoparticles upon Visible Illumination (>550 nm)

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Though both TiO_2 and ascorbic acid are noncytotoxic in the dark or under visible irradiation, ascorbic acid-modified TiO_2 nanoparticles were found to exhibit efficient DNA and cancer cell damage ability under visible light irradiation, opening a new pathway for developing highly efficient PDT sensitizers.

Inorganic nanoparticles are rapidly emerging in biomedical applications as drug delivery carriers, biosensors, imaging diagnosis, and disease therapeutics.[1] The inorganic nanoparticles that have been extensively explored in the field of biomedicine cover a variety of materials and diverse shapes, such as carbon nanotubes, silica nanospheres, metal nanoshells, and semiconductor quantum dots. As one of the most widely used semiconductor nanomaterials, TiO_2 has found applications in paint, UV protection, photocatalysis, and so on.[2] Though TiO_2 is biocompatible,[3] medical application is very limited. The most important feature that distinguishes TiO_2 from carbonic-, ceramic-, and metallic-based nanomaterials is its photocatalytic activity, i.e., the generation of reactive oxygen species (ROS, such as superoxide anion radical $O_2^{-\bullet}$, hydroxyl radical •OH, and singlet oxygen $^1O_2$) upon UV irradiation.[2] Such a property may be fully utilized to render TiO_2 nanoparticles promising therapeutic capability in photodynamic therapy (PDT), a minimal-invasive cancer treatment modality,[4] provided that its photocatalytic activities are extended to the visible region. Surface modification by organic molecules is a simple but effective strategy to extend the optical response threshold of TiO_2 nanomaterials,[5] and molecules possessing enediol structure, e.g., salicylate, catechol, dopamine, and ascorbic acid, are such surface modifiers.[6–8] Among enediol-based ligands, ascorbic acid (AA) is probably the most intriguing one. AA, namely, vitamin C, is the most frequently used supplementary medicine. Its concentration in the blood of normal individuals varies from 5 to 90 μM, and it is actively accumulated in human tissues to a concentration of micromolar level.[9,10] Moreover, the level of AA is greatly higher in neoplasms than in adjacent normal tissue.[11] As a result, AA-modified TiO_2 nanoparticles warrant scrutiny as a new type of PDT sensitizer owing to the following considerations. 1) Both TiO_2 and AA are noncytotoxic in the dark or under visible light irradiation, but their assembly may pose remarkable photodynamic capability. 2) AA-modified TiO_2 was reported to be extremely stable under irradiation,[6] therefore, avoiding photobleaching.[12] 3) Improved tumor uptake may be achieved by tuning the size of TiO_2[13] or by surface modification with targeting molecules.[14] AA itself, owing to its excess accumulation in neoplasms, might also facilitate tumor selectivity of PDT.

So far, many kinds of inorganic nanoparticles, such as silica nanoparticles, have been utilized as the delivery vehicles of PDT sensitizers,[15,16] where inorganic nanoparticles are observers in ROS generation processes of the sensitizer payload. For PDT agents based on chlorine e6-, hypocrellin B, or platinum(IV) chloride-modified TiO_2 nanoparticles, TiO_2 directly took part in ROS generation via electron-transfer interaction with the excited sensitizers.[17–19] Chlorine e6 and hypocrellin B themselves are well-known PDT sensitizers, while Pt^{IV} species present non-negligible dark cytotoxicity. In this communication, we report on the promising photodamage ability of AA-modified TiO_2 nanoparticles toward DNA and cancer cells upon visible irradiation, where AA is noncytotoxic in the dark and under visible irradiation, greatly different from chlorine e6-, hypocrellin B, or platinum(IV) chloride-modified TiO_2 nanoparticles. To the best of our knowledge, this is the first example that the combination of both noncytotoxic species under dark or visible light gives rise to photodynamic activities, opening a new pathway for developing highly efficient PDT sensitizers.

As a proof-of-concept, the most commercially available TiO_2 powder P25 (Degussa, Germany) was used to prepare TiO_2 colloid by mixing it with PBS (5.0 mM, pH 7.4) and sonicating with a supersonic probe for 10 min. In the resultant colloid, ca. 27-nm primary TiO_2 particles existed as nanoaggregates with an average diameter of about 150 nm.[22] Addition of AA made the absorption spectrum of TiO_2 extend into the visible region, though both TiO_2 and AA are only UV absorbing. The extended absorption is believed to be the excitation of localized electrons from the surface modifier AA into the conduction band continuum states of the TiO_2 particles.[6] When AA exceeded 5 mM, the spectrum red shift and absorbance increase reached saturation, implying the full coverage of TiO_2 surface.[22]

The potential of AA-modified TiO_2 colloid to cleave DNA upon visible irradiation (>550 nm) was evaluated using plasmid pBR322 DNA as target (Figure 1). AA-modified TiO_2 colloid (5.0 mM AA and 0.1 g L^{-1} TiO_2) can lead to transformation of half of plasmid pBR322 DNA cleaved from supercoiled (SC) form to nicked circular (NC) form after 0.5 h of irradiation (Figure 1A, lane 4). Prolongation of the irradiation to 1 h resulted in complete disappearance of supercoiled form, meanwhile linear form appeared (Figure 1B, lane 2). The cleavage obviously originates from the interaction between AA and TiO_2 under visible irradiation, evidenced by the fact that the cleavage ability was lost in the control experiments (Figure 1A, lane 2, 3, and 5) where any one of TiO_2, AA, and irradiation was absent. To explore the cleavage mechanism, photoreactions were carried out in the presence of *p*-benzoquinone (Figure 1A, lane 6), mannitol (Figure 1A, lane 7), and NaN_3 (Figure 1A, lane 8), the typical scavengers of $O_2^{-\bullet}$, •OH, and $^1O_2$, respectively.[21] It was found that *p*-benzoquinone, mannitol, and NaN_3 can restrict DNA cleavage efficiently, suggesting that $O_2^{-\bullet}$, •OH, and $^1O_2$ all contribute to the DNA cleavage as reactive agents.[22] Though EPR measurements were conducted to record spin adduct signals of

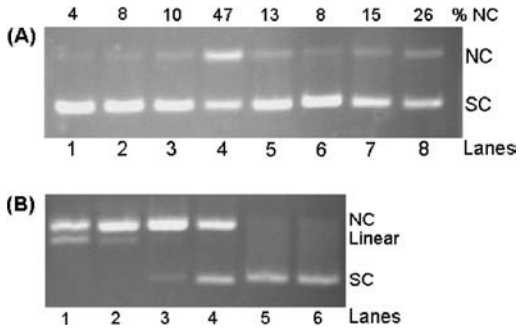

Figure 1. (A): Plasmid pBR322 DNA photocleavage upon visible irradiation for 0.5 h. Lane 1: DNA control, Lane 2: AA (5.0 mM) alone, Lane 3: TiO_2 colloid ($0.1\,g\,L^{-1}$) alone, Lane 4: AA (5.0 mM) + TiO_2 colloid ($0.1\,g\,L^{-1}$), Lane 5: similar to lane 4 but no irradiation, Lane 6–8: similar to lane 4 but in the presence of 0.1 M *p*-benzoquinone, mannitol, or NaN_3, respectively. (B): Plasmid pBR322 DNA photocleavage by AA-modified TiO_2 colloid upon visible irradiation for 1 h. Lane 1–5: TiO_2 colloid is $0.1\,g\,L^{-1}$ and the concentrations of AA are 1.0, 5.0, 10, 20, and 100 mM, respectively. Lane 6: AA (100 mM) alone.

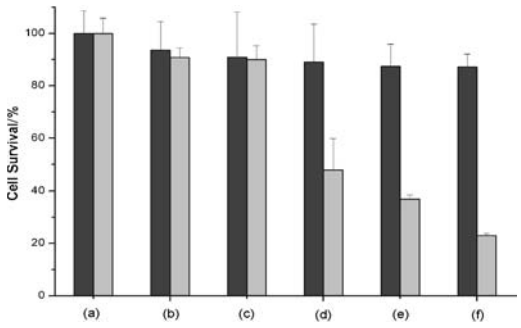

Figure 2. Phototoxicity of AA-modified TiO_2 colloid in MCF-7 cells upon irradiation for 1 h as determined by MTT assay. (a) Control, (b) AA, 2.0 mM, (c) TiO_2, $0.1\,g\,L^{-1}$, (d) $0.1\,g\,L^{-1}$ TiO_2 + 0.5 mM AA, (e) $0.1\,g\,L^{-1}$ TiO_2 + 1.0 mM AA, (f) $0.1\,g\,L^{-1}$ TiO_2 + 2.0 mM AA. The viabilities of cells with and without irradiation are shown as gray and black bars, respectively.

$O_2^{-\bullet}$, •OH, and $^1O_2$ with spin trapping agents DMPO and TEMP, no EPR signals were detected, most likely due to the efficient reduction of nitroxyl radicals by AA.[23,24]

It is worth noting that AA is a well-known antioxidant and can chemically quench ROS, especially singlet oxygen.[20] Thus, when AA exceeded 5 mM, DNA photocleavage ability of AA–TiO_2 system diminished gradually as shown in Figure 2B. In particular, no DNA cleavage was observed in the case of 100 mM AA. More interestingly, when TiO_2 particles were not fully covered by AA (the case of 1 mM AA, Figure 1B, lane 1), the DNA damage extent increased compared to full coverage cases, evidenced by the increased amount of linear DNA. Besides that ROS quenching is mitigated at low concentration of AA, the enhanced damage may, at least partly, be attributed to the binding of DNA onto the unoccupied TiO_2 surface, which improves the availability of ROS generated around the TiO_2 surface.[22]

To investigate the photodynamic properties of AA-modified TiO_2 in vitro, human breast cancer MCF-7 cells were used and MTT assay was applied. The 1-h irradiation (>550 nm) led to viability reduction by 50–80% in the cases of AA-modified TiO_2 (at the final concentration of $0.1\,g\,L^{-1}$ for TiO_2 and 0.5, 1.0, or 2.0 mM for AA, respectively) (Figure 2). In contrast, AA or TiO_2 alone exhibited negligible phototoxicity under the same conditions. The low dark cytotoxicity and remarkable phototoxicity of AA-modified TiO_2 demonstrate its promising potential as a versatile photosensitizing system in PDT application. The efforts to further enhance the photodynamic activities of AA-modified TiO_2, such as simultaneous surface modification by targeting functionalities, are in progress.

In summary, the unique property of AA to bind to TiO_2 surfaces and extend TiO_2 photocatalytic activity into the visible region was, at the first time, utilized to photodamage DNA and cancer cells, providing guidelines for developing new types of PDT agents from the biocompatible platform of TiO_2 nanoparticles.

References and Notes

1 J. M. Klostranec, W. C. W. Chan, *Adv. Mater.* **2006**, *18*, 1953.
2 X. Chen, S. S. Mao, *Chem. Rev.* **2007**, *107*, 2891.
3 R. Carbone, I. Marangi, A. Zanardi, L. Giorgetti, E. Chierici, G. Berlanda, A. Podesta, F. Fiorentini, G. Bongiorno, P. Piseri, P. G. Pehcci, P. Milant, *Biomaterials* **2006**, *27*, 3221.
4 S. Verma, G. M. Watt, Z. Mai, T. Hasan, *Photochem. Photobiol.* **2007**, *83*, 996.
5 A. Mishra, M. K. R. Fischer, P. Bäuerle, *Angew. Chem., Int. Ed.* **2009**, *48*, 2474.
6 T. Rajh, L. X. Chen, K. Lukas, T. Liu, M. C. Thurnauer, D. M. Tiedi, *J. Phys. Chem. B* **2002**, *106*, 10543.
7 T. Tachikawa, Y. Asanoi, K. Kawai, S. Tojo, A. Sugimoto, M. Fujitsuka, T. Majima, *Chem.—Eur. J.* **2008**, *14*, 1492.
8 N. M. Dimitrijevic, E. Rozhkova, T. Rajh, *J. Am. Chem. Soc.* **2009**, *131*, 2893.
9 M. Levine, C. Conry-Cantilena, Y. Wang, R. W. Welch, P. W. Washko, K. R. Dhariwal, J. B. Park, A. Lazarev, J. F. Graumlich, J. King, L. R. Cantilena, *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 3704.
10 R. W. Welch, Y. Wang, A. Crossman, J. B. Park, K. L. Kirk, M. Levine, *J. Biol. Chem.* **1995**, *270*, 12584.
11 H. Langemann, J. Torhorst, A. Kabiersch, W. Krenger, C. G. Honegger, *Int. J. Cancer* **1989**, *43*, 1169.
12 R. Bonnett, G. Martínez, *Tetrahedron* **2001**, *57*, 9513.
13 Y. N. Konan, R. Gurny, E. Allemann, *J. Photochem. Photobiol. B* **2002**, *66*, 89.
14 R. V. J. Chari, *Acc. Chem. Res.* **2008**, *41*, 98.
15 A. C. S. Samia, X. Chen, C. Burda, *J. Am. Chem. Soc.* **2003**, *125*, 15736.
16 S. Kim, T. Y. Ohulchanskyy, H. E. Pudavar, R. K. Pandey, P. N. Prasad, *J. Am. Chem. Soc.* **2007**, *129*, 2669.
17 S. Xu, J. Shen, S. Chen, M. Zhang, T. Shen, *J. Photochem. Photobiol. B* **2002**, *67*, 64.
18 Y. Tokuoka, M. Yamada, N. Kawashima, T. Miyasaka, *Chem. Lett.* **2006**, *35*, 496.
19 A. Jańczyk, A. Wolnicka-Glubisz, K. Urbanska, H. Kisch, G. Stochel, W. Macyk, *Free Radical Biol. Med.* **2008**, *44*, 1120.
20 M. L. Rooney, *Photochem. Photobiol.* **1983**, *38*, 619.
21 J. Bandara, J. Kiwi, *New J. Chem.* **1999**, *23*, 717.
22 Supporting Information is available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html.
23 Y. Lin, W. Liu, H. Ohno, T. Ogata, *Anal. Sci.* **1999**, *15*, 973.
24 Y. Sakurai, H. Sanuki, R. Komatsu-Watanabe, T. Ideguchi, N. Yanagi, K. Kawai, K. Kanaori, K. Tajima, *Chem. Lett.* **2008**, *37*, 1270.

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