Raman spectroscopic study of DNA photodamage sensitized by hypocrellin B and 5-brominated-hypocrellin B

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Abstract Hypocrellin B (HB) is a highly effective photosensitizer. One of its derivatives, 5-brominated-hypocrellin B (5-Br-HB) has better photosensitive characteristics than it. Using Raman spectroscopy, the microcosmic photodamage to DNA sensitized by HB and 5-Br-HB was studied at molecular level. The results showed that when HB and 5-Br-HB were added to the solution of DNA and irradiated, the characteristic Raman frequencies and intensities of DNA changed to various degrees. The photodamage occurred on the whole DNA molecule, including phosphate backbone, deoxyribose and four bases. Not only the conformation but also the configuration of DNA was photodamaged: breakage of some H-bonds, disappearance of B-form conformation , scission of double or single strand and serious damage of four bases. The damage to A-T pairs was stronger than that to C-G pairs and DNA became polynucleotides finally. The 5-Br-HB-photosensitized damage to DNA was stronger than HB , which suggested the better photosensitive characteristics of 5-Br-HB in bioactive environment. The molecular mechanisms of photodamage to DNA sensitized by 5-Br-HB and HB were also elucidated.

Keywords: hypocrellin B, 5-brominated hypocrellin B, calf thymus DNA, photodamage, Raman spectroscopy.

HYPOCRELLINS, including hypocrellin A and hypocrellin B, are highly effective photosensitizers and

have been used as potent phototherapeutic agents. In recent years, some of their derivatives with better photosensitive characters have been synthesized. 5-brominated hypocrellin B (5-Br-HB) is one of them. Compared with HB, the maximum absorbed wavelength of 5-Br-HB moves to red light region and the extinction coefficient is higher[1]. It has been reported that there are more $^1O_2$ and $\cdot OH$ yield in the heterogeneous solution of 5-Br-HB than that of HB[2] and the anticancer property of 5-Br-HB is better than that of HB because the nuclear envelope and nuclear morphology of Hela cells were damaged more strongly when 5-Br-HB was added and irradiated[3]. There is some progress in the studies of photodamage to DNA sensitized by photosensitizers at molecular level in recent years using Raman spectroscopy[4]. However little is known about the photodamage to DNA and molecular mechanisms of photodamage sensitized by HB and 5-Br-HB. Such studies will contribute to elucidate their anticancer molecular mechanisms and select highly effective photosensitive drugs.

In this work, Raman spectroscopy was applied for studying the photosensitive damages to calf thymus DNA sensitized by HB and 5-Br-HB. The structural information with respect to the phosphate backbone, deoxyribose and four bases of DNA could be provided before and after the photosensitive damage. The site, mode and potential degree of damages could also be understood. The characteristics and mechanisms of the photodamage can be investigated and the anticancer mechanisms of the drugs might be understood better.

1 Materials and methods

(ⅰ) Materials. Calf thymus DNA (highly pure fiber) purchased from Sigma Chemical Co. was dissolved in the buffer prepared from 0.01 mol/L sodium cacodylate and 0.001 mol/L EDTA (pH 7.0). The concentration of DNA was 4% (W/V). HB was prepared according to ref. [5] and 5-Br-HB was prepared according to ref. [1]. 5-Br-HB was synthesized first by Master Liu Wei of Institute of Photographic Chemistry, the Chinese Academy of Sciences. The two photosensitizers were solved in Dimethyl Sulfoxide which was purchased from Merck Company, Germany and their concentrations were 2 mmol/L. The solutions were diluted to 0.2 μmol/L with the above buffer and their last concentrations in DNA were 0.1 μmol/L. Other reagents were all spectroscopy and analysis pure.

(ⅱ) Methods. The samples were put into a quartz tube with an inside diameter of 3 mm and detected by Raman spectrophotometer. All Raman beam was obtained on a France JY-T 800 Raman spectrophotometer fitted with computer and Spectra-Physics Co. Argon-ion laser Model 164. The experimental conditions were as follows: exciting line 514.5 nm and power 200 mW; the width of four slits of the triple monochromator, 600, 700, 700 and 600 μm; scanning range 500—1 750 cm^{-1}; scanning speed 1.2 cm^{-1}/s; photo count; time constant 1 s; signal averaging 4 scans and room temperature (18±2)℃. The experiment was repeated three times.

2 Results

Raman spectra of the nature calf thymus DNA and DNA damaged by HB and 5-Br-HB are

shown in figs. 1—3. There are not any bands of HB, 5-Br-HB and Dimethyl Sulfoxide in figs. 2 and 3. The assignments of calf thymus DNA referred to ref. [4] . A, G, C, and T in table 1 are listed in order according to their contributions to the spectra. They indicate the vibration characteristics of adenine, guanine, cytosine and thymine bases, respectively.

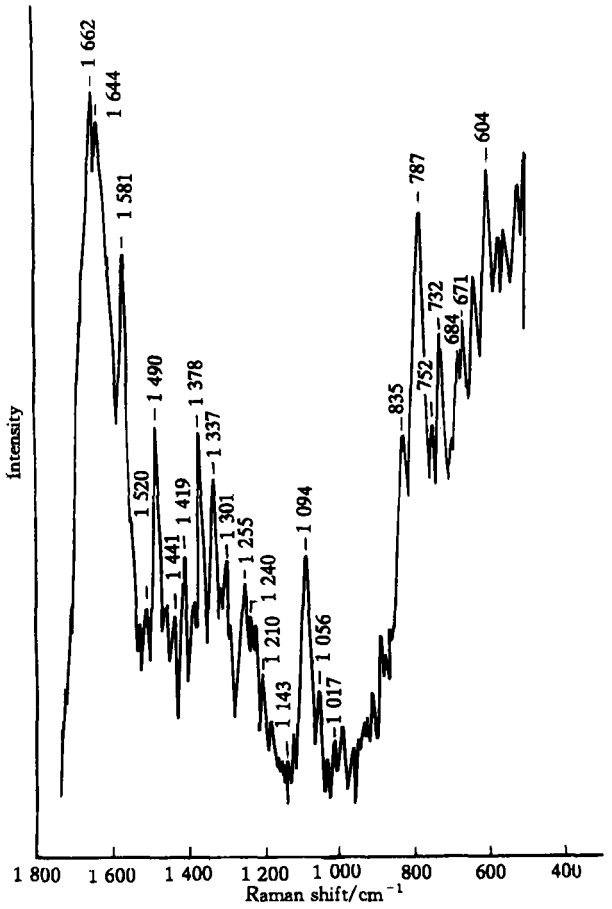

Fig. 1. Raman spectrum of calf thymus DNA (500—1750 cm^{-1}).

2.1 Photodamages to the conformation and configuration of DNA sensitized by HB and 5-Br-HB

The terminus led by photosensitization , such as cell death, mutation of chromosomes, relates to the breakage of DNA strands. Damages to deoxyribose , backbone phosphate groups are the sources of breakage of DNA strands. Comparing fig. 1 with figs. 2 and 3, it can be seen that the positions and intensities of some important bands changed clearly when HB and 5-Br-HB were added and irradiated.

The band at 835 cm^{-1} of B-form of calf thymus DNA disappeared when HB was added and irradiated, suggesting that B-form of DNA was damaged . The band at 787 cm^{-1} assigned to the symmetric stretching vibration of PO_2 became a wide peak whose center was at 803 cm^{-1}. It possibly included many symmetric stretching vibration of PO_2 of polynucleosides. This band was very sensitive to conformation change of DNA[6,7]. However the strong peak at 1 094 cm^{-1} assigned to the symmetric stretching vibration of PO_2^- was not sensitive to the conformation change of DNA[6]. It changed only when the configuration of DNA was damaged. After the photodamage of HB, it became three weak peaks belonging to the symmetric stretching vibration of PO_2^- of polynucleosides at 1 099, 1 088 and 1 076 cm^{-1}[7]. It has been reported that if ordered double-stranded DNA became unordered single-stranded DNA, the band at 787 cm^{-1} would shift to 795 cm^{-1}, B-form conformation disappear but the band at 1 094 cm^{-1} would hardly change[7]. The above results showed that after the photodamage of HB, B-form conformation of DNA disappeared, double and single strands of DNA broke and gave rise to many kinds of polynucleosides. Fig. 2 was very similar to the Raman spectra of polynuleosides, especially the line of PO_2^-, whose intensity was much lower than that of DNA[6—8]. When 5-Br-HB was added and irradiated, B-form of DNA and configuration were

damaged because the line at 835 cm^{-1} disappeared , the line belonging to the symmetric stretching vibration of PO_2^- at 1 094 cm^{-1} shifted to 1 105 cm^{-1}, its intensity decreased suddenly and the line assigned to the symmetric stretching vibration of PO_2 at 787 cm^{-1} shifted to 793 cm^{-1}. It suggested that the single and double strands of calf thymus DNA were scissored and became polynucleosides finally. Fig. 3 was also similar to the Raman spectra of polynucleosides[6—8]. But the photodamage to DNA sensitized by 5-Br-HB was different from that by HB. It was expressed as follows. (HB) and (5-Br-HB) represented the lines in fig. 2 and fig. 3 respectively.

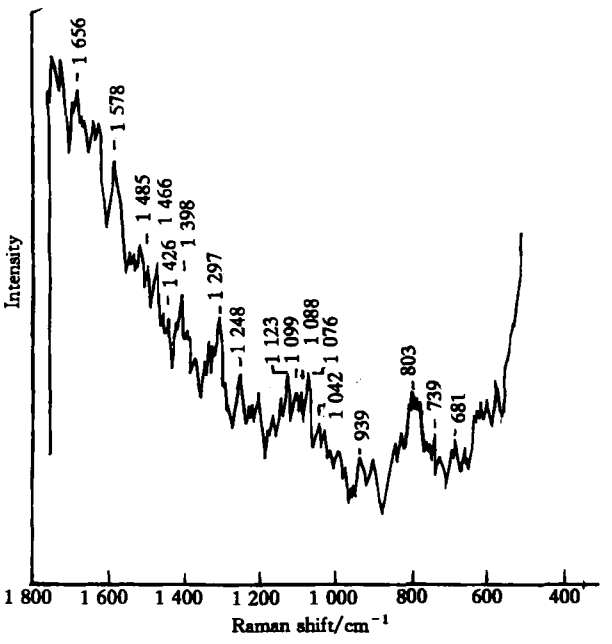

Fig. 2. Raman spectrum of calf thymus DNA after the photodamage sensitized by HB (500—1 750 cm^{-1}).

2.2 Photodamage to deoxyribose sensitized by HB and 5-Br-HB

The bands belonging to deoxyriboses shifted or disappeared after the photosensitization of HB and 5-Br-HB. But the degree was different. The lines at 1 000 cm^{-1} and 1 462 cm^{-1} shifted to 997 cm^{-1}, 1 466 cm^{-1}(HB) and 992 cm^{-1}, 1 471 cm^{-1} (5-Br-HB). The line at 934 cm^{-1} shifted to 939 cm^{-1} in fig. 2 and disappeared in fig. 3. The line at 1 441 cm^{-1} did not shift but its intensity decreased clearly in fig. 2, disappeared in fig. 3. The bands assigned to deoxyribose-phosphate at 1 143 cm^{-1} shifted to 1 152 cm^{-1} (HB) and 1 153 cm^{-1} (5-Br-HB). The bands belonging to C-O stretching vibration of deoxyribose at 1 017 cm^{-1} and 1 056 cm^{-1} shifted to 1 025 cm^{-1}, 1 042 cm^{-1} (HB) and 1 016 cm^{-1}, 1 055 cm^{-1} (5-Br-HB). The above results showed that the photodamages to deoxyribose of DNA sensitized by HB and 5-Br-HB were strong and the displacement of lines caused by 5-Br-HB was larger than that

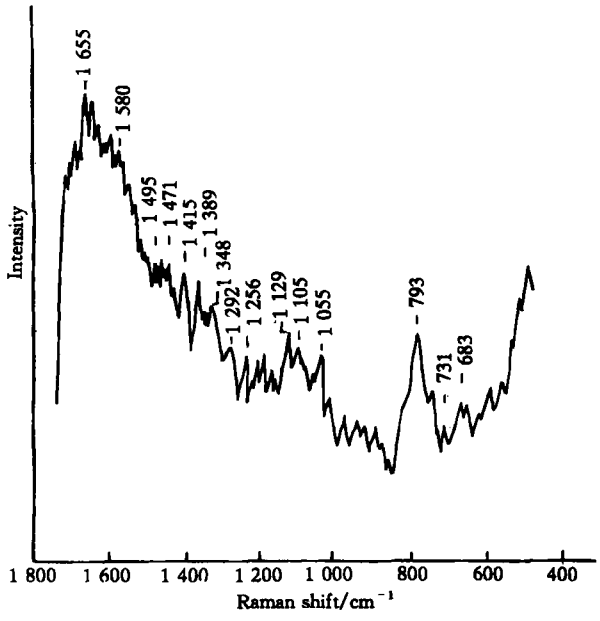

Fig. 3. Raman spectrum of calf thymus DNA after the photodamage sensitized by 5-Br-HB (500—1 750 cm^{-1}).

by HB (the lines at 1 017 and 1056 cm^{-1} excluded). There were also some lines in fig. 3 disappearing, which suggested that the photodamage to deoxyribose of DNA sensitized by 5-Br-HB was stronger than by HB.

Table 1 Raman band frequencies and assignments of calf thymus DNA before and after the photodamage sensitized by HB and 5-Br-HB (500—1 750 cm^{-1})

Frequency/cm^{-1}			Assignment[a)]
DNA	DNA*[b)]	DNA**[b)]	
671	657	658	T
684	681	683	G
732	739	731	A
752	753	755	T
787	with a center at 803	793	O—P—O symmetric stretch
835	–	–	B-form conformation
934	939	–	deoxyribose
1 000	997	992	deoxyribose
1 017	1 025	1 016	C—O stretch
1 056	1 042	1 055	C—O stretch
1 094	1 076 1 088 1 099	1 105	O···P···O symmetric stretch
1 125	1 123	1 129	
1 143	1 152	1 153	deoxyribose-phosphate
1 185	1 179	1 189	Base external C—N stretch
1 210	1 201	1 201	T
1 222	1 219	1 213	A
1 240	–	–	T
1 255	1 248	1 256	C, A
1 301	1 297	1 292	A
1 325	1 317	–	G
1 337	1 342	1 348	A
1 378	1 380	1 389	T, A, G
1 419	1 426	1 415	A, G
1 441	1 441	–	deoxyribose
1 462	1 466	1 471	deoxyribose
1 490	1 485	1 495	G, A
1 520	1 513	–	A
1 534	1 540	1 546	G, C
1 581	1 578	1 580	G, A
1 662	1 656	1 655	C=O stretch

a) A, G, C and T are listed in order according to their contributions to the spectra, which indicate the vibration characteristics of adenine, guanine, cytosine and thymine bases, respectively; b) DNA* and DNA** represent DNA after the photodamaged sensitized by HB and 5-Br-HB respectively.

2.3 Photodamage to the four bases of DNA sensitized by HB and 5-Br-HB

Many bands of four bases of DNA shifted clearly and their intensities decreased suddenly after the photodamage sensitized by HB and 5-Br-HB. The thymine line at 671 cm^{-1} shifted 14 cm^{-1} (HB) and 13 cm^{-1}(5-Br-HB), the line at 752 cm^{-1} and 1 210 cm^{-1} shifted to 753 cm^{-1}, 1 201 cm^{-1}(HB) and 755 cm^{-1}, 1 201 cm^{-1}(5-Br-HB). The line at 1 240 cm^{-1} disappeared (HB and 5-Br-HB). The bands assigned to adenine at 732, 1 222, 1 301 and 1 337 cm^{-1} shifted to 739, 1 219, 1 297, 1 342 cm^{-1}(HB) and 731, 1 213, 1 292, 1 348 cm^{-1}(5-Br-HB) respectively. The line at 1 520 cm^{-1} shifted to 1 513 cm^{-1}(HB), but it disappeared in fig. 3 (5-Br-HB). The guanine line shifted from 684 cm^{-1} to 681 cm^{-1}(HB) and 683 cm^{-1}(5-Br-HB), from 1 325 cm^{-1}

to 1 317 cm^{-1} in fig. 2 and disappeared in fig. 3. The line assigned to cytonine and adenine at 1 255 cm^{-1} shifted to 1 248 cm^{-1}(HB) and 1 256 cm^{-1}(5-Br-HB). The line belonging to T, A, G at 1 378 cm^{-1} shifted to 1 380 cm^{-1}(HB) and 1 389 cm^{-1}(5-Br-HB). The A, G line shifted from 1 419 cm^{-1} to 1 426 cm^{-1}(HB), 1 415 cm^{-1}(5-Br-HB). The G, A line at 1 490 cm^{-1}, 1 581 cm^{-1} shifted to 1 485 cm^{-1}, 1 578 cm^{-1}(HB) and 1 495 cm^{-1}, 1 580 cm^{-1}(5-Br-HB). The band assigned to G, C at 1 534 cm^{-1} shifted to 1 540 cm^{-1}(HB) and 1 546 cm^{-1}(5-Br-HB). Furthermore, the line belonging to C-N base external stretching vibration at 1 185 cm^{-1} shifted to 1 179 cm^{-1} (HB) and 1 189 cm^{-1} (5-Br-HB). The carbonyl stretching line of thymine at 1 662 cm^{-1}, involving interbase hydrogen bonding, shifted to 1 656 cm^{-1}(HB) and 1 655 cm^{-1} (5-Br-HB). All the facts above suggested that the interbase hydrogen bonds were broken, four bases of DNA were all photodamaged at different degrees and the photodamage to bases T and A were stronger than that to C and G. The photosensitization of 5-Br-HB was much more effective than that of HB because the displacement of four bases were further (the line at 1 419 cm^{-1} excluded) and some lines disappeared after the photodamage sensitized by 5-Br-HB.

In a word, the Raman characteristic frequencies and intensities of calf thymus DNA changed clearly after the photosensitization of HB and 5-Br-HB, which suggested that DNA was photodamaged and the homogeneous solution of DNA became a heterogeneous solution of polynucleosides: the interbase hydrogen bonds were broken , B-form conformation were damaged , single and double strands of DNA were scissored.

3 Discussions

The results showed that the photodamage to DNA sensitized by 5-Br-HB was stronger than that by HB, which is related to the higher yield of $^1O_2$ and $\cdot OH$ during the photosensitization of 5-Br-HB in heterogeneous system[2]. Generally, there are two reaction mechanisms between photosensitizers and DNA[9]:

(i)Direct modification of DNA.

(a) energy transfer from an excited sensitizer to DNA directly:

$$^3Sens^* + {}^1DNA \rightarrow {}^1Sens + {}^3DNA, \quad (1)$$

(b) electron or hydrogen transfer from an excited sensitizer to DNA:

$$^3Sens^* + DNA \rightarrow Sens^{+\cdot} + DNA^{-\cdot}, \quad (2)$$

$$^3Sens^* + DNA \rightarrow Sens^{-\cdot} + DNA^{+\cdot}$$

(c) the excited photosensitizer combines with DNA and an adduct is formed:

$$^3Sens^* + DNA \rightarrow adduct, \quad (3)$$

Sens represents photosensitizer; $^3Sens^*$ and ^1Sens represent triplet and singlet state of photosensitizer respectively; ^1DNA and ^3DNA represent singlet and triplet state of DNA respectively; $Sens^{+\cdot}$, $DNA^{+\cdot}$ and $Sens^{-\cdot}$, $DNA^{-\cdot}$ represent the canion and anion radicals of photosensitizer and DNA.

In this work, HB and 5-Br-HB cannot transfer energy to DNA directly because the exciting light is in the visible range of the spectrum[9]. It is known that the photosensitization of HB and 5-Br-HB can give rise to $HB^{-\cdot}$ and 5-Br-$HB^{-\cdot}$ [1,8]. So electron transfer could occur between DNA

and HB, 5-Br-HB and DNA radicals were formed. It has been reported that phenol can interact with the A-T plane[8] of DNA, which can be confirmed from the experimental results that the photodamage to A, T is stronger than to C. It has been reported that drugs attached to the N-7 and NH_2 positions of the adenine base[8]. The combination of DNA and HB, 5-Br-HB was possibly completed by the hydrogen bond between OH group of HB, 5-Br-HB and NH_2 position of adenine base. The lines belonging to C-O stretching vibration of deoxyribose at 1 017 cm^{-1} and 1 056 cm^{-1} shifted clearly and their intensities decreased obviously, suggesting that the hydrogen bond was formed between OH of phenol and C-O group on the ring of deoxyribose. The experimental results showed that the adenine and furan nucleus of deoxyribose which easily combine with photosensitizers were photodamaged stronger. The combination degree affecting the photodamage degree to DNA sensitized by photosensitizers suggested the direct modification to DNA of HB and 5-Br-HB.

(ii) Modification of DNA mediated by molecular oxygen. $^1O_2$, O_2^- and ·OH yield in the photosensitive reaction of HB and 5-Br-HB in a medium saturated with air[1,10]. OH and $^1O_2$ are important mediators in the photodamage to DNA caused by perylenequinonoid photosensitizers. ·OH is the most important mediator damaged to DNA[11]. $^1O_2$ can oxidate bases and possibly take part in DNA strand scissoring[7]. O_2^- cannot modify DNA directly but it can give rise to ·OH by so-called Fenton reaction to lead to DNA damage[9]. ·OH can modify bases, deoxyribose and deoxyribose-phosphate[12] by hydrogen abstract or other reaction to yield substrate radicals to cause DNA damage. ·OH can abstract hydrogen from C-1 or C-4 position of deoxyribose to give rise to deoxyribose radicals. The following reaction will occur on bases or phosphate groups or the furan nucleus of deoxyribose. It could be confirmed from the change of lines assigned to backbone phosphate groups, deoxyribose-phosphate and deoxyribose. The photodamage to bases is attacking on bases by ·OH[9].

It can be seen that the lines at 1 017 cm^{-1} and 1 056 cm^{-1} assigned to C-O stretching vibration of deoxyribose shifted more in fig. 2 than that in fig. 3. The lines at 1 419 cm^{-1} belonging to A, G bases shifted more and its intensity also decreased more in fig. 2 than that in fig. 3. Why does the phenomina occur? The reason is that the combination of 5-Br-HB with DNA must be harder than HB because of the atom Br space occupation. $^1O_2$ and ·OH were the main mediators in the indirect modification to DNA because of most of lines in fig. 3 changing more than that in fig. 2 and higher yield of $^1O_2$, ·OH of 5-Br-HB. It was confirmed that 5-Br-HB had better photosensitive characteristics than HB.

The assignment of the line at 1 125 cm^{-1} shifting to 1 123 cm^{-1}(HB) and 1 129 cm^{-1}(5-Br-HB) is not known.

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