

## Effects of different levels of vitamin C on UV radiation-induced DNA damage

Dianfeng Zhou (周殿凤)<sup>1,2</sup>, Hang Heng (衡航)<sup>1</sup>, Kang Ji (籍康)<sup>1</sup>, and Weizhong Ke (柯惟中)<sup>1</sup>

<sup>1</sup>*Jiangsu Provincial Key Lab for Photoelectric Technology, Analysis and Test Centre, Nanjing Normal University, Nanjing 210097*

<sup>2</sup>*Physics Department of Yancheng Normal College, Yancheng 224002*

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The Raman spectra of DNA in different levels of vitamin C with 10- and 30-min ultraviolet (UV) radiations were reported. The intensity of UV radiation was 18.68 W/m<sup>2</sup>. The experimental results proved that vitamin C could alone prevent UV radiation from damaging DNA, but the effects depended on the concentration of vitamin C. When the concentration of vitamin C was about 0.08–0.4 mmol/L, vitamin C decreased UV radiation-induced DNA's damage. When the concentration of vitamin C exceeded 0.4 mmol/L, vitamin C accelerated DNA's damage instead. Maybe the reason is that when DNA in aqueous solution is radiated by UV, free radicals come into being, and vitamin C can scavenge free radicals, so vitamin C in lower concentration can protect DNA. The quantity of free radicals is finite, when vitamin C is superfluous, free radicals have been scavenged absolutely and vitamin C is residual. Vitamin C is a strong reductant. When the mixture of DNA and residual vitamin C is radiated by UV, vitamin C reacts with DNA. The more residual vitamin C and the longer time of UV radiation, the more DNA is damaged.

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Exposure of cells to ultraviolet (UV) irradiation leads to the induction of a variety of chemical changes in the genetic material. These changes are manifested in biological effects, such as mutations, aging, carcinogenesis, and lethality. The breakage of ozonosphere leads more UV radiation to reach ground. UV radiation can increase the output of active oxygenic free radicals such as <sup>1</sup>O<sub>2</sub>, O<sub>2</sub><sup>-</sup>, and HO·, which are harmful to DNA. Some antioxidant can reduce the probability of mutations and carcinogenesis that were induced by UV radiation. Vitamin C became more and more important due to its antioxygenation. Each stuck to his own view about whether vitamin C could alone protect DNA or not<sup>[1,2]</sup>. In a previous paper<sup>[3]</sup>, we studied the effects of UV radiation on DNA in aqueous solution. Up to now, no one has studied the effects of different levels of vitamin C on UV radiation-induced DNA's damage by Raman spectroscopy. To study whether vitamin C can alone protect DNA, DNA in different concentrations of vitamin C was radiated by UV in different time, and then their Raman spectra were tested.

The calf thymus DNA is from Serva Company in German. Six mixtures of DNA, Na<sub>2</sub>SO<sub>4</sub>, and vitamin C were dissolved in twice distilled H<sub>2</sub>O at pH7.0. The final concentration of DNA was 5 wt.-%, and Na<sub>2</sub>SO<sub>4</sub> (its 981-cm<sup>-1</sup> band was used as a standard line) was 0.045 mmol/L, and vitamin C was 0.08, 0.15, 0.35, 0.45, 0.65, and 1 mmol/L, respectively. The samples were put in a refrigerator for 48 h at a temperature of 4 °C. Then the samples were irradiated with UV radiation with the wavelength of 200–400 nm, and were detected with Raman spectroscopy. The intensity of the UV radiation was 18.68 W/m<sup>2</sup>, which was measured with a digital laser power meter (EG&G, Model 460-1).

Raman spectra were obtained by using the 514.5-nm line of a Coherent Series Innova 70 argon-ion laser. Laser power of 200 mW was employed to record spectra between 600 and 1700 cm<sup>-1</sup>. Raman scattering was collected at 90° to the exciting laser beam and obtained by

a Jobin Yvon HRD-2 double monochromator fitted with a photomultiplier detector. Raman spectra were manipulated, including background subtraction. The slit widths of the spectrometer were 500-500-500 μm. Each Raman spectrum was the result of 5–6 measurements, which were averaged in order to minimize effects due to differences in local DNA composition. Line positions of well-resolved lines are accurate within ±2 cm<sup>-1</sup>. All spectra were obtained at 23 °C.

Raman spectra of different concentrations of vitamin C in aqueous solution with 10- or 30-min UV radiation were similar. They were also similar to vitamin C in aqueous solution without UV radiation. So only one of the Raman Spectra of vitamin C was shown (Fig. 1). Raman spectra of damaged DNA can be obtained by using Raman spectra of the mixture of DNA and vitamin C subtracted relevant Raman spectra of vitamin C. Raman spectra of DNA in 0.15-mmol/L vitamin C with UV radiation were similar to that in 0.08-mmol/L vitamin C, but the fluorescence background noise of the latter was higher. Raman spectra of DNA in 0.45-mmol/L vitamin C with UV radiation were similar to that in 0.08-mmol/L vitamin C. Raman spectra of DNA in 1-mmol/L vitamin C with UV radiation had a very high fluorescence background noise, and any Raman characteristic bands could not be seen. There only some Raman spectra of damaged DNA are shown in Figs. 2 and 3. The band of 981 cm<sup>-1</sup> which is corresponding to SO<sub>4</sub><sup>2-</sup> provides an internal intensity standard. All bands' intensities were calculated by it.

In Ref. [3], we studied the influence of UV irradiation on DNA, and concluded that UV radiation for 10 min damaged the conformation and configuration of DNA. And it also damaged the hydrogen bonds and groups among the Purine, pyrimidine bases, and deoxyribose. UV radiation for 20 min caused DNA very serious damage, and any Raman characteristic bands could not be seen (see Fig. 4).

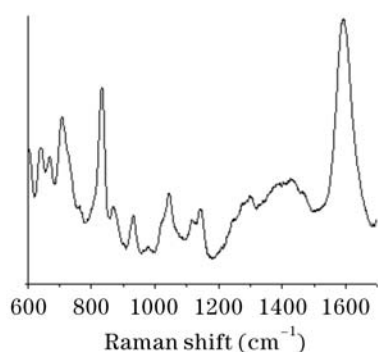


Fig. 1. Raman spectrum of 0.2-mmol/L vitamin C aqueous solution with 30-min UV radiation.

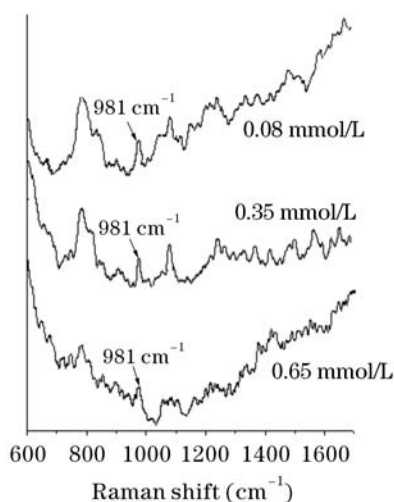


Fig. 2. Raman spectra of DNA in different concentrations of vitamin C with 10-min UV radiation.

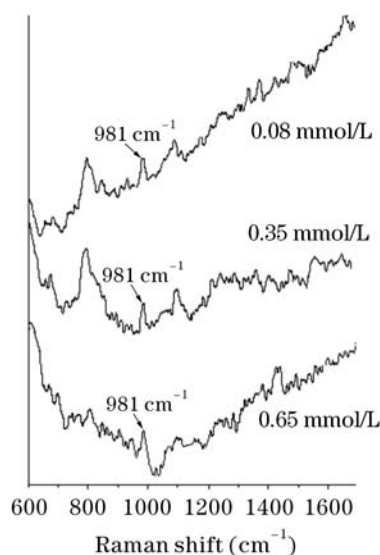


Fig. 3. Raman spectra of DNA in different concentrations of vitamin C with 30-min UV radiation.

The phosphonic bond stretching vibration which appears at about  $1094\text{ cm}^{-1}$  is sensitive to configuration changes. So we define the intensity of  $1094\text{-cm}^{-1}$  band of DNA in aqueous solution without UV radiation as 1. Comparative intensities of  $1094\text{-cm}^{-1}$  band of DNA

in different levels of vitamin C with different time UV radiation were shown in Table 1. The correlation curve between the intensity of  $1094\text{-cm}^{-1}$  band with UV radiation and the concentration of vitamin C fitted with the data of Table 1 (see Fig. 5).

Figure 5(a) shows that when DNA in  $0.16\text{--}0.36\text{ mmol/L}$  vitamin C, the comparative intensity of  $1094\text{-cm}^{-1}$  band was higher than 0.9, which indicated that UV radiation for 10 min did not damage DNA nearly. When vitamin C concentration was about  $0.04\text{--}0.16\text{ mmol/L}$  or  $0.36\text{--}0.4\text{ mmol/L}$ , the intensity of  $1094\text{-cm}^{-1}$  band was  $0.8\text{--}0.9$ , which suggested that DNA was damaged slightly. When the concentration of vitamin C was higher than  $0.4\text{ mmol/L}$ , the intensity of  $1094\text{-cm}^{-1}$  band was lower than 0.75, which implied higher concentration of vitamin C accelerated UV radiation-induced DNA's damage. The higher concentration of vitamin C, the more DNA was damaged.

We can also deduce the above conclusions from the fluorescence background noise of Raman spectra. When DNA in  $0\text{--}0.35\text{ mmol/L}$  vitamin C was radiated by UV, the higher concentration of vitamin C, the lower fluorescence background noise in the higher wave number region was. It indicated that DNA was protected better and better with the rising of concentration of vitamin C. When the concentration of vitamin C is higher than  $0.35\text{ mmol/L}$ , the fluorescence background noise in the higher wave number region became higher with the rising of the concentration of vitamin C. UV radiation caused fracture of many hydrogen bonds and generation of single chains. When DNA was radiated by UV, some damaged bases became other substances. When these mixed molecules and groups were irradiated by the laser, the fluorescence background noise increased<sup>[4]</sup>. The greater damage, the higher fluorescence background noise was.

Figure 5(b) shows the influence of UV radiation for 30 min on DNA was not the same as 10-min UV radiation. In a pervious paper<sup>[3]</sup>, UV radiation for 20 min damaged DNA badly, and any Raman characteristic bands could not be seen. When DNA was in  $0.35\text{-mmol/L}$  vitamin C aqueous solution, even UV radiation time was 30 min, DNA was just damaged slightly. It was well enough to prove that vitamin C can protect DNA. When the concentration of vitamin C was  $0.22\text{--}0.35\text{ mmol/L}$ , the comparative intensity of  $1094\text{-cm}^{-1}$  band was higher than 0.9. It indicated that UV radiation for 30 min did not damage DNA nearly. When the concentration of vitamin C was higher than  $0.4\text{ mmol/L}$ , DNA was damaged badly with the increase of the concentration of vitamin C.

Table 1. Comparative Intensity of  $1094\text{-cm}^{-1}$  Band with UV Radiation

Concentration of Vitamin C (mmol/L)	10-min UV Radiation	30-min UV Radiation
0	0.75	0
0.08	0.86	0.82
0.15	0.92	0.83
0.35	0.98	0.99
0.45	0.59	0.52
0.65	0.45	0.39
1	0.15	0

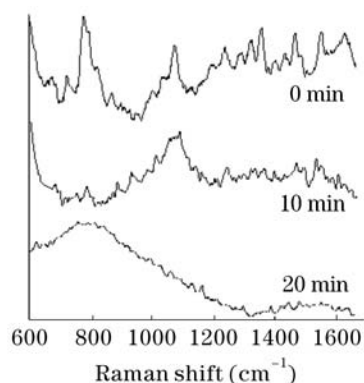


Fig. 4. Raman spectra of calf thymus DNA in aqueous solution after different time UV radiation.

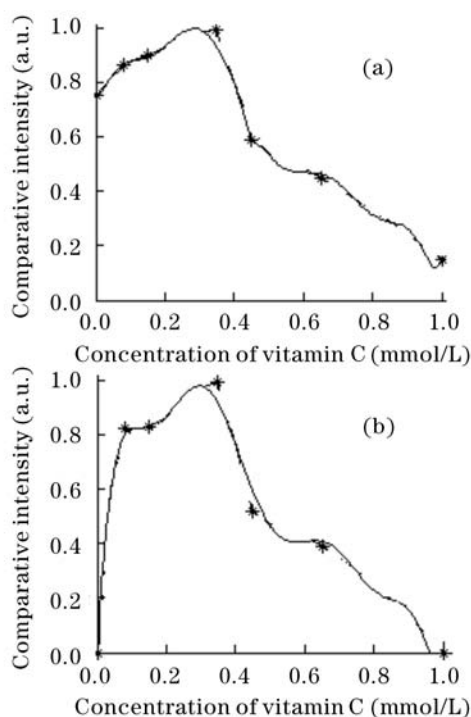
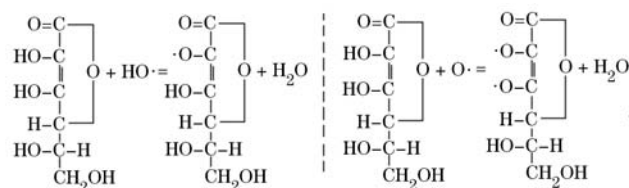


Fig. 5. Comparative intensity of  $1094\text{-cm}^{-1}$  band of DNA in different concentrations of vitamin C with (a) 10-min and (b) 30-min UV radiation.

With the existence of oxygen, free radicals maybe come into being when the mixture of DNA and vitamin C in aqueous solution was radiated by UV<sup>[5]</sup>. Most of free radicals are chemically active. Oxidative free radicals can cause DNA damage such as the breakages of single and double bonds, cross-link of base, and the generations of 5-methylol-uracil and 8-hydroxydeoxyguanine<sup>[6]</sup>. The reaction between DNA and  $\text{HO}\cdot$  causes the generations of 8-hydroxyl-adenine, 5-hydroxyl-cytosine, and breakages of single and double bonds. The effect of  $\text{HO}\cdot$  on deoxyribose was realized by H-abstraction reaction. Superoxide anion radical can degrade DNA.  $^1\text{O}_2$  can cause base oxidative damage and cut off the chains of DNA.

Free radicals such as  $\text{HO}\cdot$  are oxidative. Vitamin C has reducibility and it can react to oxidative free radicals.

Vitamin C is a very important antioxidant in vivo. Vitamin C can clean off  $\text{O}\cdot$ ,  $\text{HO}\cdot$ , superoxide, ozogen, and other water-solubility peroxide radicals. The reactions of vitamin C to  $\text{HO}\cdot$  or  $\text{O}\cdot$  are



Vitamin C tends to lose electrons. When it loses an electron, it became semi-dehydroascorbic acid radical. When it continues to lose another electron, it became dehydroascorbic acid radical<sup>[6]</sup>. These semi-dehydroascorbic acid and dehydroascorbic acid radical were prone to react to oxidative free radicals, so they prevented oxidative free radicals from harming DNA. The effects of vitamin C on UV radiation-induced DNA's damage depended on its concentration. Perhaps it is because that the quantity of free radicals is finite with certain time of UV radiation, so lower concentration of vitamin C can protect DNA. When the concentration of vitamin C was higher, free radicals generated by UV radiation had been cleaned off, but vitamin C was residual. Residual vitamin C possibly reacted to DNA with UV radiation, so DNA was damaged. The more residual vitamin C and the longer time of UV radiation, the more DNA was damaged.

On the other hand, we find vitamin C has a strong absorbed peak at 260 nm in absorbance spectrum. DNA can also absorb 260-nm UV light because both purine and pyrimidine have conjugated double bond. DNA absorbed UV light without vitamin C. When UV radiation time was long enough, DNA was damaged. When vitamin C existed in DNA aqueous solution, vitamin C had stronger ability of absorbing UV light, so DNA was protected. But it cannot explain why higher concentration vitamin C accelerated DNA damage instead.

W. Ke is the author to whom the correspondence should be addressed, his e-mail address is keweij@jlonline.com.

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