THE PRESENT APPLICATIONS OF IR FEL AT PEKING UNIVERSITY *

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Abstract

In this study the sections of human tissues were treated under 9.5 μm FEL in the BFEL based on previous vibrational spectroscopic investigations that significant differences occur between normal and malignant tissues. Under the defocus condition, one part of the tissue section is burned while another part remains unchanged, suggesting that the FEL can selectively destroy some parts of the tissue. Vibrational spectroscopic and microscopic methods have shown that the FEL can induce decomposition of malignant tissues. The application of FEL whose wavelength is on the characteristic bands of malignant tissues may provide a new method to kill cancer cells with higher selectivity. For understanding the interactions between FEL and biological tissues, structure changes of substances under irradiation by FEL of about 9.4 and 11 μm were measured using FTIR spectroscopy. The samples include ATP, ADP, AMP, and D-ribose, etc. The FTIR spectra of the molecules before and after irradiation of FEL indicate molecular structure variations of the samples after irradiation of FEL, especially the rearrangement of their hydrogen bond networks, which may be caused by multiple photons process induced by FEL.

INTRODUCTION

Infrared laser, which stimulates vibrational levels of specific chemical groups in molecules and induces a variation of chemical reaction that can not occur by conventional thermal treatment, is regarded as "alchemy in chemistry" and received extensive investigation since 1970. However, the selection of wavelength in conventional infrared laser is limited. This hampers the understanding of the nature of the interaction between infrared laser and molecules.

Free Electron Lasers (FEL), as a powerful tool, are used for researches in material science, photochemistry, chemical technology, biophysical science, medical applications and surface studies, etc. [1-4]. The advent of FEL makes infrared laser with continuous tunable wavelength possible. This brings abundant opportunity for further understanding on the nature of the interaction between infrared laser and molecules. Especially the investigation on the changes of structures of bio-molecules induced by FEL provides information for the various biomedical applications [5-12].

The BFEL (Beijing Free Electron Laser, constructed by the Institute of High Energy Physics, CAS), whose wavelengths cover the fingerprint region of bio-molecules/organic molecules, provides a powerful tool for modification of molecular structure at chemical bonds, conformation as well as crystalline lattice levels. Recently, we take advantage of the mid-infrared light outputted by BFEL and FTIR to investigate on the sections of human tissues and some organic substances. The sections of human tissues were treated under 9.5 μm FEL in the BFEL due to the significant spectroscopic differences found between normal and malignant tissues [13]. For understanding the interactions between FEL and biological tissues, structure changes of substances under irradiation by about 9.4 and 11 μm FEL were measured using FTIR spectroscopy. Taking the sorts of benzoic acid as examples, after irradiation under some specified condition of FEL, the samples are characterized before and after irradiation with FTIR (Fourier Transform Infrared) spectroscopy, which indicate that some changes are observed [5]. In this work, significant structural variations of ATP, ADP, AMP, tRNA, cholic acid, deoxycholic acid, sodium cholate and sodium deoxycholate, D-ribose and its metal complex were observed in their FT-IR spectra upon the irradiation of FEL.

EXPERIMENTAL

The principal laser used in these experiments was BFEL, which has a complex pulse structure that consists of a train of macropulses, each containing a train of ultra short micropulses. The time structure of laser pulse is shown in the reference [5]. The width of the macropulses is 4 μs and the repetition rate is ca. 3 Hz. The separation between micropulses is 350 ps. The width of the micropulses is 4 ps.

The sections of human stomach tissues obtained from the third hospital, Peking University were treated under 9.5 μm FEL. The samples, analytical reagents from commercial source, including ATP, ADP, AMP, tRNA, cholic acid, deoxycholic acid, sodium cholate, sodium deoxycholate, ribose and a metal-saccharide complex were put under the FEL exposure in air at room temperature. The exposure time to the sample surface is 30 min. The size of light spot FEL was unfocused and controlled around 1 mm in diameter. The light parameters of irradiation are listed in Table 1. The FEL was tuned at

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the wavelength regions about 9, 6 and 11 μm. FT-IR spectra of the samples before and after irradiation of FEL were measured using micro IR method on a Nic-plan Nicolet Magna IR 750-II spectrometer at 4 cm^{-1} resolution and 128 scans.

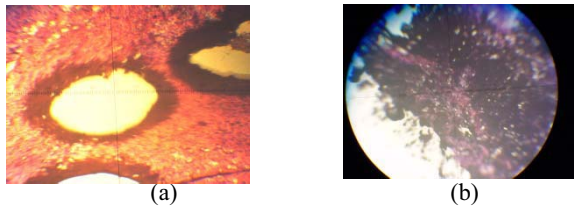
Table 1: The light parameters of irradiation

	Irradiation wavelength (μm)	Energy (mJ)	Time of irradiation (min)
AMP	11.04	1.56	30
ADP	11.02	1.5	30
ATP	11.06	1.62	30
tRNA	9.32	2.35	30
cholic acid	9.3	2.4	30
deoxycholic acid	9.4	2.2	30
NaC	9.3	2.4	30
Na(DC)	9.4	2.2	30
D-Ribose	9.92	2.8-3	30
$SmCl_3$-D-ribose	6.72	2.9-3	30

RESULTS AND DISCUSSION

The application of FEL on cancer treatment [6]

The sections of human stomach tissues were treated under 9.5 μm FEL in the BFEL because significant differences occur between normal and malignant tissues. The power of FEL is strong enough to destroy malignant tissues (Fig. 1(a)). Thus application of FEL whose wavelength is on the characteristic bands of malignant tissues may provide a new method to kill cancer cells with higher selectivity. In addition, combination of FEL with fiber optic techniques into endoscope may provide surgeons a powerful tool to perform minor-trauma surgery process to improve the surviving rate of terminal cancer patients. Under the defocus condition, one part of the tissue section is burned, but another part remains unchanged, suggesting that the FEL can selectively destroy some part of tissue. The photo was shown in Fig. 1 (b). Vibrational spectroscopic and microscopic methods have shown that the FEL can induce decomposition of malignant tissues. If this approach is feasible, then the FT-IR spectroscopic studies of cancer will come into a new era. The methods will extent from diagnosis cancer at molecular level into cure cancer at molecular level. The characteristic bands of malignancy tissue provide us an alternative selectivity. It can be imaged that surgical treatment of cancer in the future is performed by the interactive combination of spectroscopic diagnosis and spectroscopic treatment. The malignant and peripheral region are scanned by FEL laser, spectroscopic diagnosis information from each spot of the region are used as feedback that controls the laser power, so that it is possible to destroy malignant tissue as much as possible and protect normal tissue as much as possible. The application of fiber optics renders the surgical operation to be of minor trauma. For understanding the interactions between FEL and biological tissues, the interactions between FEL and biomolecules were investigated. The structure changes of substances under irradiation by FEL of about 9 and 11 μm were measured using FTIR spectroscopy.



(a) (b)

Fig.1 (a) The malignant tissue destroyed by FEL (b) Under the de-focus condition, one part of the tissue section is burned and another part remains unchanged, suggesting that the FEL can selective destroy some part of the tissue.

FTIR spectra of AMP, ADP, ATP and tRNA before and after FEL irradiation [7]

Adenosine 5'-phosphate (AMP), Adenosine 5'-diphosphate (ADP), Adenosine 5'-triphosphate (ATP) and transfer RNA (tRNA) are important bio-molecules. The four samples were irradiated at different wavelengths of free electron laser (FEL) obtained by Beijing Free Electron Laser (BFEL), and they were characterized with FT-IR spectroscopy before and after irradiation. The light parameters of irradiation are listed in Table 1. The FTIR spectra of AMP before and after irradiation of FEL are shown in Fig. 2. The characteristic frequencies in the FTIR spectra of AMP, ADP, ATP and tRNA before and after irradiation of FEL are listed in Table 2.

For AMP and ATP, some changes were observed in the region of 3500-3000 cm^{-1} with the OH stretching bands become even broader after irradiation. It also indicated that irradiation of FEL has great influence on P=O and P-O-C. The stretching band of P=O in both AMP and ATP splits into two bands. For AMP, the P=O stretching band is a broad band at 1234 cm^{-1}, while after irradiation it divides into two sharp bands at 1218 cm^{-1} and 1205 cm^{-1}; For ATP, the irradiation of FEL induces the P=O stretching band to split from 1248 cm^{-1} to 1261 cm^{-1} and 1221 cm^{-1}. These results indicate that the hydrogen bond networks are dissociated partly and rearranged with the irradiation of FEL. Considering the obvious variation in 1100 cm^{-1} and 1010 cm^{-1} of P-O-C and C-O vibration, it is suggested that the molecular skeletons of AMP and ATP vary after irradiation. However, for ADP, only minor changes in skeleton vibration can be observed in FT-IR spectra after irradiation. For tRNA, interesting change can be observed after irradiation. That is, the variation of intensity rate of peaks at 1085 cm^{-1} and 1073 cm^{-1}. Before irradiation the intensity rate is 0.965, while after irradiation, the rate is 1.083. Considering the P=O band from 1234 cm^{-1} to 1236 cm^{-1}, the irradiation of FEL induces the conformational variation of lecithoid group. The same phenomenon can also be observed in AMP. The

intensity rate of peaks at 1085 cm^{-1} and 1065 cm^{-1} is 1.167 before irradiation, after irradiation the rate of peaks at 1084 cm^{-1} and 1058 cm^{-1} is 0.875. Considering the irradiation wavelength of FEL at tRNA is 9.32 μm that is 1073 cm^{-1}, which is near the stretching band of P-O-C, at AMP and ATP the irradiation wavelengths of FEL are 11.04 μm and 11.06 μm respectively, which are about 909 cm^{-1}. We can get the conclusion that the irradiation induced the conformation change of the biomolecules with lecithoid group.

Table 2: The characteristic frequencies in the FTIR spectra of AMP, ADP, ATP and tRNA

Sample	Original	After FEL irradiation	Assignment
AMP	3333,3208	3388,3094	OH
	1693	1685	
	1234	1218,1205	P=O
	1085,1065	1084,1058	P-O-C, C-O
ADP	3339	3335	OH
	1660	1659	
	1223	1222	P=O
	1081	1079	P-O-C, C-O
ATP	3347,3151	3344,3154	OH
	1698	1716	
	1248	1261,1221	P=O
	1104,1051 ,1018,965	1101,998,9 70	P-O-C, C-O
tRNA	3360,3238	3336,3244	OH
	1686,1650	1688,1651	
	1234	1236	P=O
	1085,1073	1085, 1073	P-O-C, C-O

FTIR spectra of cholic acid, deoxycholic acid and their sodium salts before and after FEL irradiation [8]

As the predominant ingredients of bile, bile salts are one of the most important bio-surfactants in vivo. Cholic acid, deoxycholic acid and their sodium salts were irradiated using FEL about 9.4 μm and characterized with FT-IR spectroscopy before and after irradiation. Their FTIR spectra are shown in Fig. 3. The frequencies are listed in Table 3. For cholic acid and deoxycholic acid, the fine structures in OH stretching region (3000-3700 cm^{-1}) disappear, and the CH stretching bands become even broader after irradiation. For cholic acid, the C=O of COOH stretching band is a sharp band at 1715 cm^{-1}, while after irradiation it becomes a broad band at 1708 cm^{-1}. For deoxycholic acid, the COOH stretching bands are two sharp bands at 1714 cm^{-1} and 1695 cm^{-1}, while after irradiation they appear a broad band at 1709 cm^{-1}. These results indicate that the hydrogen bond networks are dissociated and rearranged after irradiation. Considering the obvious variation in skeleton vibration, it is suggested that the molecular skeletons of these two bile

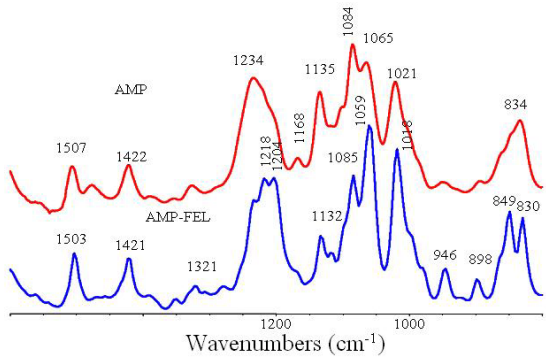


Fig. 2 IR spectra of AMP before and after irradiation (1600-800 cm^{-1})

acids vary after irradiation. However, for sodium cholate and sodium deoxycholate, there is no obvious alternation in FTIR spectra after irradiation. Only minor changes in skeleton vibration can be observed. In conclusion, for cholic acid and deoxycholic acid, the interaction of FEL can induce the dissociation and rearrangement of H-bond structure. However, the effect of FEL to sodium cholate and sodium deoxycholate is not so obvious.

For above molecules, a mechanism of multiple photons process induced by FEL is suggested. FT-IR spectra of the samples treated with FEL represent stable/meta-stable structures of the samples and FEL provide energy to help the molecular system to overcome the energy barrier on the potential surface and reach a stable/meta-stable structure. When FEL makes the molecules reach their excited states, vibrational energy rapidly flow and have rapid redistribution, then have stable/meta-stable structures. The variation of the FT-IR spectra of the molecules proves that the spectral variations of the samples induced by FEL are closely related to hydrogen bond networks.

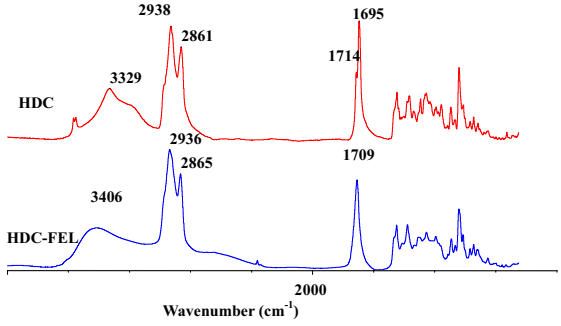


Fig. 3 The FTIR spectra of HDC before and after FEL irradiation

Table 3: The characteristic frequency data of FTIR spectra of HC and HDC before and after FEL irradiation

	HDC		HC	
	Original (cm^{-1})	After FEL (cm^{-1})	Original (cm^{-1})	After FEL (cm^{-1})
OH	3567, 3553, 3329	3406	3523, 3321, 3192	3405
CH	2928, 2861	2936, 2865	2966, 2935, 2872	2935, 2868
COOH	1714, 1695	1709	1715	1708

FTIR spectra of D-ribose and its metal complex before and after FEL irradiation

The above results indicate that hydrogen bond networks rearrange after irradiation of FEL. Carbohydrates are important biomolecules and often used as model systems to study hydrogen bonds, therefore, the structure changes of carbohydrates after irradiation of FEL were investigated. The FTIR spectra of D-ribose and $SmCl_3$-D-ribose complex before and after irradiation of FEL are shown in Fig. 4 and Fig. 5. The results indicate that the fine structures disappeared and hydrogen bond networks were broken and rearranged for $SmCl_3$-D-ribose complex ($SmCl_3 \cdot C_5H_{10}O_5 \cdot 5H_2O$) after irradiation of FEL. The bands of OH and CH vibrations become broader after irradiation. Two sharp bands at 1634 and 1619 cm^{-1}, corresponding to the deformation vibrations of water molecules, become a broad band located at 1639 cm^{-1} after irradiation of FEL. In the 1500-900 cm^{-1} region the bands also become broad, which indicate that the hydrogen bond networks formed by hydroxyl groups of D-ribose, chloride ions and water molecules broken and rearranged after irradiation of FEL. For D-ribose, the OH stretching vibrations also have shifts about 10 cm^{-1}. Most of the bands in the 1500-650 cm^{-1} region have differences in peak positions before and after irradiation of FEL, which indicate the changes of the structure. The different changes for D-ribose and $SmCl_3$-D-ribose complex may be caused by the different excited wavelength of FEL.

CONCLUSIONS

The H-bond networks of a series of molecules, including ATP, ADP, AMP, tRNA, cholic acid, deoxycholic acid, sodium cholate, sodium deoxycholate, D-ribose and $SmCl_3$-D-ribose complex exhibit significant re- arrangement upon the irradiation of FEL at both about 9.4 and 11 μm. Free electron lasers provide a powerful tool for modification of molecular structure at chemical bonds, conformation as well as crystalline lattice levels. Structure changes were observed after irradiation of FEL for some molecules, which indicate that FEL may be used to control reactions. The results show that FELs would have extensive applications in the fields of chemistry and biology.

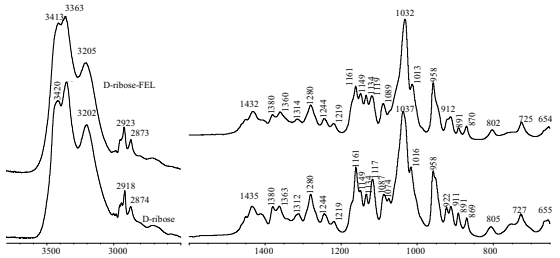


Figure 4: The FTIR spectrum of D-ribose in the 3800-2500 and 1600-650 cm^{-1} region before and after irradiation of 9.92 μm FEL.

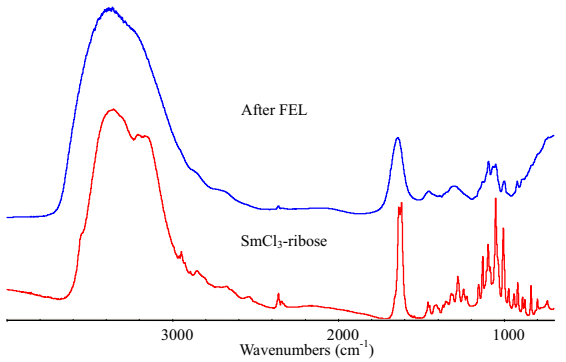


Figure 5: The FTIR spectra of $SmCl_3$-D-ribose before and after irradiation of 6.72 μm FEL.

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