Raman spectroscopic study on structure of human immunodeficiency virus (HIV) and hypericin-induced photosensitive damage of HIV

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Received February 17, 2004; revised July 15, 2004

Abstract The first Raman spectra of HIV1- HIV2 in human sera and hypericin-induced photosensitive damage of the virus have been obtained. The prominent Raman lines in the spectra are assigned respectively to the carbohydrates of viral glycoprotein, RNA, protein and lipid. The spectra are dominated by Raman scattering of the carbohydrates. The lines of D-Mannose and N-acetylglucosamine in carbohydrates are obvious and there is a β-configuration in the anomeric C1 position in D-Mannose. The viral RNA duplexes bound assumes an A-form geometry. The lines of backbone phosphate group, bases (involving interbase hydrogen bonding) and ribose of the RNA are complete and distinct. The secondary structure of the viral protein maintains α-helix, β-sheet, β-turn and random coil. Its side chains are rich and vary from tryptophan, phenylalanine and "buried" tyrosine; the stable conformation of the S-S bond of gauche-gauche-gauche; the two forms of C-S bonds of gauche and trans ; to sulfhydrl group and ionized and unionized carboxyl groups. The viral lipid bilayer molecules are probably in the liquid ordered phase or the gel phase. It was observed that the hypericin-induced photosensitive damage of HIV1-HIV2 in human sera changed various components of HIV1-HIV2 in different degrees : The orderly A-form viral RNA would become a disordered viral RNA. There were a breakage of interbase hydrogen bonds and disruption of vertical base-base stacking interactions. In addition, the groups of ribos and four bases were damaged obviously. A decrease in ordered structure (α-helix and β-sheet) of viral protein is accompanied by an increase in random coil. The Tyr buried in the three-dimensional structure of protein was damaged, but it was still "buried" and the damage of C-S bond of trans form was stronger. The groups of carbohydrates, including D-Mannos and N-acetyl glucosamine, in viral envelope glycoprotein had also been changed. The hydrophilic C-N bond of choline in viral lipid was damaged, which was the possible binding site to hypericin, whereas the viral lipids bilayers were still probably in the liquid ordered phase or the gel phase. So the space structure of HIV1-HIV2 was damaged under the experimental conditions, which might block viral infection and inhibit its growth and breeding. It is apparent that the laser Raman spectra have provided certain direct evidence at the molecular level for photosensitive damage of HIV1-HIV2.

Kewords: HIV1-HIV2, structure, hypericin, photosensitive damage, Raman spectroscopy.

DOI: 10.1360/04yc0015

Viruses are agents of some of the most destructive diseases afflicting plants and animals[1]. Viruses also play a central role in experimental methods of molecular and cellular biology, especially in modern genetic engineering[1]. Raman spectroscopy is a powerful tool for studying the structure of the whole virion. A number of researches are limited to the conformation of viruses, involving only nucleic acid (RNA or DNA) and its coat protein[1]. Literatures can be found concerning Raman spectroscopic study of single-stranded RNA viruses, such as tobacco mosatic virus (TMV), trurnip yellow mosaic virus (TYMV), belladonna mottle virus (BDMV), cowpea chlorotic mottle virus (CCMV) and bacteriophages R17 and MS2[1,2]. Raman spectroscopic study can also be found on filamentous DNA bacteriophages (fd, If1, Iki, Pf1, Xf, Pf3) in single-stranded DNA viruses and that of double- stranded DNA viruses bacteriophages P22 and T7[1,2]. The filamentous phage subunit conformation was also analyzed quantitatively[2]. In 1996, we reported for the first time "Raman spectroscopic study on human immunodeficiency virus (HIV1—HIV2) space structure and microcosmic and photosensitive damage of hypericin to the virus"[3].

The Acquired Immunodeficiency Syndrome (AIDS) was identified more than 20 years ago[4—6]. It is HIV1 and HIV2, two variants, that cause the majority of AIDS in human bodies and constitute one of the principal threats to human life. Aiming to understand and eventually find a cure for AIDS, the HIV virus becomes the most intensively studied virus in human history[7] Great progress has been made in obtaining an outlining sketch of how genes and proteins in HIV virion or virus particles operate and in understanding the biochemical specificity of HIV, the factors controlling its replication, the pathology of how it destroys the human immune system and the molecular bases of HIV infection and immunosuppression[7—13].

In general, the relative amounts of each component of retrovirus are estimated to be about 60%—70% proteins, 30%—40% lipids, 1%—2.5% nucleic acids and 2%—4% carbohydrates[14,15]. HIV is a retrovirus. The whole structure of HIV1 is the same as that of HIV2[6]. The outer coat of the HIV virion consists of a lipid bilayer assembled partly from lipid molecules taken from membrane of surrounding host human cells. The lipid bilayer is filled with many proteins, including the viral envelope glycoproteins gp120 and gp41[7]. An extensive part of the virion surface is predicted to be covered by carbohydrate[16]. The gp120 glycoproteins can bind to the membrane glycoprotein CD_4 on the surface of T lymphocytes of the host and the gp41 glycoprotein plays a crucial role in controlling the process of how HIV binds to and enters target cells[7,16,17]. The N-linked oligosaccharides chain of gp120 may participate in the interaction with CD_4[16]. However, it is not clear whether the mannose binding protein, which is bound to gp120, and the carbohydrate mannose play any role in mediating the attachment of the virus to oligosaccharides of host tissues[16,17]. There are many other important protein molecules in the HIV virion such as a matrix protein (P17), several capsid proteins (P24, P7) and different enzymes[7,8]. The genetic material of HIV consists of two strands of RNA of about 9200 nucleotide bases long each of which fits within the viral core[4]. The structures of some of the viral proteins and other components and the relationship between the structure and the function are known[18—26]. Their secondary strutures or three dimensional structure inside the HIV virion are also studied extensively. It is of interest, for example, to find out the configuration of viral carbohydrates, the state of the lipid bilayer, and the secondary structures of the viral RNA and protein molecules.

Raman spectroscopy is capable of answering structural questions at the molecular level and has been used successfully to study viruses with proteins and nucleic acids as their major components [27,28]. We report here for the first time the Raman spectra of human immunodeficiency virus HIV1-HIV2 in human sera. These are the first Raman spectra of complex virus with an RNA, protein, carbohydrate and lipid. The abstract of this paper was presented at the XVth International Conference On Raman Spectroscopy[3]. It was demonstrated that HIV1 contains the viral RNA duplexes bound with helix structure[29]; the viral pro-

tein maintains α-helix, β-sheet, β-turn and random coil and there were Tyr, Trp and Phe and so on in HIV1[29], which is in agreement with part of the results in our manuscript[3].

Hypericin (HC), a polycyclic quinone derived from plant genus Hypericum (saint-John wort), has been used to prevent uninfected T-cells from being infected with the AIDS virus in cells culture[30,31]. It has been known that light plays an important role in some biological events[30–38]. HC and some of polycyclic quinines irradiated by light could inhibit HIV-1 replication in cultured cell, inactivated reverse transcriptase and protein kinase C activity *in vitro*[32–34], and inhibit viral fusion[35–37] . Degar et al. found evidence for photochemical alterations of HIV1 capsid P24 and a block in uncoating of HIV1[38]. An animal test showed that HC had low toxicity at therapeutic doses[32,39]. Experiments are needed to elucidate the light-induced interactions between HC and the viral RNA, potein, carbohydrate and lipids.

We first studied the hypericin-induced photosensitive damage of HIV1-HV2 in human sera by laser Raman spectroscopy at the molecular level. Structural information on photosensitive damage of four components——viral RNA, protein, carbohydrat and lipids was obtained. So the characteristics of photosensitive damage of the virus can be deeply investigated, and the anti-AIDS mechanism of the drug further probed. However, it is apparent that more understanding of the mechanisms of photoinactivation should be useful for the development of photodynamic therapies for treatment of AIDS[40–46].

1 Materials and methods

1.1 Materials

HIV: HIV1 and HIV2 in human serum and negative human sera with 0.1% sodium azide in Target cassettes for the detection of HIV1-HIV2 antibodies (patent No. 4797260) were obtained from V-Tech, Inc. (Pomona CA, U.S.A.). Target HIV1-HIV2 is a rapid immunoenzymatic assay that detects and differentiates anti- HIV1 and anti-HIV2 antibodies in specimens of serum, plasma, or whole blood. The HIV1-HIV2 in human serum is an enriched liquor required as strong positive control in the test.

Hypericin was purchased from Carl Roth Co. Germany. 15 μL HIV1-HIV2 in human sera was mixed with 1 μL 32 μmol/L hypericin in the buffer to final concentration of 2 μmol/L hypericin.

1.2 Spectroscopic methods

The Raman spectra of all samples were obtained with a Jobin Yvon model JY-T 800 Raman spectrophotometer (Paris, France) fitted with microcomputer. An argon ion laser (Model 164 from Spectra Physics, CA, U.S.A.) was used as excitation. The experimental conditions were as follows: exciting line 514.5 nm and power 500 mW; the widths of the four slits of the triple monochromator 700, 800, 800, 700 μm; step 1 cm^{-1}; integral time and signal average: 0.1 s and 7 scans (450—1750 cm^{-1}), 0.2 s and 5 scans (2500—2600 cm^{-1}), 0.2 s and 2 scans (2800—3100 cm^{-1}), room temperatures 18±2℃. The vibrational frequencies in cm^{-1} and tentative assignments are listed in table 1.

2 Results and discussions

2.1 The space structure of protein, RNA, carbohydrate and lipid bilayer in HIV

Fig. 1 shows the Raman spectra of HIV1-HIV2 in human serum and the negative human serum used as a control in selected regions of interest between 450 and 3100 cm^{-1}. More details of the Raman spectra in fig. 1 are shown in figs. 2—4 in different spectral regions. It is clear from fig. 1 that the major contribution of the Raman signal from the HIV1-HIV2 sample is from the virus. Their characteristic Raman frequencies in cm^{-1} and assignments are listed in table 1.

In contrast, the prominent Raman lines in the Raman spectra of HIV1-HIV2 in human serum have contributions from all four major components of the virus. Peaks can be assigned to the carbohydrates of the viral glycoprotein, proteins including different enzymes, the two strands of the RNA, and lipids, respectively (figs. 1—3)[28,29,47–60]. In some cases, a peak has contribution from more than one component.

Table 1 Raman spectra of HIV1-HIV2 in human serum at 18℃ (450–3100 cm^{-1})

Frequency/cm^{-1}	Assignment			
	carbohydrate	RNA	protein	lipid
465				
493	Man			
*503	skeletal modes	r	S–S str	
515	GlcNac		S–S str	
548			Trp	
581		C, G	Trp	
596		C		
602	Man			
613				
620			Phe	
*631	GlcNac	r		
640			Tyr	
669	Man	G		
676				
684				
692				
703	GlcNac		C–S str	
717			C–S str	C–N sym
728		A		
736	GlcNac			
* 743	Man		Ile, Asn	
751		C		
759			Trp, Val	
769	Man			
778				
786		C, U	Thr	
814		O—P—O	Asn	
* 832	Man		Tyr, Val	
* 851			Tyr	
859				
880	β-C1 confign. Man		Trp, Val	
907	Man		C–C str	
* 926	COH bending		C–C str	
933	GlcNac		C–C str	
952	Man		C–C str	
974	Man	r		
994	CH_2 rock			
*1004		r	Phe	
1048	C1-H bending,Man C–O, C–C, GlcNac	r	C–N str	
1089	COH bending		C–N str	C–C str
1106	Man	O---P---O		
* 1123	Man, GlcNac	C, U	C–N str	C–C str
1132	Man		C–N str	
1157		r	C–N str	
1172				
1178		G, A, C	Tyr	
1193				
*1205			Tyr, Phe	

(*To be continued on the next page*)

(*Continued*)

Frequency/cm^{-1}	Assignment			
	carbohydrate	RNA	protein	lipid
*1226		A	Amide III	
1237		U, C	Amide III	
1244			Amide III	
*1250		A, C	Amide III	
1264			Amide III	
1277	COH, Man		Amide III	
1282				
1303		A, C	Amide III	
1322	GlcNac	G	C–H def	
1330	C(6)–H_2, CH_3, COH			
*1341		A	C–H def, Trp	
1348	COH, Man			
1356			Trp	
1379	CH_3, GlcNac, Man	G, U, A		
1394				
1408	Man		CO_2-sym str	
1419		G, A		
1433	GlcNac		Trp(N–H def)	
*1456	CH_2 bending	CH_2 bending	CH_2 bending	
1484		G, A		
1494				
1508		A		
1520				
1527		C		
1537		G		
1555	GlcNac(Amid II)		Amide II, Trp	
1567				
1581		G, A	Trp	
1613				
1620	Man	C═O str, U	Trp, Tyr, Phe	
1628				
1635	GlcNac(AmideI)			
1644			Amide I	
1660		C═O str, U, G, C	Amide I	
1669			Amide I	
1696				
1725			C═O str	
*2552			S–H str, Cys	
2578			S–H str, Cys	
2853				CH_2 sym str
2883			C–H str	CH_2 asystr
2900	C–H str			
*2935	C–H str	C–H str	C–H str	C–H str
*2979	C–H str	C–H str	C–H str	

*The common vibration mode of both HIV1-HIV2 and negative human serum is noted. +Abbreviations: Man, D-Mannose; GlcNac, N-acetylglucosamine; Tyr, tyrosine; Trp, tryptophan; Phe, phenylalanine; O—P—O, PO_2; O⁻⁻P⁻⁻O, PO_2^-; r, ribose; A, adenine; G, guanine; C, cytosine; U, uracil; sym, symmetric; asym, asymmetric; str, stretching; def, deformation.

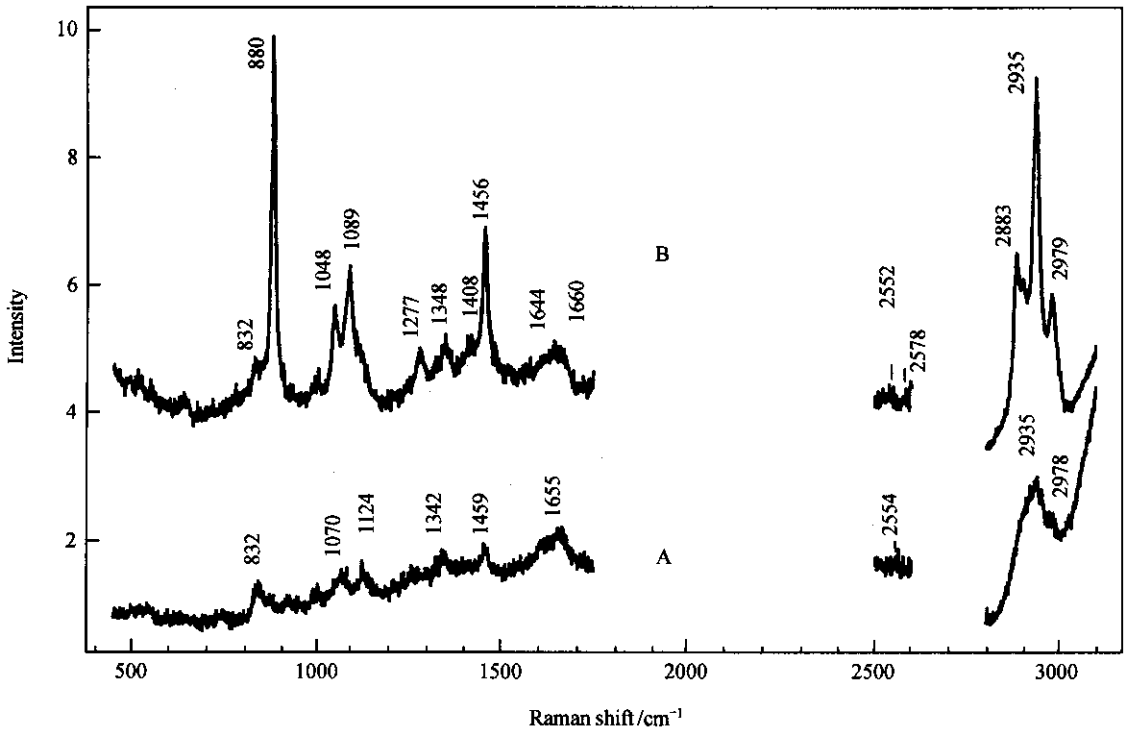


Fig. 1. Raman spectra of negative human serum (A) and HIV1-HIV2 in human serum (450–3100 cm^{-1}) (B).

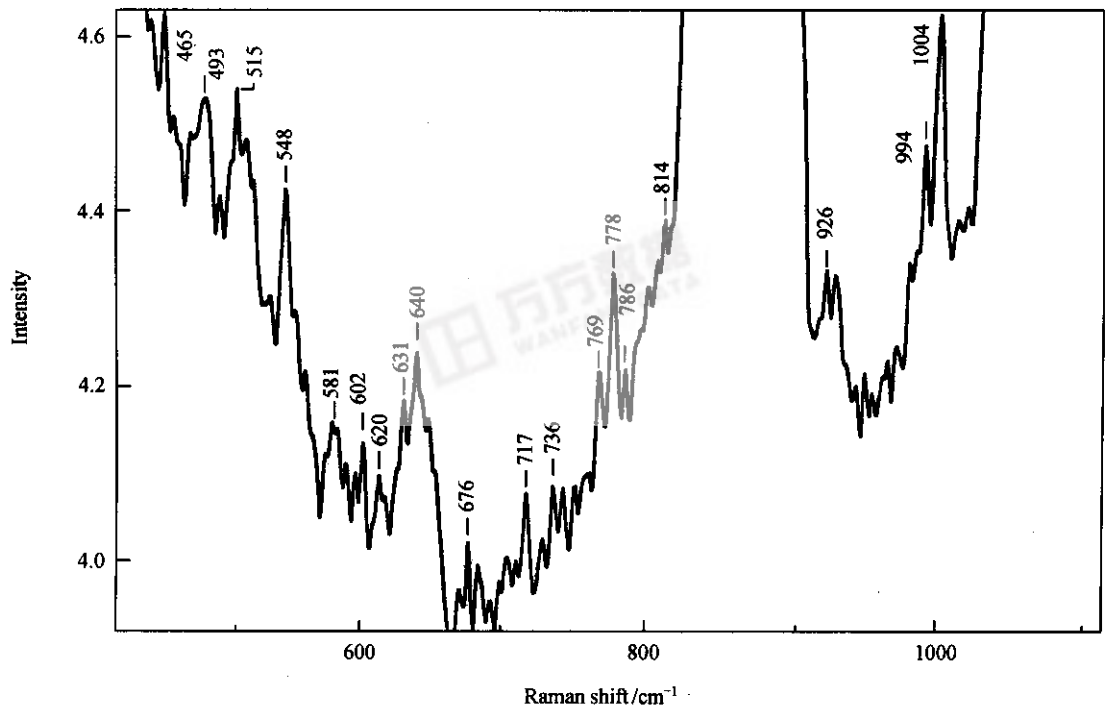


Fig. 2. Raman spectra of HIV1-HIV2 in human serum (450—1100 cm^{-1}).

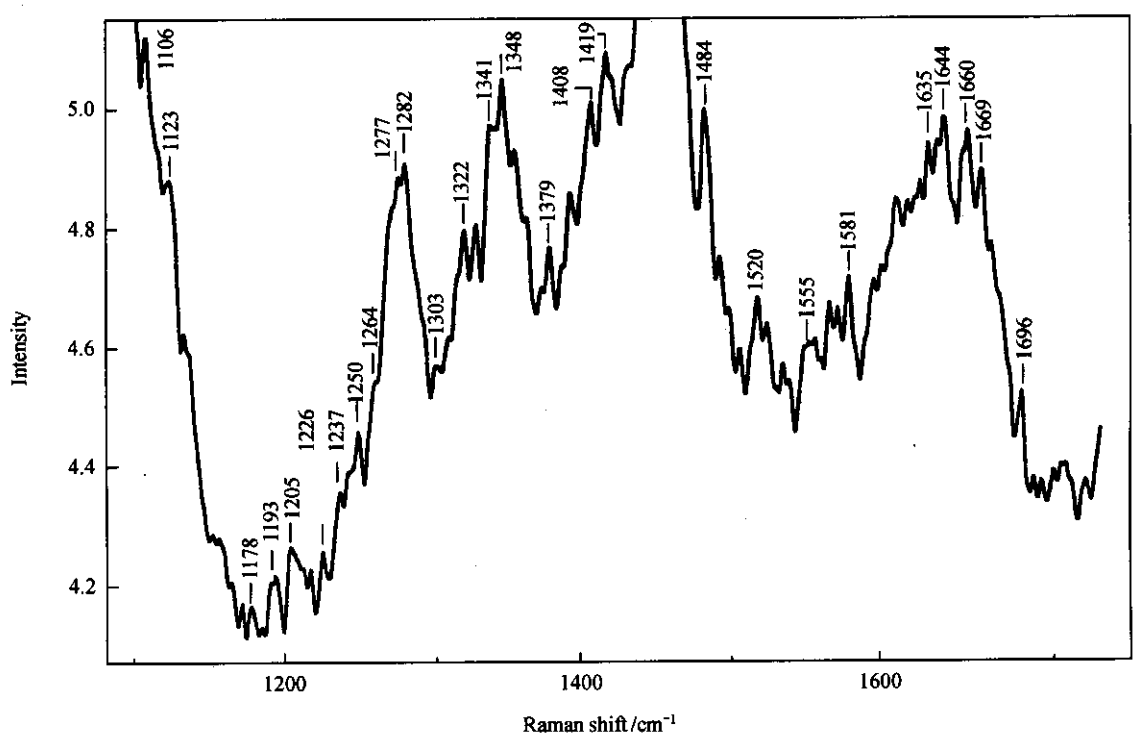


Fig. 3. Raman spectra of HIV1-HIV2 in human serum (1100—1750 cm^{-1}).

(i) Raman spectra of the carbohydrates of HIV1-HIV2 in human sera. 50% of the molecular mass of the major envelope glycoprotein is carbohydrate in the form of an array of oligosaccharide structures attached to the polypeptide backbone[16]. An extensive part of the virion surface is predicted to be covered by carbohydrates[16]. In addition, their Raman activities are stronger, so the lines intensities of carbohydrates are very high. Raman spectra of HIV1-HIV2 in human serum are dominated by Raman scattering of the constituent carbohydrates. The strong lines occurring at 880, 1048, 1089, 1277, 1348, 1456 cm^{-1} and 2935, 2979 cm^{-1} belonged to carbohydrates molecules in HIV1-HIV2[50—52,56]. The band at 1048 cm^{-1} is assigned to C1—H bending vibration. The vibrational mode may be associated with either the CCH group or the OCH group, or a combination of the two[50,51]. There was the line assigned to COH bending at 1089 cm^{-1} [50,51]. The lines belonging to the vibrational mode of the COH, COH and CH_2 bending appeared at 1277, 1348 and 1456 cm^{-1}, respectively[50,51]. The lines assigned to C-H stretching vibration at 2935 and 2979 cm^{-1}[51]. And two lines at 503 and 515 cm^{-1} probably belonged to the skeleton of the carbohydrate[52]. Mannose and N-acetylglucosamine were the main component of gp120's oligosaccharide[16]. The strong lines occurring at 880, 1048, 1277 and 1348 cm^{-1} clearly also were assigned to D-Mannose[52]. The strong line at 880 cm^{-1} belonged to the β-configuration in the anomeric C1 position of D-Mannose[52]. The strong line belonging to N-acetyl glucosamine appeared at 1048 cm^{-1}, and middle or weak lines that occurred at 631, 736, 1322, 1330 (C(6)—H_2, CH_3, COH), 1379 (CH_3), 1555(Amid II) and 1635 cm^{-1} (Amide I), respectively[56].

(ii) Raman spectra of the protein of HIV1-HIV2 in human serum. There are encoding 15 distinct proteins[29] including a matrix protein (P17) that forms a layer underneath the envelope, a capsid protein (P24) that makes up the core, protein P6, nucleocapsid protein P7 and several important enzymes including a protease, a reverse transcriptase and an integrase[7,8,29], in addition, the protein in the two major glycoproteins

gp41 and gp120[7,29] .

Main chain conformation: The amide I and amide III modes of the backbone are known to be sensitive to the secondary structure of proteins[47–49,53,54]. In the amide III region, the peak at ca. 1226 and 1237 cm^{-1} can be assigned to β-sheet and those at ca. 1264 and 1277 cm^{-1} to α-helix[47–49]. Similarly the peak at ca. 1303 cm^{-1} is due to β-turn[53–54] and those at ca. 1244 and 1250 cm^{-1} to random coil[47,49]. The main chain conformation of HIV1's protein was also researched through the crystal structure analysis of protein and NMR technique. It was indicated that α-helix, β-sheet, β-turn and random coil also existed in HIV1[29].

C–C and C–N backbone stretching vibrations are also sensitive to protein conformation. For example, the C–C stretching modes at 907, 926, 933 and 952 cm^{-1} suggest the presence of α-helix and random coil, respectively[47–50]. The lines assigned to C–N stretching vibrations occur at 1048, 1089, 1123, 1132 and 1157 cm^{-1} [47]. Some of the strongest lines are due to the main chain also. The CH_2 bending mode at ca. 1456 cm^{-1} and C–H stretching vibrations at 2935 and 2979 cm^{-1} are very clear and strong[47]. Finally, the lines at 1322 and 1341 cm^{-1} can be assigned to C–H deformation with major contribution expected from the main chain[47–49].

Side chain conformation: Most of the peaks due to side chain of the protein are from the aromatic residues[47–49]. For example, the indole ring mode of trytophan can be found in the Raman spectra of HIV1-HIV2 at ca. 548, 581, 759, 880, 1341, 1356, 1433, 1555, 1581 and 1620 cm^{-1}, the p-hydroxy phenyl ring mode of tyrosine at ca. 640, 832, 851, 1178, 1205 and 1620 cm^{-1}, and the mono-substituted phenyl ring modes of phenylalanine at ca. 620, 1004, 1205 and 1620 cm^{-1} [47–49]. The double peaks of tyrosine at 832 and 851 cm^{-1} come from the Fermi resonance between a ring breathing vibration and an overtone of an out-of-plane ring bending vibration[48]. The intensity ratio (I_{850}/I_{830}) is sensitive to the nature of hydrogen bonding and/or the state of ionization of the phenolic hydroxyl group[48]. The ratio of two peaks in our spectra is 0.91, suggesting that these tyrosine residues are "buried"[48].

The disulfide bond and sulfhydrl group can also be observed. The cysteinyl S–H stretching mode can be found at 2552 and 2578 cm^{-1} [47–49]. The peaks from the S-S bond stretching vibrations are at 503 and 515 cm^{-1}, indicating that the conformation of the C–S–S–C is the gauche-gauche-gauche[47–49]. Furthermore, the peaks at 631, 640 cm^{-1} and 703, 717 cm^{-1} can be assigned to the two conformations of gauche and transforms of C–S stretching vibration, respectively[47–49].

Modes from the carboxyl group of the aspartic acid and glutamic acid residues can also be observed. The peak at ca. 1725 cm^{-1} is likely to be due to the unionized –COOH group[47] while the peak at ca. 1408 cm^{-1} is from the ionized $-COO^-$ group[47]. The information of side chain conformation of viral protein has not been reported till now.

(iii) Raman spectra of the RNA of HIV1-HIV2 in human serum. Frankal et al. reported HIV1 contains two identical RNA with a six-base pair helix that forms "hairpins" structure[29] in 1998. We demonstrated that the viral double helical RNA exists in HIV1—HIV2 in human serum by Raman spectroscopy in 1996[3].

Space structure of backbone phosphate group and ribose: The line assigned to backbone phosphate diester (PO_2) symmetric stretching at 814 cm^{-1} is clear. It is indicated that the viral RNA duplexes bound assumes an A-form geometry[3,28]. The line assigned to backbone phosphate ion (PO_2^-) symmetric stretching occurred at 1106 cm^{-1} [27,28]. The average conformation of backbone phosphate ion in the virus is apparently different from that of protein-free RNA. The characteristic frequency is 1106 cm^{-1} in the former, whereas the characteristic frequency is 1100 cm^{-1} in the latter[28]. The lines belonging to C–O and C–C stretching vibration of ribose in the viral RNA appeared at 974, 1048 and 1157 cm^{-1}, respectively[27,28].

Conformation of bases: The lines assigned to ring stretching vibration of bases in the viral RNA occurred

at 669(G), 728(A), 751(C), 786(C, U), 1123(C, U), 1178(G, A, C), 1226(A), 1237(U, C) 1250(A, C), 1484(G, A), 1508(A), 1527(C) and 1537(G) cm^{-1}, respectively[28,55]. The lines asssigned to ring stretching and CH deformation vibrations occurred at 1303(A, C), 1322(G), 1341(A), 1379(G, U, A), 1419(G, A) cm^{-1}, respectively[28,55]. The line at 1581(G,A) cm^{-1} is assigned to C═N and C═C stretching vibrations[28,55]. The lines appearing at 1620(U) and 1660(U, G, C) cm^{-1} belonged to C═O stretching vibrations[28,55], involved in interbase hydrogen bonding[28].

(iv) Raman spectra of the lipids of HIV1-HIV2 in human serum. The lines assigned to lipids of the viral membrane appeared at 717, 1089, 1123, 1456, 2853, 2883 and 2935 cm^{-1}, respectively[56–60]. They are assigned to C—N symmetrical stretching of choline group, C—C skeletal (gauche and trans), CH_2 bending and C—H stretching vibrations[56–60], respectively. Comparing Raman spectra of HIV1-HIV2 in human serum with negative human serum at 2800—3100 cm^{-1}, it was found that the former was of these bands appearing at 2853 and 2883 cm^{-1} while the latter not. The intensity ratio (I_{CH}) I_{2883}/I_{2853} of the two bands assigned to asymmetrical and symmetrical C—H stretching vibration of methylene (—CH_2—) at 2883 and 2853 cm^{-1}, respectively, is quite sensitive to the changes of conformation of lipid. The order parameter for the lateral packing of lipids S_{lat} which reflected the state of lateral packing of the lipid bilayer was used widely to study the structure of membranes[56–60]. The band at 2883 cm^{-1} is involved in C—H stretching vibration of the viral protein[28]. The intensity ratio is about 5.6, so S_{lat} was 3.3, therefor the lipid bilayer molecules in HIV1-HIV2 are probably in liquid ordered phase or gal phase[56–60]. The fine and close outer coat-envelope consisting of lipid bilayers would effectively protect various viral proteins, enzymes and RNA genomes.

To sum up, the prominent lines in Raman spectra of HIV1-HIV2 in human serum are respectively assigned to carbohydrates of the viral glycoprotein, RNA, protein and lipid. The spectra are dominated by Raman scattering of the carbohydrates. The lines of D-Mannose and N-cetylglucosamine in carbohydrates are obvious and there is β-configuration in the anomeric C1 position in D-Mannose. The viral RNA duplexes bound assumes an A-form geometry. The lines of backbone phosphate group, bases (involving interbase hydrogen bonding) and ribose of the RNA are complete and distinct. The secondary structure of the viral protein maintains α-helix, β-sheet, β-turn and random coil. Its side chains are rich and vary from tryptophan, phenylalanine and "buried" tyrosine; the stable conformation of the S—S bond of gauche-gauche-gauche; the two forms of C—S bonds of gauche and trans; to sulfhydrl group and ionized and unionized carboxyl groups. The viral lipid bilayer molecules are probably in the liquid ordered phase or the gel phase.

(v) Raman spectra of the negative human serum. The strong lines in the Raman spectrum of the negative human serum can be assigned to be due to protein (see fig. 4). For example, the backbone modes of amide I and III can be found in the region around 1660 cm^{-1} and 1220 to 1300 cm^{-1} respectively. The peaks at ca. 1655 cm^{-1} are typical amide I peak of α-helix[47–49]. Similarly, in the amide III region, the 1226 and 1232 cm^{-1} peaks can be assigned to β-sheet, the 1253 cm^{-1} peak to random coil and 1299 cm^{-1} to α-helix[47–49], respectively. It is interesting to note that there seems to be no β-turn[47–49]. The other strong peaks can also be assigned to protein such as the peaks at ca. 500, 521, and 543 cm^{-1} to S-S stretch, 832 and 852 cm^{-1} to tyrosine, 1003 cm^{-1} to phenylalanine, a weak peak at 2554 cm^{-1} to S—H stretch[26–28], 1070 cm^{-1} to C—N stretch, 1342 cm^{-1} to C—H deformation, 1459 cm^{-1} to CH_2 bending, and 2935 and 2978 cm^{-1} to C—H stretch, respectively[47–49]. The proteins in the negative human serum do not contain tryptophan. In addition, there is no contribution from lipid, carbohydrate and RNA in the Raman spectra of the negative human serum.

2.2 Hypericin-induced photosensitive damage of HIV

The structure of hypericin is shown in Scheme 1.

The Raman spectra of HIV1-HIV2 in human serum before and after being exposed to light in the

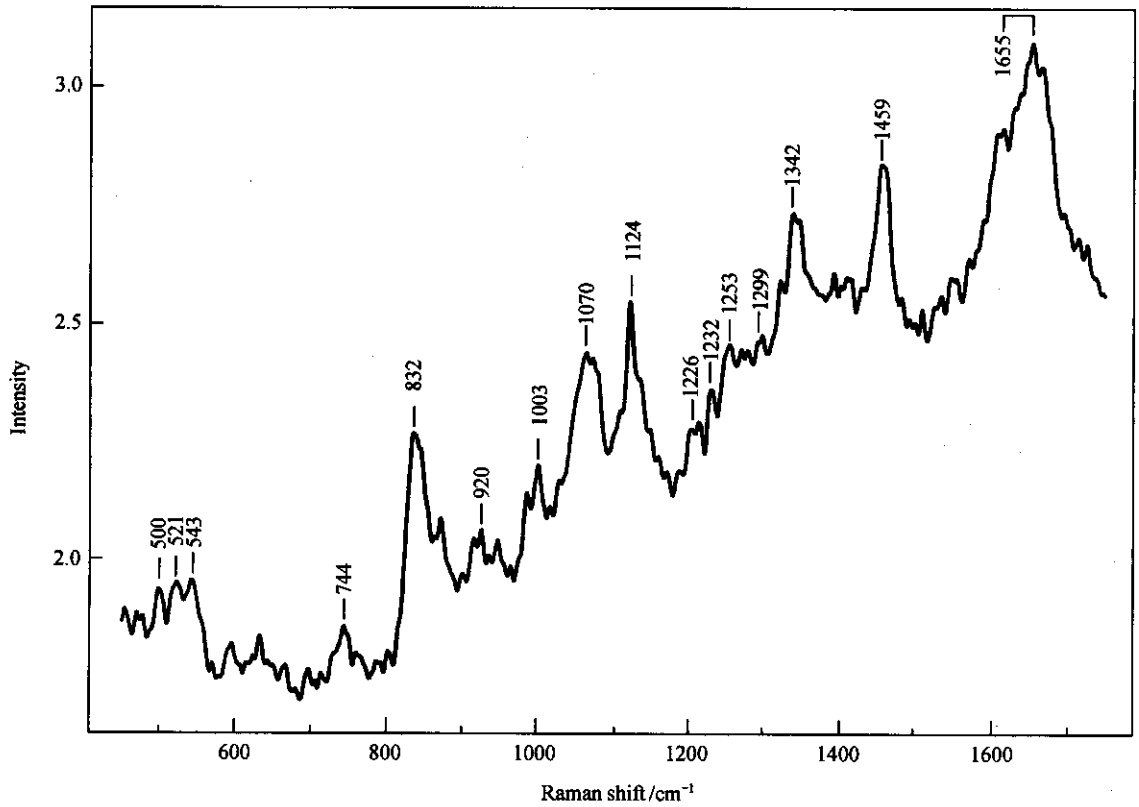
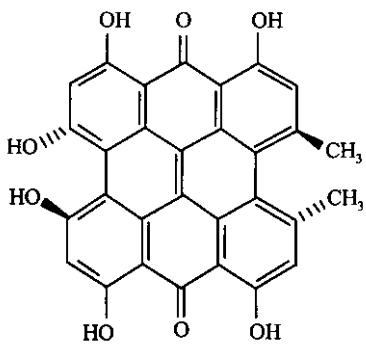


Fig. 4. Raman spectra of the negative human serum (450—1750 cm^{-1}).



Scheme 1. Structure of hypericin.

presence of hypericin (HC) are shown in fig. 5. More details of the Raman spectra in fig. 5(b) are shown in figs. 6 and 7 in different spectral regions. It is obvious that the major contribution of the Raman signal is from the HIV1-HIV2. There was no signal of HC observed. We have compared Raman spectra of HIV1-HIV2 in human serum before and after the photosensitive damage. The intensity and position of the line assigned to the breathing vibration of the monosubstituted phenyl ring of phenylalanine at 1004 cm^{-1}, which is insensitive to conformational change[47,48], was not changed by the photosensitive damage under the experimental conditions. Therefore this special line was used as the inner standard to determine the relative intensity of other lines of HIV1-HIV2 in human serum. The intensity of Raman line is proportional to the number of scattering centers[61,62]. Therefore, when line intensity decreased, it meant the decrease of the number of scattring centers, such as the groups and chemical bonds. Change rates in line intensities of various groups of HIV1-HIV2 were correlated to the damaged degree. The shoulder peak was not determined. The common lines of some groups were also recorded to investigate the structure changes of HIV1-HIV2 damaged.

(i) The photosensitive damage of the viral car-

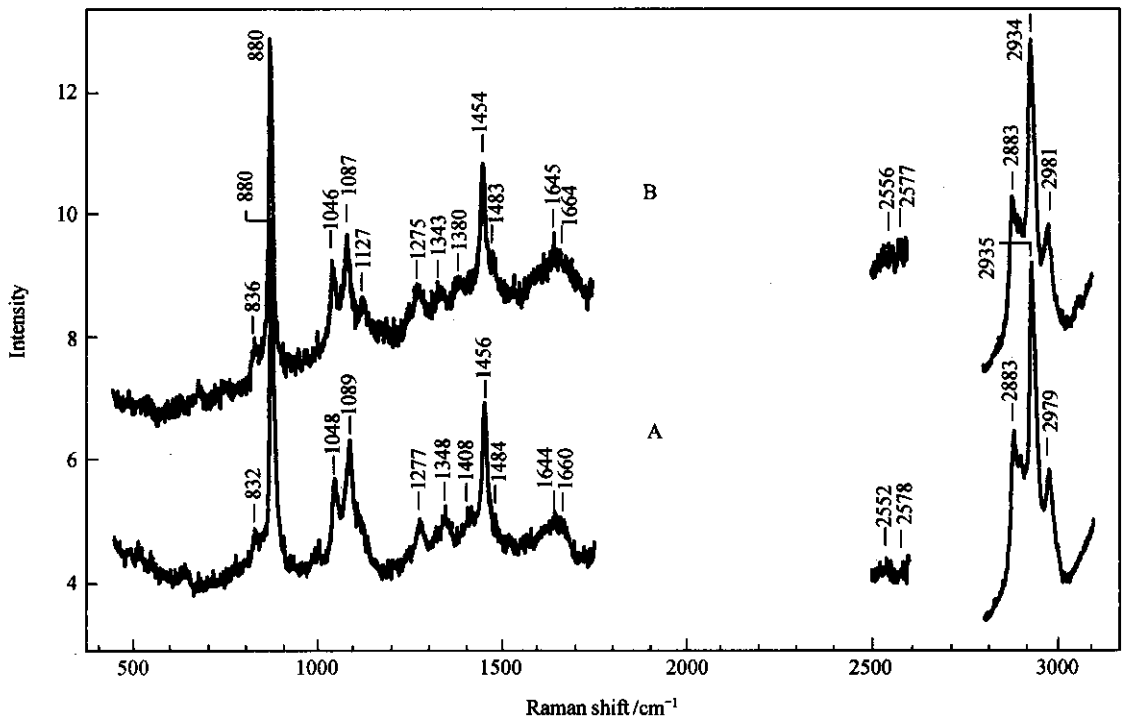


Fig. 5. Raman spectra of HIV1-HIV2 in human serum before (A) and after (B) hypericin-induced photosensitive damage (450–3100 cm^{-1}).

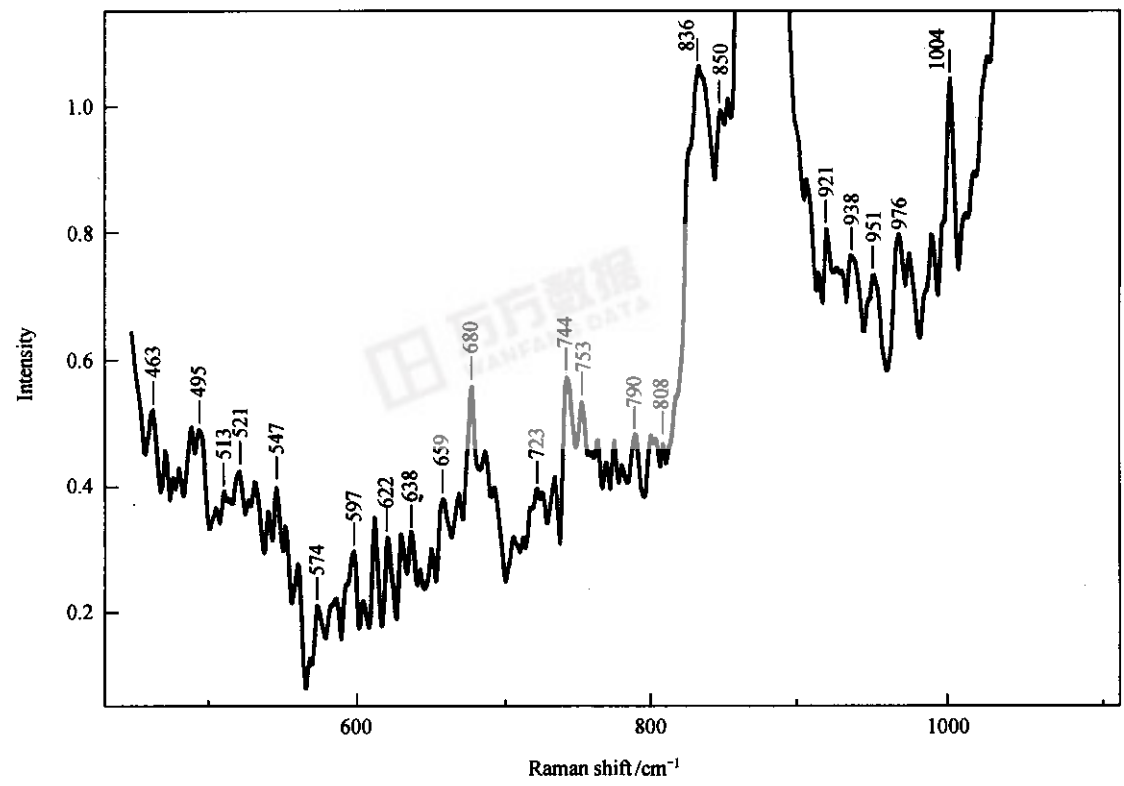


Fig. 6. Raman spectra of HIV1-HIV2 in human serum after hypericin-induced photosensitive damage (450–1100 cm^{-1}).

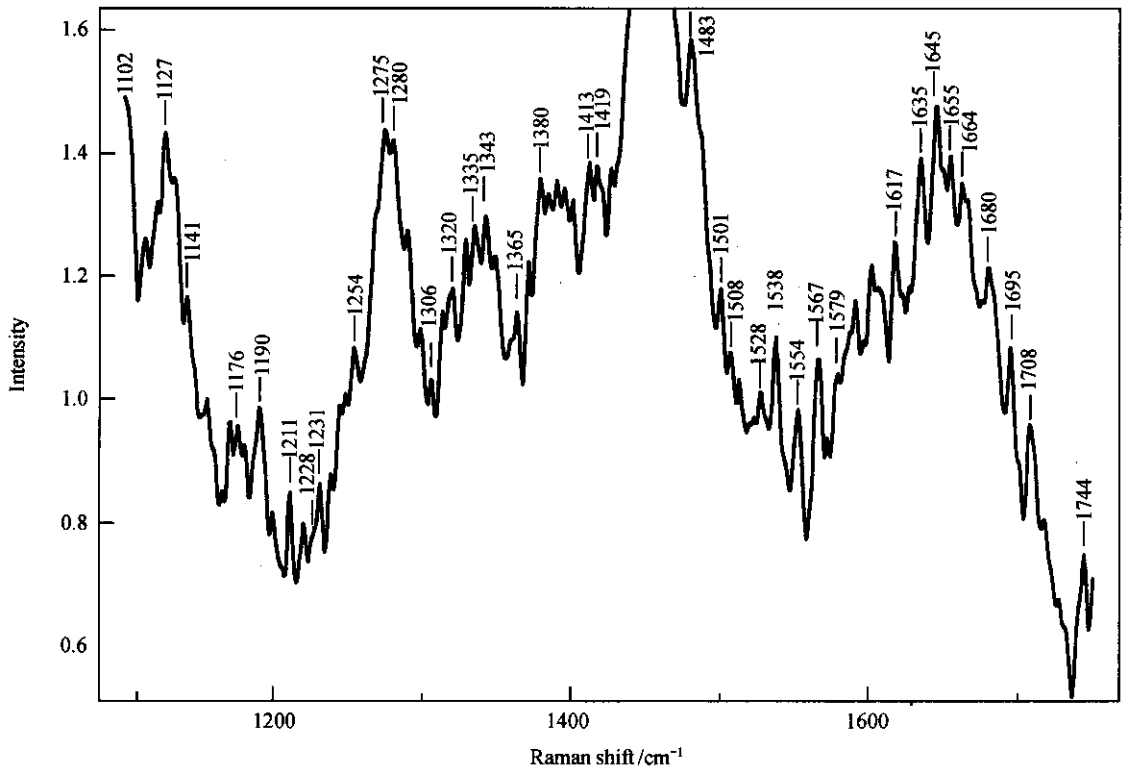


Fig. 7. Raman spectra of HIV1-HIV2 in human serum after hypericin-induced photosensitive damage (1100–1750 cm^{-1}).

bohydrates. It is important to understand HC-induced photosensitive damage of the carbohydrates, since 50% of the molecular mass of the major envelope glycoprotein is carbohydrate[20]. The intensities of the strong lines at 880, 1048, 1089, 1277, and 1456 cm^{-1}, respectively, assigned to β-configuration in the anomeric C1 position of D-Mannos[50–52], C1—H bending viration, the vibrational mode may be associated with either the CCH group or the OCH group, or a combination of the two. COH bending, COH and CH_2 bending[50–52] somewhat decreased, whereas strong lines belonging to COH[52] at 1348 cm^{-1} shifted to 1343 cm^{-1} and its line intensities decreased by 41%. The line at 513 cm^{-1} is probably due to the skeleton of the carbohydrates[50,51] and decreased by 72%. It suggested that damage of the skeletons of the viral carbohydrates was stronger. S—S bond of the protein also contributed to the line at 513 cm^{-1}[47]. The line intensities assigned to GlcNac, CH_2 rock and GlcNac(C(6)-H_2, CH_3, COH) at 631, 994 and 1330 cm^{-1}[51,56,63] decreased by 52%, 38% and 9%, respectively. The ribose of RNA also contributed to the line at 631 cm^{-1}. It is evient that the photosensitive damage of viral carbohydrates would affect the interaction of CD_4 with gp120 that plays important roles in viral infectivity[16,8].

(ii) The photosensitive damage of the viral protein

Main chain conformation: The lines assigned to the main chain amide I and amide III of the viral protein are sensitive to the change in protein conformation[47–49]. The line assigned to α-helix[47–49] at 1264 cm^{-1} disappeared. The peak belonging to β-sheet at 1226 cm^{-1} changed into shoulder peak which shifted to 1228 cm^{-1} and the line assigned to β-turn at 1303 cm^{-1} (Amide III)[53,54] shifted to 1306 cm^{-1}. The line assigned to α-helix or random coil at 1660 cm^{-1} [47–49] shifted to 1664 cm^{-1}, which was assigned to random coil[47–49]. The line at 1669 cm^{-1} assigned to β-sheet or random coil[47,49] disappeared. It was noted that a decrease in orderly structure (α-helix and β-sheet) was

followed by an increase in random coil in the viral proteins.

C—C and C—N backbone stretching vibrations are also sensitive to the changes in protein conformation[47—49]. The lines of α-helix of C—C stretching vibration shifted from 907, 926 and 933 cm^1 to 908, 928 and 938 cm^{-1} [47—49]. The line intensity at 951 cm^{-1}, which was assigned to random coil of C—C stretching vibration, increased by 123%. The lines of C—N stretching vibrations at 1046, 1123, 1132 and 1157 cm^{-1} shifted to 1048, 1127, 1141 and 1155 cm^{-1}[47—49], respectively. Their lines intensities also changed in different degree.

Side chain conformation: The lines of S—S bond stretching vibration at 503 and 515 cm^{-1} shifted to 506 and 513 cm^{-1}, respectively. The line intensities at 513 cm^{-1}, which were assigned to gauche-gauche-gauche[47—49], decreased by 72%. The skeleton of the carbohydrates also contributed to the line at 513 cm^{-1}, in addition conformation of S—S bond had no change, so its damage might be weaker whereas damage of the skeleton of the carbohydrates might be stronger. The lines at 703 and 717 cm^{-1},which belonged to trans form of C—S stretching vibration[47—49], shifted to 708 and 723 cm^{-1} and their lines intensities decreased by 38% and 40%, respectively. It suggested that damage of the C—S bond of trans form was stronger. The double peak of Tyr at 836 and 850 cm^{-1}, which arose from the Fermi resonance between a ring-breathing vibration and overton of an out-of plan ring bending vibration[47—49], was changed upon the photosensitive damage. The decrease rates of line intensities at 836 and 850 cm^{-1} were 9% and 24%, and shifted from 832, 851 cm^{-1} to 836, 850 cm^{-1}. The intensity ratio (I_{850}/I_{830}) was 0.78 , so the Tyr was still " buried"[47—49].

As mentioned above the space structure of viral proteins has been changed clearly, which would directly affect the biological functions of reversed transcriptase, integrase and protease and various capsid proteins in the viral protein. The laser irradiation of HC generates $^1O_2$, $O_2^{-\bullet}$, •OH and $HC^{-\bullet}$ [40—44], which damage hydrogen bonds that support the space structure of viral protein molecule. Additionally the organization of the hydrogen bonds might be affected by the HC-induced photosensitive damage of the side-chain of some amino acids located in the α-helix and β-sheet regions[49]. These effects eventually damage α-helix, β- sheet and β-turn. The photosensitive damage of the main-chain changs the environment of the side-chain, which in turn affects the conformation of the side- chain[49].

(iii) The photosensitive damage of the viral RNA

The backbone phosphate groups: The line assigned phosphate diester (PO_2) symmetric strectching, which is sensitive to RNA conformation change, shifted from 814 cm^{-1} to 808 cm^{-1}. This indicated that the A form viral RAN becomes disordered[3,28]. The line assigned to backbone phospha ion (PO_2^-) symmetric stretching[27,28] shifted from 1106 cm^{-1} to 1102 cm^{-1}. The line was found to be relatively insensitive to conformation of RNA[28] , then it was usually considered as an internal standard[28]. The obvious shift of the line implied that the phosphate ions (PO_2^-) might be the sites of interaction of RNA and hypericin.

The ribose: The line intensities of ribose at 631 cm^{-1} [27,28] decreased by 52%, GlcNac also contributed to the line at 631 cm^{-1}, whereas that at 974 cm^{-1} [27,28] increased by 206% and shifted to 976 cm^{-1}. HC is known to generate. •OH free radical under the irradiated condition[40]. If •OH free radical abstracts a hydrogen atom from the sugar in C-1 and C-5 positions, the covalent bond between ribose and base as well as the phosphate diester bond would be broken and the ribose would be damaged[64]. This would change the ribose and its force bearing environment to make its Raman activity increase so the intensity of the Raman line of the ribose increases.

The bases: The line intensities belonging to various groups of bases had changed. The lines intensities assigned to the cytosine and uracil ring at 786 cm^{-1}, to the guanine ring at 1322 cm^{-1}, to the adenine ring at 1341 and 1520 cm^{-1}, to the guanine and adenine ring at 1419 cm^{-1} [27,28] changed –17%, –32%, –35%,

–54% and –25%, respectively, and their lines shifted to 790, 1320, 1335 and 1514 cm^{-1}, while the line of C=N, C=C stretching vibration of the guanine and adenine ring at 1579 cm^{-1} [27,28] almost disappeared. The line intensities at 751(C), 1226(A), 1379(G, U, A), 1484(G, A), 1537 cm^{1}(G)[27,28] increased by 47%, 37%, 146%, 20% and 177%, respectively, and their lines shifted to 753, 1231, 1380, 1483 and 1538 cm^{-1}. The line of the guanine ring at 1365 cm^{-1} [27,28] changed from shoulder to sharp peak. Two lines of the guanine at 669 cm^{-1} and cytosine, uracil at 1123 cm^{-1} [27,28] changed from shoulder peaks to moderate and very strong lines, and their lines shifted to 670 and 1127 cm^{-1}, respectively. It showed the occurrence of hyperchromic effect, which resulted from disruption of vertical base-base stacking interactions[28,65]. While the carbonyl stretching vibration line of the uracil, guanine and cytosine at 1660 cm^{-1}, involving interbase hydrogen bonds[28,65], shifted to 1655 cm^{-1}. This shift was related to the breakage of interbase hydrogen bonds[28,65].

The common line of the groups split into two lines by the HC-induced photosensitive damage at various components of the virus, e.g., the line at 1226 cm^{-1} split into two lines assigned to β-sheet of the protein at 1228 cm^{-1}[47–49] and to adenine of the RNA at 1231 cm^{-1}[27,28]. The line belonging to α-helix or random coil of the viral protein[47–49] and the uracil, guanine and cytosine, involving interbase hydrogen bonds[28,65], at 1660 cm^{-1} became two lines assigned to random coil of the viral protein[47–49] at 1664 cm^{-1} and to the carbonyl stretching vibration of the uracil, guanine and cytosine, involving interbase hydrogen bonds[28,65] at 1655 cm^{-1}.

As indicated above the backbone phosphate group, ribose and bases of the viral RNA were damaged obviously. Since the viral RNA is an important genetic matter, damages on it would extremely affect the growth and breeding of HIV.

(iv) The photosensitive damage of the viral lipids

The two bands assigned to symmetrical and asymmetrical C—H stretching vibration of methylene ($-CH_2-$) occurred at 2883 and 2848 cm^{-1} [56,66]. The intensity ratio (I_{CH}) I_{2883}/I_{2848} was about 5.6, and the order parameter for the lateral interaction of lipid—S_{lat} was about 3.3[56,66] after photosensitive damage, so viral lipids bilayers still were probably in the liquid ordered phase or gel phase[60,66]. The line assigned to C–N symmetrical stretching vibration of choline group of lipids at 717 cm^{-1} [58,66] shifted to 723 cm^{-1} and its intensity decreased by 40%, which was followed by an increase of line width. This suggested that the C–N group was the possible binding site to HC[57,58]. The C–N group is located in hydrophilic head of lipid and has positive changes on it. It might be attacked easily by highly active oxygen species, such as the superoxide anion radical ($O_2^{-\bullet}$), the hydroxide radical $^{\bullet}OH$, etc. produced by HC photosensitization[40,58].

In summary, hypericin-induced photosensitive damage of HIV1-HIV2 in human serum changed various components of HIV1-HIV2 in different degrees: The orderly A-form viral RNA becomes a disordered viral RNA. There were a breakage of interbase hydrogen bonds and disruption of vertical base-base stacking interactions. In addition, the groups of the ribos and four bases were damaged obviously. A decrease in orderly structure (α-helix and β-sheet) of viral protein is accompanied by increase in random coil. Damage of the C–S bond of trans form was stronger. The Tyr buried in the three-dimensional structure of protein was also damaged, but it was still "buried". The groups of carbohydrates, including D-Mannos and N- acetyl glucosamine, in viral envelope glycoprotein had been changed. The hydrophilic C–N bond of choline in viral lipid was damaged, which is the possible binding site to hypericin, whereas the viral lipids bilayers were still probably in the liquid ordered phase or the gel phase, so the space structure of HIV1-HIV2 was damaged under the experimental conditions, which might block viral infection and inhibit its growth and breeding. It is apparent that the laser Raman spectra have provided certain direct evidence at the molecular level for photosensitive damage of HIV1-HIV2.

Acknowledgements This work was supported by the National Natu-

ral Science Foundation of China (Grant No. 39570189). We thank Profs. Chen Jenweng and Li Gouhua for assistance in the experiment. We also thank Mr. Xia Guang, Dr. G.T.Yu and Dr. Diwu Zhenjun for their revision on English manuscript and their helpful discussion.

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