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Raman Spectroscopic Analysis of the Effect of Ultraviolet Irradiation on Calf Thymus DNA

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Abstract Raman spectroscopy was used for the first time to detect the effect of independent UVA (ultraviolet-A: 320–400 nm) and UVB (ultraviolet-B: 280–320 nm) irradiation on the calf thymus DNA in aqueous solution. After both UVA and UVB irradiation for 1 h or 3 h, the damage to the conformation of DNA was moderate, but the reduction of the B-form DNA component was obvious. Both UVA and UVB caused significant damage to the deoxyribose moiety and bases, among which the pyrimidine base pairs were more seriously affected. There appeared to be preferential damaging sites on DNA molecules caused by UVA and UVB irradiation. UVA irradiation caused more damage to the deoxyribose than UVB irradiation, while UVB irradiation caused more significant damage to the pyrimidine moiety than UVA irradiation. After UVB irradiation for 3 h, unstacking of the AT base pairs and the cytosine ring took place, severe damage to the thymine moiety occurred, and some base pairs were modified. Moreover, with either UVA or UVB irradiation for 3 h, the photoreactivation of DNA occurred. The damage to the DNA caused by UVB was immediate, while the damage caused by UVA was proportional to the irradiation duration. The experimental results partly indicate the formation of some cyclobutane pyrimidine dimers and (6-4) photoproducts.

Key words Raman spectroscopy; deoxyribonucleic acid (DNA); ultraviolet irradiation (UV); base pair

It is well known that solar UV light is a carcinogen. Dramatic increases in the incidence of skin cancer have been recorded in recent years, and this is believed to be related largely to increased selective exposure to sunlight. Many studies have shown that UVA (ultraviolet-A: 320–400 nm) and UVB (ultraviolet-B: 280–320 nm) components are responsible for the induction of human skin cancers [1–3], but the molecular mechanisms of the biological action of UV irradiation on DNA have not been completely made clear yet.

With the development of instruments and detection methods, Raman spectroscopy has become a very useful method for studying the structures and properties of DNA because of the large amount of information it provides on DNA structure, the high speed of detection and the convenience of operation. With this technique, unique information on hydrogen bonding, local conformation, and base stacking of nucleic acid residues in DNA [4] can be obtained. Ke *et al*. [5,6] have studied the effect of UV irradiation on herring sperm DNA with Raman spectroscopy. However, no experiment has been carried out with independent UVA and UVB, so not much is known about the damage caused to DNA after having been subjected to a single interval of UV irradiation.

The present work aims to investigate the structural changes to the calf thymus DNA in solution after separate irradiation by UVA and UVB, and to compare the different effects caused by the two UV irradiation intervals.

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Materials and Methods

Materials

Calf thymus DNA purchased from Sigma (Beijing

Dingguo Biotech. Co., Ltd.). was used without further purification. Solutions of DNA were prepared by mixing weighted amounts of DNA and 0.04 M Na_2SO_4 in three-distilled water at pH 7.0, then placed in a glass capillary tube. The final concentration of DNA was 3% by weight. Freshly prepared aqueous solutions of DNA were centrifugated at 10,000 rpm for 1 s and the samples were put in a refrigerator for 24 h at a temperature of 4 °C. After taking Raman measurements, the samples were irradiated with the corresponding UV irradiation and the Raman spectra were then detected again.

UV irradiation and spectral detecting

The DNA solution was irradiated with UV irradiation from a 75 W short-arc xenon lamp equipped with three bandpass filters, with which UVA, UVB and UVC can be obtained. The parameters of the three filters are listed in **Table 1**. The intensity of the ultraviolet irradiation, which is adjustable in a certain range, was kept at 0.5 W/m^2 and measured with a digital photometer (SG-501) made in Zhejiang University (Hangzhou, China). The prepared sample solutions were divided into two groups. One group was exposed to UVA irradiation and the other was irradiated by UVB irradiation, for 1 h and 3 h, respectively.

The Raman spectra were excited with 514.5 nm irradiation from an INNOVA 750 argon ion laser (Coherent Co., Santa Paula, USA) and recorded by a T64000 Raman spectrometer (Jobin-Yvon Co., Longjumeau, France) equipped with a liquid nitrogen-cooled charge-coupled device detector (EEV CCD-37, Princeton Instruments, Trenton, NJ, USA). The sample solution was put into a glass capillary and irradiated with the power at about 200 mW. In order to reduce the background fluorescence, all samples were irradiated with the laser irradiation for 30 min before the signal was collected. The reported DNA Raman frequencies were calibrated using the 459.5 cm^{-1} Raman spectrum of CCl_4. The peak wavenumber values of Raman bands were reproducible to within ±1 cm^{-1} except for broad bands. The spectra were the accumulated averages of 10–20 exposures of 30 s each.

Results

The Raman spectra of the calf thymus DNA in the aqueous solution and after UV irradiation are shown in **Fig. 1** and **Fig. 2**, and the Raman shift and tentative assignment [6–10] are presented in **Table 2**. The Raman band of SO_4^{2-} at 982 cm^{-1} was used as an internal intensity standard, and all the spectra were normalized with respect to it [11]. On these bases, the ratio of the spectral intensity change of each DNA group after UV irradiation was calculated from the following equation:

$$\Delta I_n = \frac{I_n - I_0}{I_0} \times 100\%$$

where I_0 denotes the intensity of a band before UV irradiation and I_n denotes the intensity of a band after UV irradiation. A negative ratio means there is damage to the corresponding groups or chemical bonds, and a spectral line displacement means there is a change in them [6].

Effect of UVA irradiation

UVA wavelengths are less efficient in inducing DNA damage because native DNA cannot directly absorb UVA irradiation, but UVA irradiation can still produce secon dary photoreactions of existing DNA photoproducts or damage DNA via indirect photosensitizing reactions [1].

Backbone and conformation The Raman peaks centered at 830 cm^{-1}, 1093 cm^{-1} and 1427 cm^{-1} are the main characteristic bands of the B-DNA nucleic acid backbone in the aqueous solution [5,8]. After UVA irradiation for 1 h or 3 h, no obvious changes to these bands were found. At the same time, the guanine nucleoside markers at 626 cm^{-1} and 676 cm^{-1}, the diagnostic of the sugar pucker and glycosyl torsion of dG (deoxyribose-guanine) residues [12], shifted by only one or two wavenumbers without any change in intensity. These results show that the B form of the DNA was retained.

However, the strong band at 1093 cm^{-1}, which is due to the symmetric stretching vibration of the phosphodioxy (PO_2^-) moiety, shifted to 1097 cm^{-1} and 1098 cm^{-1} after

Table 1 Technology parameters of the three bandpass filters

Peak wavelength (λ_0)	Bandwidth ($1/2\Delta\lambda_0$)	Peak transmissivity (T_{max})	Background
(254 ± 2) nm	<15 nm	>20%	=10^{-4}
(297 ± 3) nm	=30 nm	>25%	=10^{-4}
(365 ± 2) nm	=13 nm	>25%	=10^{-4}

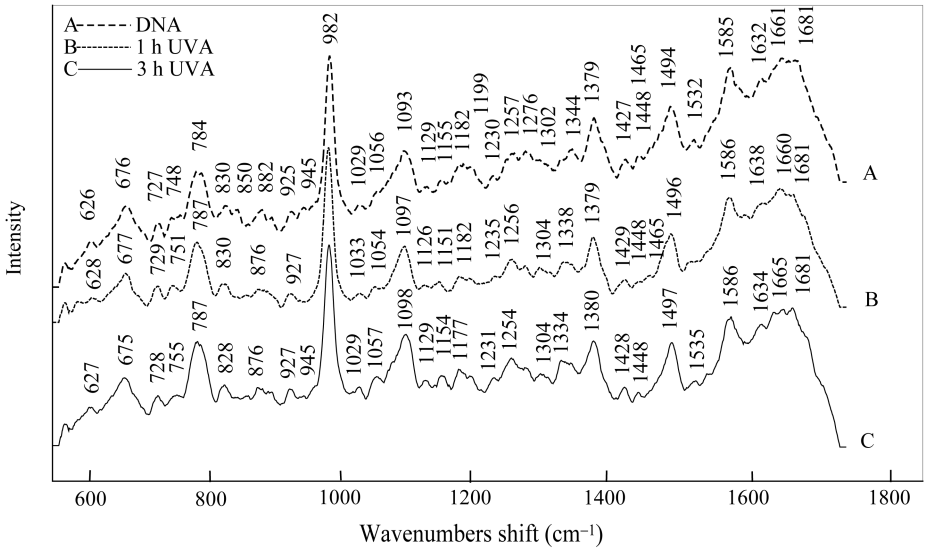

Fig. 1 Raman spectra of DNA in aqueous solution and after UVA irradiation for different intervals

DNA, in aqueous solution; 1 h UVA, 1 h of UVA irradiation; 3 h UVA, 3 h of UVA irradiation.

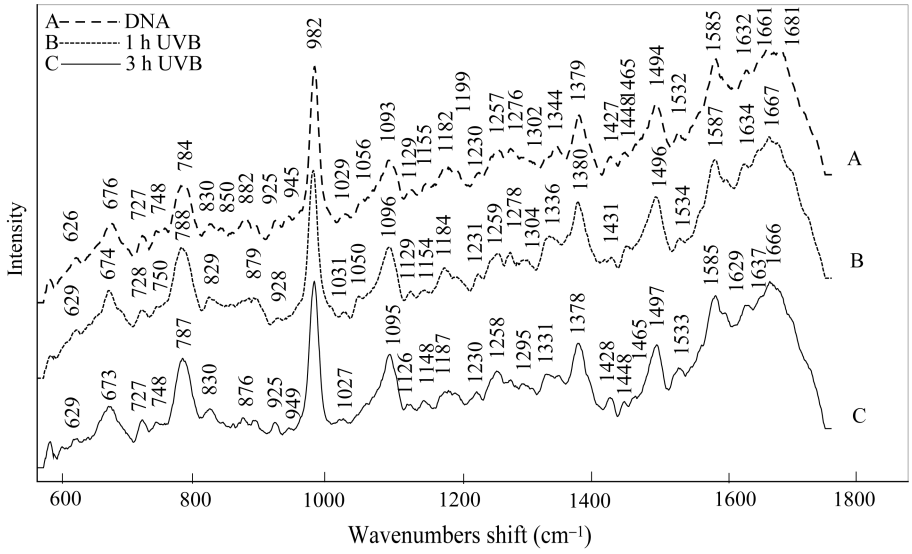

Fig. 2 Raman spectra of DNA in aqueous solution and after UVB irradiation for different intervals

DNA, in aqueous solution; 1 h UVB, after 1 h of UVB irradiation; 3 h UVB, after 3 h of UVB irradiation.

1 h and 3 h of UVA irradiation, respectively. At the same time, the band at 784 cm^{-1}, corresponding to the phosphodiester symmetric stretching mode of the B-form DNA backbone and cytosine ring, shifted to 787 cm^{-1} with an increase in intensity of more than 20% after either 1 h or 3 h of UVA irradiation. The changes to these two bands show that the main chain spatial structure of the DNA was damaged somehow. Further information can be obtained from the band of the ring-breathing vibration of thymine at 748 cm^{-1}, a marker for the C2*'-endo-anti* conformation of the nucleotides, which shows the typical structure of B-DNA [5]. After 1 h and 3 h of UVA irradiation, this band moved to 751 cm^{-1} and 755 cm^{-1}, respectively. These observations suggest that UVA irradiation can affect the DNA structure to some degree, which may be due to the single-strand breaks caused by UVA [13].

Table 2 Raman frequencies and assignments of calf thymus DNA before and after UVA and UVB irradiation [a]

In aqueous solution	UVA irradiation		UVB radiation		Tentative assignment [b]
	1 h	3 h	1 h	3 h	
626m	628w	627m	629w	629w	G, A
676vs	677	675	674	673	G
727s	729	728	728	727	A
748w	751m	755w	750sh	748w	T
784vs	787	787	788	787	T, C, O-P-O, bk
830s	830	828	829	830	γ(O-P-O), bk, B-type
850s	–	–	–	–	ψ-DNA, B-type
882m	876w	876w	879 w	876w	d-p
925s	927	927	928w	926s	d
945m	–	945w	–	949 w	d
1029m	1033	1029	1031	1027	Weak band
1056w	1054	1057s	1050w	sh	γ(C-O), d
1093vs	1097	1098	1096	1095	γ(PO_2^-), bk
1129m	1126w	1129m	1129m	1126w	d-p
1155m	1151	1154s	1154w	1148s	d-p
1182w	1182	1177m	1184s	1187w	T, C
1199w	1198	1199	1200	1198	T
1230m	1235	1231	1231	1230	A
1257m	1256s	1254s	1259s	1258s	C, A
1276m	1276	1276	1278	1279	Weak band
1302w	1304m	1304m	1304m	1295m	A
1344s	1338	1334	1336m	1331broad	A
1379vs	1379	1380	1380	1378	T, A, G
1427s	1429	1428	1431w	1428s	A, G
1448(overlap)	1448	1448m	overlap	1448	d(CH_2δ)
1465(overlap)	1465	–	overlap	1465	d(5′-CH_2δ)
1494vs	1496	1497	1496	1497	G, A
1532s	sh	1535m	1534m	1533s	G, C
1585vs	1586	1586	1587	1585	G, A
1632w	1638sh	1634w	1634	1629	C
				1637	
1661w	1660m	1665w	1667m	1666s	T, C, γ(C=O)
1681w	1681	1681m	sh	–	G

[a] sh=shoulder; s=strong; m=medium; vs=very strong; w=weak. [b] abbreviations: γ and δ indicate stretching and deformation vibrations, respectively, of the atoms listed; bk indicates a vibration of the DNA backbone; d indicates a vibration localized in the deoxyribose moiety; d-p indicates a vibration of the deoxyribose-phosphate moiety; A, T, G, and C indicate vibrations of adenine, thymine, guanine and cytosine, including modes either localized in the purine and pyrimidine or delocalized (base plus furanose moieties).

Moreover, the moderately strong band at 850 cm^{-1}, corresponding to a compact form, ψ-DNA, which belongs to the family of B-DNA [14], completely disappeared after just 1 h of UVA irradiation. This clearly shows that some B-form DNA was reduced after UVA irradiation.

Deoxyribose **Fig. 1** clearly showed that the UVA irradia-

tion damaged the deoxyribose groups (spectra B and C). The bands at 925 cm^{-1}, 945 cm^{-1} and overlap bands at 1448 (1465) cm^{-1}, assigned to the deoxyribose [7], all changed significantly after the UVA irradiation. The band at 925 cm^{-1} shifted to 927 cm^{-1} after either 1 h or 3 h of UVA irradiation. The 945 cm^{-1} band disappeared completely after 1 h of UVA irradiation, while the overlap bands at 1448 (1465) cm^{-1}, which were difficult to distinguish, separated into two independent bands after 1 h of UVA irradiation. These results clearly show that irradiation damage to the deoxyribose moiety of DNA was obviously caused by the UVA irradiation. Moreover, the 882 cm^{-1} band, corresponding to the deoxyribose-phosphate, shifted to 876 cm^{-1} and became a broad band, and its intensity dropped by about 70% after 1 h of UVA irradiation. Another band at 1155 cm^{-1}, also due to the deoxyribose-phosphate, shifted to 1151 cm^{-1} and 1154 cm^{-1}, and its intensity decreased initially by 25%, which rose to 75%. The analysis of these two bands also indicates that the deoxyribose unit was significantly damaged by UVA irradiation, and that some phosphodiester bonds might be cut off, which is consistent with the damage done to the backbone of the DNA.

An interesting phenomenon is that some bands, such as those corresponding to deoxyribose or deoxyribose-phosphate at 882 cm^{-1}, 945 cm^{-1}, 1155 cm^{-1} and 1448 cm^{-1}, showed much less change after 3 h of UVA irradiation compared with after 1 h of UVA irradiation. This can be interpreted as being the result of photoreactivation [15], which means that with the increase in the irradiation duration, the damage to the DNA caused by UV irradiation will be reduced by lower-frequency irradiation.

Bases The effect of UVA irradiation on the four bases of the calf thymus DNA was different. The bands at 1379 cm^{-1}, 1427 cm^{-1}, 1494 cm^{-1} and 1585 cm^{-1}, assigned to in-plane ring vibrations of guanine and adenine, did not shift much, except that the 1494 cm^{-1} band appeared at 1497 cm^{-1} after 3 h of UVA irradiation. However, these bands all decreased moderately in intensity. The bands at 1230 cm^{-1}, 1302 cm^{-1} and 1344 cm^{-1}, corresponding to adenine, all changed to some extent after UVA irradiation. The 1230 cm^{-1} band shifted to 1235 cm^{-1} and 1231 cm^{-1} after 1 h of UVA irradiation and became a shoulder band; the weak band at 1302 cm^{-1} shifted to 1304 cm^{-1} after UVA irradiation and increased greatly in intensity, indicating the unstacking of the adenine base pairs [16]; the 1344 cm^{-1} band shifted to 1338 cm^{-1} and 1334 cm^{-1} after 1 h and 3 h of irradiation respectively, and became a broad band, with its intensity decreasing significantly. These results suggest that the UVA irradiation damaged the adenine moiety significantly, the stacking of the AT base pairs decreased to some extent, and some base pairs were modified, but the damage to the guanine moiety was not clear.

The band at 1532 cm^{-1}, corresponding to guanine and cytosine, almost disappeared after 1 h of UVA irradiation. Another band at 1632 cm^{-1}, belonging to cytosine, moved to 1638 cm^{-1} and 1634 cm^{-1}, and became a shoulder band after 1 h of UVA irradiation. Similarly, the band at 1257 cm^{-1}, corresponding to cytosine and adenine, also showed an increase of 60% in intensity, and shifted to 1254 cm^{-1} after 3 h of UVA irradiation. These results clearly show that the UVA irradiation damaged the cytosine moiety severely and that unstacking of the cytosine ring occurred. The damage to the thymine moiety caused by UVA irradiation was also significant. For example, the intensity of the thymine band at 748 cm^{-1} increased by more than 60% after 1 h of UVA irradiation, and it shifted to 755 cm^{-1} after 3 h of UVA irradiation, almost becoming a shoulder band. At the same time, the 1182 cm^{-1} band, corresponding to thymine and cytosine, shifted to 1177 cm^{-1} after 3 h of UVA irradiation.

In general, UVA irradiation only damaged the conformation of B DNA slightly, but it reduced some B-form DNA content and some phosphodiester bonds were cut off. Comparatively, the damage to the deoxyribose and base pairs was more serious. Some base pairs were modified, and some groups were disrupted.

Effect of UVB irradiation

The adverse effects of solar irradiation on living systems are mostly attributed to the small amount of UVB that is absorbed by cellular DNA [1]. The Raman spectra of calf thymus DNA in the aqueous solution and after UVB irradiation for 1 h and 3 h are shown in **Fig. 2**.

Backbone and conformation The band centered around 830 cm^{-1} is ascribed to an antisymmetric O-P-O stretching mode of the B-form DNA backbone which shows that the furanose rings are in C2′-*endo* conformation [16]. This peak did not present a large change after 1 h or 3 h of UVB irradiation, suggesting that the B conformation of DNA was still retained. However, it was obvious that some other characteristic bands of B DNA had changed. The cytosine band at 784 cm^{-1}, which gives rise to the symmetric phosphodiester stretching mode of the B-form DNA backbone, shifted to 788 cm^{-1} and 787 cm^{-1} after 1 h and 3 h of UVB irradiation respectively, accompanied by an increase in intensity of more than 20%. The strong peak centered at 1093 cm^{-1}, corresponding to the symmetric stretching vibration of the phosphodioxy moiety, shifted to 1096 cm^{-1} after 1 h of UVB irradiation and to 1095 cm^{-1}

after 3 h of UVB irradiation, with an increase in intensity of about 40%. These results clearly indicate that the main chain structure of the B-form DNA was moderately damaged. Further information can be obtained from the peak centered at 1427 cm^{-1} that is attributed to the C2′-*endo-anti* conformation of the nucleotides, representing the typical structure of B-DNA. This peak appeared at 1431 cm^{-1} and decreased by 60% in intensity after 1 h of UVB irradiation. Moreover, the moderately strong band at 850 cm^{-1}, corresponding to a compact form, ψ-DNA (B-DNA) [14], disappeared completely after either 1 h or 3 h of UVB irradiation. From these results, it can be concluded that the UVB irradiation really damaged the conformation of B-DNA to some degree, and some B-form DNA was reduced.

Furthermore, the Raman bands of the ring-breathing vibrations of guanine (676 cm^{-1}) and thymine (748 cm^{-1}), corresponding to the C2′-*endo-anti* conformation of the nucleotides, which is typical for B-DNA [16], both showed some changes after UVB irradiation. The guanine line shifted to 674 cm^{-1} and 673 cm^{-1} after 1 h and 3 h of UVB irradiation respectively, which is associated with the base rotation around the glycoside bond [8]. Such a shift can be interpreted as a slight decrease of the angle of guanine rotation around the glycoside bond in the direction of the *syn* conformation, suggesting that UVB irradiation might have some influence on DNA nucleotide conformation. The thymine band almost disappeared after 1 h of UVB irradiation. These results confirm again that the DNA backbone conformation was damaged moderately by UVB irradiation, which is in agreement with the carcinogenesis of solar UV irradiation.

Deoxyribose The damage to the deoxyribose moiety was comparatively more serious. As can be seen in **Fig. 2**, the bands at 925 cm^{-1}, 945 cm^{-1} and 1448 (1465) cm^{-1}, assigned to the deoxyribose, all showed significant changes after UVB irradiation. The 925 cm^{-1} band shifted to 928 cm^{-1} and its intensity decreased by 50% after 1 h of UVB irradiation. The 945 cm^{-1} band disappeared completely after 1 h of UVB irradiation. The overlap bands at 1448 (1465) cm^{-1} could not be distinguished even after 1 h of UVB irradiation, but after 3 h of irradiation, the two bands separated into two independent bands completely. These results suggest that the damage to the deoxyribose caused by UVB irradiation was significant. This can be confirmed from two other bands at 882 cm^{-1} and 1155 cm^{-1}. The band at 882 cm^{-1} (deoxyribose-phosphate) moved to 879 cm^{-1} and 876 cm^{-1}, and almost disappeared after 1 h of UVB irradiation. The similar band at 1155 cm^{-1} shifted to 1154 cm^{-1} and 1148 cm^{-1} after 1 h and 3 h of UVB irradiation, respectively. Furthermore, the 1056 cm^{-1} band, which is due to the stretching vibration of the C-O bond of deoxyribose, moved to 1050 cm^{-1} after 1 h of UVB irradiation, and disappeared completely after 3 h of irradiation, suggesting that the deoxyribose moiety was significantly damaged.

At the same time, after 3 h of UVB irradiation, the photoreactivation of DNA also occurred. For example, the 925 cm^{-1} and 945 cm^{-1} bands did not show much change after 3 h of UVB irradiation, while after 1 h of UVB irradiation, their changes were very drastic.

Bases As far as the four bases are concerned, the damage caused by UVB irradiation was more serious than that caused by UVA irradiation.

The bands centered at 1427 cm^{-1} and 1494 cm^{-1} are due to in-plane ring vibrations of guanine and adenine. The former shifted to 1431 cm^{-1} and 1428 cm^{-1} after 1 h and 3 h of UVB irradiation respectively, and with 1 h of UVB irradiation, this band almost disappeared. The latter moved to 1496 cm^{-1} and 1497 cm^{-1}, with an increase in intensity of about 20%. Hence, it can be inferred that the UVB irradiation caused a certain amount of damage to the DNA purine base pairs. Further information can be obtained from the bands at 1230 cm^{-1} and 1344 cm^{-1} (adenine). The 1230 cm^{-1} band increased by 50% in intensity with 3 h of UVB irradiation, indicating the unstacking of the adenine base pairs [16]. The 1344 cm^{-1} band shifted to 1336 cm^{-1} and 1331 cm^{-1} after 1 h and 3 h of irradiation respectively, and its intensity decreased by 43% with 3 h of UVB irradiation. These results confirm that the purine base pairs had been damaged. Furthermore, the band at 1532 cm^{-1} (guanine and cytosine) separated into a shoulder band (1520 cm^{-1}, A) after 3 h of UVB irradiation, suggesting that a change had taken place in the corresponding base pairs' structure.

The UVB irradiation damaged the pyrimidine moieties even more seriously. In **Fig. 2**, the band of cytosine at 1632 cm^{-1} shifted to 1634 cm^{-1} after 1 h of UVB irradiation, and separated into two peaks at 1629 cm^{-1} and 1637 cm^{-1} after 3 h of irradiation, indicating that unstacking of, and damage to, the cytosine ring had occurred. The unstacking also happened due to the change in the band at 1257 cm^{-1} (cytosine and adenine), which increased by 60% in intensity after either 1 h or 3 h of UVB irradiation. At the same time, the thymine ring was also seriously damaged: The band centered at 748 cm^{-1} (thymine) almost disappeared after just 1 h of UVB irradiation, while the band at 1182 cm^{-1} (thymine and cytosine) shifted to 1184 cm^{-1} and 1187 cm^{-1} after 1 h and 3 h of UVB irradiation respectively, with an increase in intensity of 50% after 1 h of UVB irradiation. These observations suggest that the UVB irra-

diation caused severe damage to the pyrimidine base pairs, and some cyclobutane pyrimidine dimers and (6–4) photoproducts may have formed.

Comparing **Fig. 1** and **Fig. 2**, it can be concluded that UVB irradiation damaged DNA immediately while the damage caused by UVA irradiation was accumulated over time. This is clearly demonstrated by the bands centered at 925 cm^{-1} (deoxyribose), 1056 cm^{-1} (deoxyribose), 1155 cm^{-1} (deoxyribose-phosphate), 1182 cm^{-1} (T, C) and 1302 cm^{-1} (A), which all changed in a completely different way after being irradiated by UVA, compared with the changes caused by UVB irradiation. After UVA irradiation, the longer the irradiation duration, the more changes there were to these bands. On the contrary, with UVB irradiation, the variation of these characteristic lines after 3 h of irradiation was much less than that after just 1 h irradiation, and this might be related to the direct absorption of UVB irradiation by DNA. This result is consistent with the reported literature [1,13].

Another difference in the effect of UVA and UVB irradiation on DNA is that UVA irradiation damaged the deoxyribose moiety more seriously than UVB irradiation, while UVB irradiation damaged the base pairs more seriously than UVA irradiation. For instance, the bands at 945 cm^{-1}, 1155 cm^{-1} and the overlap bands at 1448 (1465) cm^{-1}, corresponding to the deoxyribose and deoxyribose-phosphate, all showed a larger change with UVA irradiation than with UVB irradiation for 3 h. On the other hand, the bands centered at 748 cm^{-1} (T), 1230 cm^{-1} (A), 1302 cm^{-1} (A) and 1532 cm^{-1} (G, C) all showed a more significant alteration with UVB irradiation than with UVA irradiation for 3 h. Thus, UVA and UVB irradiation damage the DNA macromolecule in a selective way.

Discussion

Raman spectroscopy has been used to obtain qualitative information about the changes in molecular composition and structure of calf thymus DNA in aqueous solution after UV irradiation. In this experiment, the effect of UVA and UVB irradiation on the conformation of the calf thymus DNA in aqueous solution is moderate, although some phosphodiester bonds were cut off by UVA irradiation. However, both UVA and UVB irradiation reduce the B-form DNA content effectively. As far as the deoxyribose groups are concerned, the damage caused by UVA and UVB irradiation is significant, and UVA irradiation results in more prominent damage. UVA and UVB irradiation causes damage to all base pairs, but pyrimidine base pairs are by far the most easily affected, and UVB irradiation causes more significant damage than UVA radiation. After UVB irradiation for a certain period of time, unstacking of the AT base pairs and the cytosine ring takes place, severe damage to the thymine moiety occurs, and some base pairs are modified. Thus, some cyclobutane pyrimidine dimers (CPDs) and (6-4) photoproducts must have formed.

Attention should be paid to the photoreactivation of DNA, which occurs after UVA or UVB irradiation for 3 h. Moreover, UVB irradiation damages DNA immediately, while the damage caused by UVA irradiation accumulates over time. In addition, different intervals of UV irradiation seem to damage the DNA in preferential regions.

In conclusion, both UVA and UVB irradiation can damage calf thymus DNA in aqueous solution after a certain period of irradiation, but the type, speed and degree of the damage are different.

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Correction

2004, 36(12): 832–839

Identification and Analysis of Genes Present in *Leptospira interrogens* serovar lai but Absent in *L. biflexa* serovar monvalerio

Ping HE, Xiang-Yan ZHANG, Xiao-Kui GUO*, Bao-Yu HU, Xiao-Tian HUANG, Yang YANG, and Guo-Ping ZHAO[1,2]*

Leptospira interrogens in the whole text should be spelled as *Leptospira interrogans.*