

# Fourier Transform Infrared Emission Spectroscopy in the Study of Biological Molecules

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**Abstract**—Infrared spectroscopy is a powerful analytical method that is not completely developed in relation to biological systems as yet. Previously, this method has been successfully used to analyze secondary structures. This mini-review shows the latest advances in the study of proteins using the Fourier transform infrared technique. The application of Fourier transform infrared emission spectroscopy to the study of photophysical and photochemical reactions in photoreceptor proteins is discussed.

**Keywords:** FTIR spectroscopy, stimulated infrared emission, visible light, infrared light, protein, pigment-protein complex, amino acids

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## INTRODUCTION

Vibrational spectroscopy is a powerful tool for studying biological samples [1–5]. Infrared spectroscopy (IR spectroscopy) and Raman scattering spectroscopy (Raman spectroscopy) are two complementary methods of vibrational spectroscopy, which are based on two fundamentally different physical processes. However, excitation of molecules to higher vibrational energy states is observed in both cases. IR spectroscopy is associated with absorption of IR quanta, as a result of which vibrational and rotational motions of the molecules, determined by the chemical composition of the sample, are enhanced [3, 5]. Chemical bonds undergo various forms of vibration, such as stretching, bending, and rotation. The energy of most molecular vibrations corresponds to the energy of quanta with the wavelength of 25–2.5  $\mu\text{m}$  or wave numbers of 4000–400  $\text{cm}^{-1}$ . The bands in the IR absorption spectrum occur as a result of transitions between vibrational sublevels of the ground electronic state and are associated with a change in the dipole moment, selection rules, molecular symmetry, and the degree of vibrational anharmonicity. The information presented in the IR spectrum is unique; it cannot be obtained using spectroscopy in the ultraviolet and visible regions.

*Abbreviations:* IR, infrared; ATR, attenuated total reflection; IRES, IR emission spectroscopy.

## MATERIALS AND METHODS

**Preparation of studied sample films.** L-lysine monohydrochloride (Aldrich-Sigma, United States) was used without preliminary purification. A film was obtained by precipitating the sample from a 1 M aqueous solution onto a crystalline silicon substrate. The film layer thickness was approximately 1  $\mu\text{m}$ . The substrate material has a transparency region in the infrared range.

A sample uniformly distributed over the smooth surface of the substrate in a volume of 200–300  $\mu\text{L}$  was dried in air at room temperature and relative humidity of approximately 60% for several hours.

Deionized water at pH 7.0 was used to prepare the solution. The deionized water used in these experiments was obtained on a Nanopure Diamond ultra-pure system (Barnstead International, United States). The water purity is confirmed by the conductivity of 18 MOhm.

**Spectroscopic studies.** The general spectroscopic studies were carried out using a number of spectroscopic methods: Fourier transform infrared difference spectroscopy, low temperature Fourier transform infrared difference spectroscopy, and Fourier transform IR emission spectroscopy.

The spectrum of the light-adaptive form, the parent bR<sub>568</sub> form, of bacteriorhodopsin was initially recorded in differential and low temperature measurements, which were saved in the computer's memory. The spectrum of the studied intermediate was then recorded. The difference spectrum was obtained by subtracting the spectrum of the parent form from the

obtained spectrum of the studied intermediate. The spectrum of the  $M_{412}$  intermediate was obtained at the temperature of 190 K.

Emission measurements were performed based on a domestic FS-02 FT-IR spectrometer by modifying its optical scheme. To do this, the source of infrared radiation was removed from the device and replaced by the sample that was continuously irradiated with moderate power visible light; in fact, the sample itself was an immediate source of infrared radiation that was directed to the collecting mirror and further to the interferometer. The geometry of the sample irradiation with visible light was such that the exciting light fell almost normally to the surface of the sample and was focused into a spot with a diameter of 2–4 mm using a short-focus lens. Secondary IR radiation was recorded in the reflection geometry. The exciting (stray) light did not reach the detector. Accidental penetration of visible light was cut off by a plate made of germanium, which was installed on the input window of the detector. Germanium transmits infrared light and does not transmit visible light.

The power of the excitation radiation from the xenon lamp was varied using absorption light filters (LOMO, Russia) during study of the intensity dependence of the IR emission spectrum. The filters were calibrated using a power meter; band pass infrared filters were also used.

The emission spectra were recorded in the single channel mode with a spectral resolution of  $4\text{ cm}^{-1}$ , which was chosen for the better reproducibility of the spectra.

## RESULTS AND DISCUSSION

**IR spectroscopy in the study of globular proteins.** IR spectroscopy began to be used in structural studies of proteins in the 1950s [6–8]; it is still an excellent tool when studying the conformation and dynamics of protein structures [2, 9–11]. The usefulness of infrared spectroscopy stems from its ability to detect changes in the relative orientation and hydrogen bonds of peptide C=O groups, which are the main oscillators associated with the structure. One of its main applications is the analysis of the secondary structure of the protein based on the fact that there is a close correlation between the position of specific IR bands in the spectrum and the secondary structure [12–15]. These correlations were determined by associating the frequencies of structurally sensitive amide bands with specific types of the structure, such as  $\alpha$ -helices,  $\beta$ -sheets or loops [12, 13, 16]. Despite the large number of degrees of freedom of a protein molecule its IR spectrum does not have many bands. This is determined by the fact that in the IR spectra of protein polymers only the normal vibrations are active for which the atoms in the repeating units of the polymer chain oscillate in phase [2, 7].

The protein vibration associated with the Amide I band, which is localized in the spectral region of  $1700\text{--}1620\text{ cm}^{-1}$ , has been the most studied. The Amide I band is almost entirely composed of C=O stretching vibrations associated with stretching of the peptide bond and is highly sensitive to the secondary structure. Depending on the type of the secondary structure in which peptide C=O groups are involved, normal modes tend to localize within certain spectral regions, creating frequency bands, each of which has its own spectral parameters (position, bandwidth, and attenuation coefficient).

The Amide II band, in contrast, results from plane vibrations associated with bending of the N–H bond, and from stretching vibrations associated with stretching of the C–N bond. The Amide II vibration demonstrates a much lower sensitivity to the protein conformation [2]. Other amide bands are very complex and strongly depend on the magnitude of force fields as well as the nature of side chains and hydrogen bonds, and are practically not used in the analysis of conformational studies.

One of the most significant practical advantages of detailed structural analysis is that the Amide I band contains more information than just the data on the conformation of globular proteins. The exact position of this vibrational band depends on the nature of hydrogen bonds involving C=O and N–H groups [14]. In turn, this is determined by the packing of the polypeptide chain, which reflects the conformation of the main chain and the pattern of hydrogen bonds. Thus, the observed contours of the amide bands of proteins consist of overlapping bands of various components representing  $\alpha$ -helices,  $\beta$ -sheets, bends, and disordered structural elements [2, 7, 17]. The Amide I band often shows a complex and unresolved shape of a line with a large number of wide bands with unclear maxima. Its shape can change under the influence of a solvent [16, 18, 19], due to local deformations [9], size [9], and the structural organization of local elements in the secondary structure [20]. These phenomena increase the internal information content but complicate the interpretation. Thus, the interpretation of IR spectra, which includes the identification and characteristics of the bands of the main components that determine the profile of the Amide I band, has become the main aspect in the study of proteins using infrared spectroscopy [21–24].

The emergence of new devices, Fourier transform IR spectrometers, in the 1980s introduced a new era in protein studies. The fundamentally new design of IR spectrometers in combination with the development of the Fourier transform method for collecting IR data and reliable digital subtraction allowed rapid and efficient processing and converting interferograms using the Fourier transform algorithm, as a result of which it became possible to quickly obtain the dependences of the transmission coefficient on the energy in wave

numbers [25]. The spectrum obtained as a result of mathematical processing indicates the presence of chemical bonds and, therefore, gives information on the composition and structure of the substance. Fourier transform IR spectroscopy is recognized as a valuable tool for studying protein conformation in a solution based on ordinary water, as well as its deuterated forms; it is also used to study structural stability, folding, and aggregation of proteins [26–32].

However, in the beginning the practical use of this technique was greatly limited by factors such as the low sensitivity of devices and lack of understanding of the correlation between specific types of packing of the main chain and bands of individual components [22]. It was then believed that the IR spectrum of a protein in an aqueous solution is difficult, if not impossible, to obtain unless heavy water is used as a solvent, since water strongly absorbs in the most important spectral region at approximately  $1640\text{ cm}^{-1}$  and can completely mask protein bands. Even in a  $\text{D}_2\text{O}$  solution, only qualitative information was usually obtained, because the components of the absorption bands associated with specific substructures, such as  $\alpha$ -helix and  $\beta$ -sheets, could not be resolved [11].

The high sensitivity of modern IR detectors, the stability of lasers, and the computerized instrumentation of modern Fourier transform IR spectrometers improved the signal-to-noise ratio and allowed extensive manipulation of data. Spectra can be obtained in a wide range of media with less time and material, which is an exceptional advantage of the method. In this case, direct correlations between the frequencies of the Amide I band and the components of the secondary structure can be detected based on established experimental protocols for analyzing the secondary structure of polypeptides and proteins [33–36]. Such opportunities significantly expanded the use of Fourier transform IR spectroscopy in recent years in studies of the secondary structure and dynamics of proteins [32].

Fourier transform IR spectroscopy is used not only to study the secondary structure, but also to study structural dynamics and conformational changes (this reflects ligand binding [37] and the dependence on temperature, pH, and pressure [38–40]); it is also used to study structural stability, folding, and aggregation of proteins [26, 27].

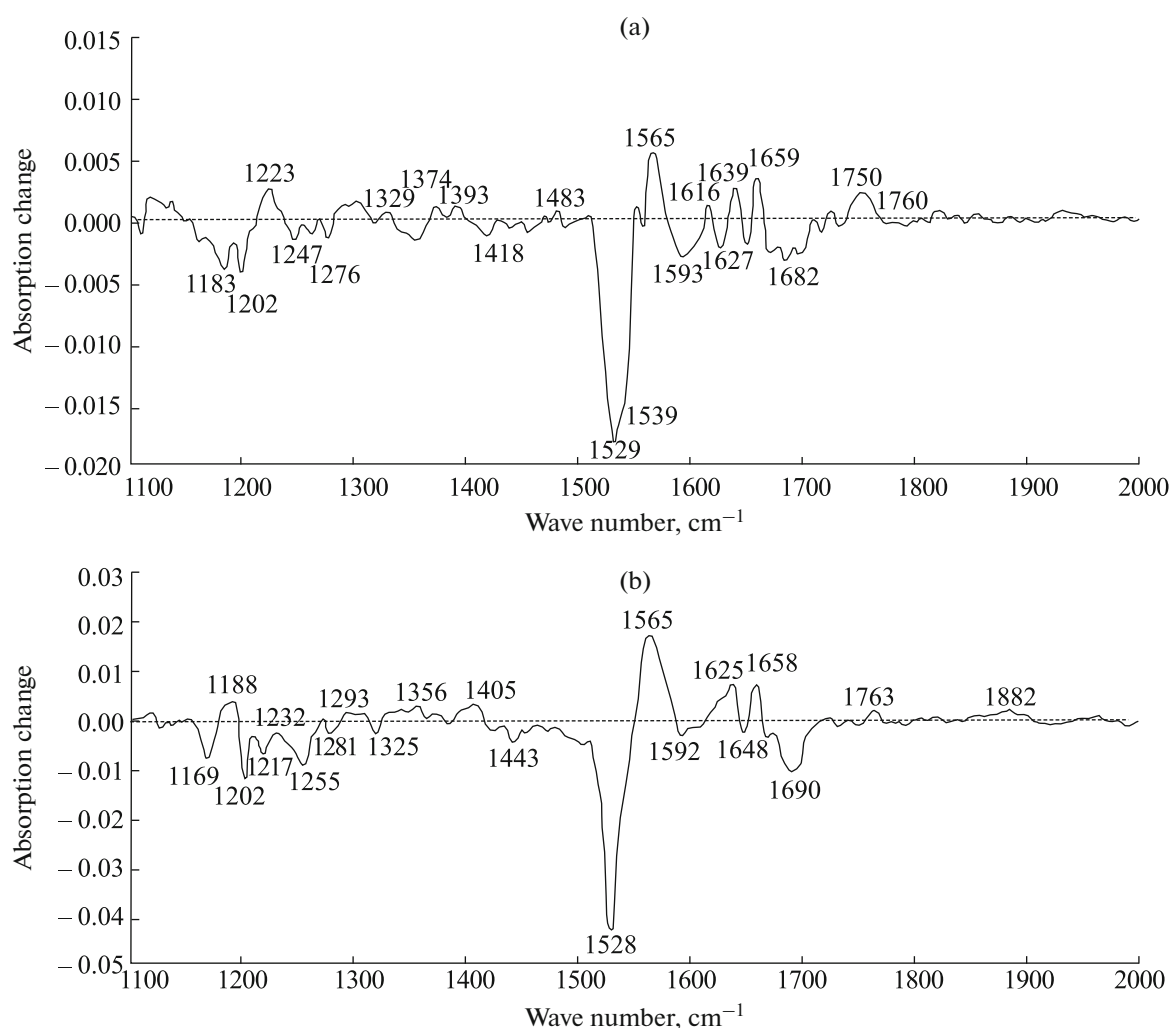
**Fourier transform infrared difference.** Although IR spectroscopy allows one to study the conformation and dynamics of the protein, in practice it is difficult to determine local structural changes in the protein core. This problem arises from the strong overlapping between vibrational modes of many identical groups in the protein. As an example, the Amide I ( $1620\text{--}1690\text{ cm}^{-1}$ ) and Amide II (approximately  $1550\text{ cm}^{-1}$ ) bands (whose exact frequency depends on the secondary structure of the protein), as well as absorption of side chain amino-acid residues, such as aspartate, glutamate, tyrosine, tryptophan, lysine, and arginine

(which absorb in the Amide I and Amide II band regions [28–31]) contribute to the  $1700\text{ cm}^{-1}$  and  $1500\text{ cm}^{-1}$  regions in the IR spectrum. Vibrations of the chromophore can also fall into this region. In this case, protein absorption will dominate in the typical infrared absorption spectrum of the pigment–protein complex in the mid-infrared range, while the absorption of chromophore molecules usually makes only a very insignificant contribution ( $\sim 10^{-3}$  to  $10^{-4}$ , depending on the size of the protein). Therefore, to obtain information about the chromophore or other components of the biological system (proteins, membranes, and so on) involved in the given photoreaction, a new method, that is, Fourier transform infrared difference IR spectroscopy, was developed in the 1980s. It was first applied to bacteriorhodopsin [41–43]. Recently, this method has been especially widely used for the study on early photophysics, energy transfer processes, and molecular mechanisms of functioning for photosystems of various complexities (see, for example, reviews [44, 45] and references therein).

The main experiment consists in comparing two IR spectra recorded before and after this reaction (see Fig. 1). All of the vibration bands that belong to molecular groups that participate in the reaction will appear in the difference spectrum, while all other vibrations will be suppressed. As a result, the sensitivity of the method manifests itself at the level of individual molecular groups and even chemical bonds. The information obtained in the difference spectrum relates to important events such as protonation/deprotonation of the side chain of one amino acid, oxidation/reduction of a pigment or cofactor, displacement of a water molecule, and conformational changes in a chromophore or protein. This information is also structural in nature: for example, in the spectra one can observe not only the formation of a hydrogen bond, but also obtain a qualitative estimate of the bond strength [44, 45].

With the development of step-scan Fourier transform IR spectrometers, the state of a biological system can be monitored with a time resolution of nano- to femtoseconds [46–48].

As mentioned above, the first studies using Fourier transform infrared difference IR spectroscopy were performed on bacteriorhodopsin. These studies allowed determination of the involvement of individual groups at different stages of the photocycle [49, 50]. In many respects, the successful practical application of this method was facilitated by the development of new techniques related to sample preparation. In addition to isotope labeling and hydrogen–deuterium exchange, special protocols such as the genetic substitution method or site-directed mutagenesis [51], as well as isotope selective staining of side chain amino-acid residues or internal water molecules [52–54], were developed. In this way, catalytically important



**Fig. 1.** Fourier transform infrared difference spectra between the initial form of bacteriorhodopsin BR<sub>568</sub> and M<sub>412</sub> form recorded at a temperature of 190 K and the relative humidity of films of 0% (a) and 93% (b). The spectra were recorded in the single-channel mode using mercury–cadmium–tellurium (MCT) detectors cooled with liquid nitrogen, with the accumulation of 600 scans and a spectral resolution of 4 cm<sup>-1</sup>.

amino acids in rhodopsins were identified [55–58]. In this regard, the specificity of sample preparation for Fourier transform infrared difference IR spectroscopy deserves separate consideration.

First, one should consider the fact that the molar extinction coefficients of vibrational modes are much smaller than those of electronic transitions. This leads to the need to use very concentrated samples. In addition, given that the envelope of the water absorption band strongly masks important bands of the protein components, it is necessary to reduce the water content while providing the preservation of the nativeness of the protein or membrane and its biochemical reactions under a change in the hydrated state. More serious problems arise in the study of water-soluble proteins. In order to solve this problem, it was proposed to use samples in the form of pressed tablets using potassium bromide powder, which is transparent in the IR

region of the spectrum. This method of pressing tablets using potassium bromide was introduced in 1952 and is still widely used. It consists in thoroughly mixing a finely grinded sample with potassium bromide powder, pressing it, and forming a translucent tablet. Despite the advantage of the absence of most interfering absorption bands, the possibility of changing the crystal structure of polymorphic substances during grinding and pressing of the tablets is among the disadvantages of this method. As well, due to the high hygroscopicity of potassium bromide it is not possible to completely avoid the presence of adsorbed water bands near 1640 and 3450 cm<sup>-1</sup> in the IR spectra. Chemical interaction of the sample substance with the tablet material is possible.

**The method of attenuated total reflection.** Since 1963, the IR reflection method, which is known as the method of attenuated total reflection (ATR), has been

used for the analysis of aqueous solutions of biological samples as an alternative to IR absorption [59]. The method of attenuated total reflection requires minimal effort for sample preparation; it also allows one to explore the surfaces of a wide variety of materials.

The ATR method is based on absorption of the incident radiation of a light wave emerging from the prism of total internal reflection, which is in optical contact with the studied surface, by the surface layer of the studied sample. The method was described in detail in [59]. Special accessories are required in Fourier transform IR spectrometers to obtain the ATR spectra. In this case, infrared spectra obtained by the ATR method are almost identical to the absorption and the determination of the secondary structure of proteins from the ATR spectra showed similar results. The method is also not without drawbacks. One of them is associated with serious errors in the interpretation of the spectra. The ATR method is often used to determine the orientation of proteins in the membrane.

**Fourier transform IR emission spectroscopy.** While these methods of Fourier transform IR spectroscopy, transmission (absorption), and reflection (attenuated total reflection) are most soundly and widely used in biological research, this cannot be said about the emission method, which is not used in biology.

Modern studies using Fourier transform IR emission spectroscopy (IRES) in most cases relate to the study of the spectra of natural and artificial light sources or laboratory samples excited by thermal methods [60–63]. In addition to the spectroscopy of celestial bodies studied in the field of astrophysics, there are two main areas of emission spectroscopy in laboratory conditions: atomic emission spectroscopy, as one of the most widely used methods of elemental analysis of substances, and IRES, which is focused mainly on thermal emissions of molecular systems. IRES can be applied to the analysis of a wide range of substances of organic and inorganic natures in the solid, liquid, and gaseous states, as well as to the analysis of substances such as bulk materials, surfaces, catalysts, and thin films and minerals [62, 63].

Although IRES has the potential to become a very sensitive, universal molecular specific technique for analyzing the molecular composition and structure of a substance, it is still rarely used in practice. One of the main reasons for this is the lack of specialized equipment that allows routine measurements. All measurements are carried out based on Fourier transform IR spectrophotometers, which have specialized accessories or a modified optical scheme, which allows adapting the device for emission experiments. These experiments are often state of the art [61]. The IRES method is in demand due to the lack of alternatives to its application in such scientific fields as astrophysics, chemical catalysis, and mineralogy [63]. Therefore, the IRES method continues to improve and develop.

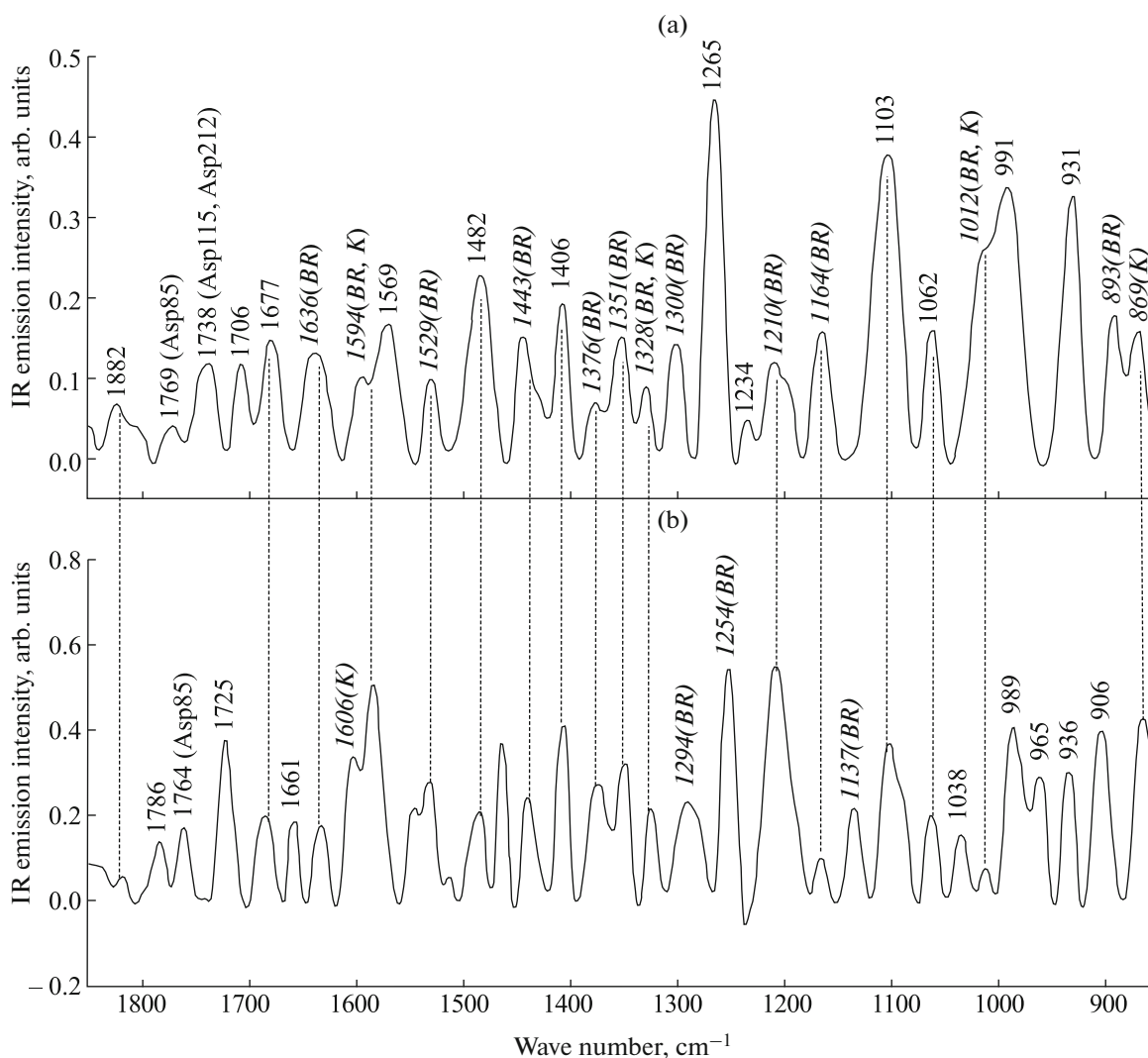
One of these achievements is the ability to use it in relation to biological samples, when direct contact heating is excluded, and emissions are excited by visible light with an energy that does not cause heating of the sample.

The IR emission spectrum, like the Raman or IR absorption spectra, contains detailed information on the chemical bonds that are present; therefore, it gives a representation on the chemical composition of the sample. The advantage of the method is that it has no restrictions for aqueous solutions, since water molecules do not have an IR signal that can mask important protein bands. Therefore, measurements can be carried out in aqueous solutions or directly on undried samples. The sample does not require special preparation, which allows one to study whole cells and even tissues [64]. The Amid I ( $1600\text{--}1700\text{ cm}^{-1}$ ) and Amid II ( $1480\text{--}1575\text{ cm}^{-1}$ ) spectral regions contain many well resolved bands, rather than single wide bands, which allows one to obtain more detailed information about studied processes. However, the analysis of emission spectra includes spectral regions that have rarely been studied thus far ( $>1800\text{ cm}^{-1}$  and  $<1000\text{ cm}^{-1}$ ). Their study in photosensitive proteins is becoming increasingly popular (see, for example, [65–67]) (Fig. 2). As a rule, in the IR absorption or differential spectra, the spectral window between  $1800$  and  $1900\text{ cm}^{-1}$  is devoid of any spectral contribution, while well resolved peaks occur in this region in IR emission spectra (Fig. 2) Their identification was carried out in experiments using model compounds of retinal and amino acids.

Figure 2 illustrates the ability of the method to obtain information on chromophore retinal and protein components of bacteriorhodopsin in aqueous solution and wet films. The identification of bands related to retinal was carried out based on the comparison with the data of Raman scattering and IR absorption of the model retinal compounds. Identification of protein bands was carried out based on the analysis of the spectra of amino acids used as models. It turned out that under nonresonant conditions, amino acids, like retinal, are able to generate infrared radiation in the mid-IR range under the influence of visible light. This may be important in deciding the origin of vibrational excitation in opsin in the primary photophysics of bacteriorhodopsin.

Detailed description of IR emission experiments based on a FS-02 Fourier transform IR spectrometer was presented earlier [68]. IRES combines the use of a visible light source and Fourier transform IR adsorption technique to record IR emission spectra in a wide IR range. In this case, the standard source of IR is replaced by the sample itself, as shown schematically in Fig. 3.

As one can see, the spectrum consists of two components: a wide smooth background curve and separate sharp peaks located on its background. The real



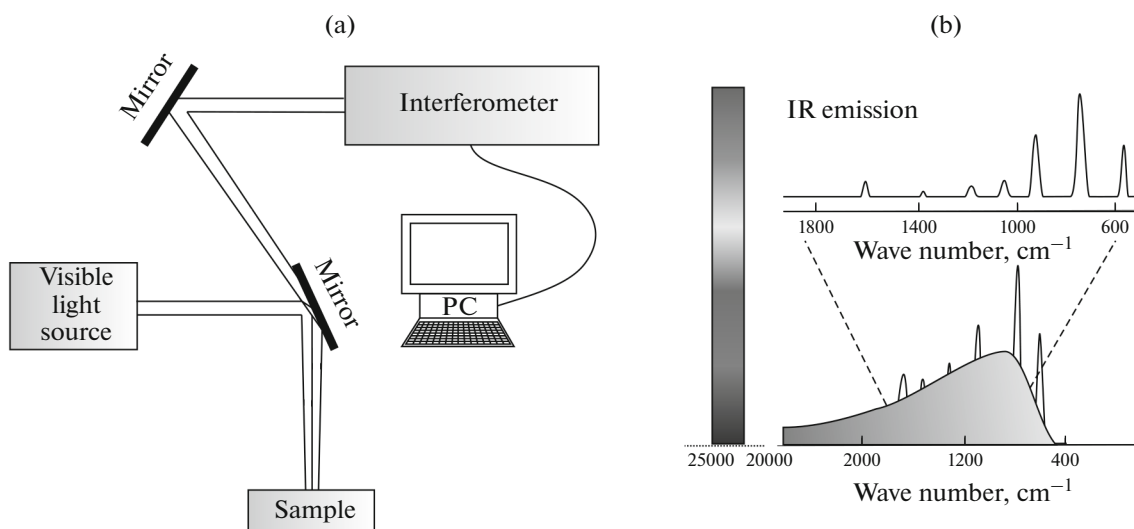
**Fig. 2.** The IR emission spectrum of bacteriorhodopsin in a film (a) and an aqueous solution (b). The spectra were recorded using visible light that resonates with the electron absorption band of the retinal chromophore ( $\lambda_{\max} = 568$  nm), thus providing selectivity of the process with respect to the retinal chromophore. The spectra were recorded at room temperature with accumulation of 400 scans and spectral resolution of  $4\text{ cm}^{-1}$ . Vibrations of the retinal chromophore ( $\text{cm}^{-1}$ ) are indicated in italics; protein vibrations are indicated in a normal font.

IR emission spectrum of the sample was obtained by subtracting the broad component of the background radiation using procedures of smoothing and fitting with a 25th order polynomial by the Origin 6 computer program. The spectra were recorded with the maximum level of useful IR signal and the minimum influence of background radiation from heated surfaces. The optimal conditions for recording the spectra were set by the software. Visible light in the form of stray light was cut off by optical filters and the germanium filter, which were set on the input window of the IR detector.

The fact that bacteriorhodopsin can emit IR radiation in the mid-IR range ( $4000\text{--}400\text{ cm}^{-1}$ ) under the conditions of resonance absorption by retinal chromo-

phore [68–70] has also been confirmed by other authors [71–74].

In the course of the study, we showed that multifrequency (broadband) visible light can excite stimulated IR radiation in pigments according to a mechanism of the combination type in addition to intrinsic fluorescence [70, 75, 76]. In this case, as in stimulated Raman scattering, IR emission is observed with the same resonance conditions that occur when optical waves are mixed in a nonlinear medium [77–80]. The possibility of a nonlinear optical mixing process using an incoherent source was shown as early as 1965 [81]. Although the probability of such a process is not large, especially when using low intensity light sources, the sensitivity of modern Fourier transform IR spectrometers is sufficient to reliably record IR emission spectra



**Fig. 3.** (a) The scheme of device used for emission excitation; (b), a schematic representation of recorded spectra. IR emission spectra are recorded using low-temperature mercury–cadmium–tellurium detector cooled with liquid nitrogen.

under these conditions. Spectra of stimulated IR emission can be obtained under nonresonant and resonant conditions. In the latter case, one can obtain a significant signal gain (by more than ten times) [82]. The structural information extracted from the IR emission spectra is similar to that extracted from the IR absorption and Raman spectra, since the same vibrational modes are observed in both cases [61, 83].

This paper presents the results of experiments on the selective excitation of individual reporter groups in lysine in order to demonstrate the capabilities of the method for obtaining information on energy states and mechanisms of absorbed energy dissipation.

In these experiments, a globar, whose radiation was passed through infrared narrow-band filters, was used as an external light source (Fig. 4). Lysine samples were used as films.

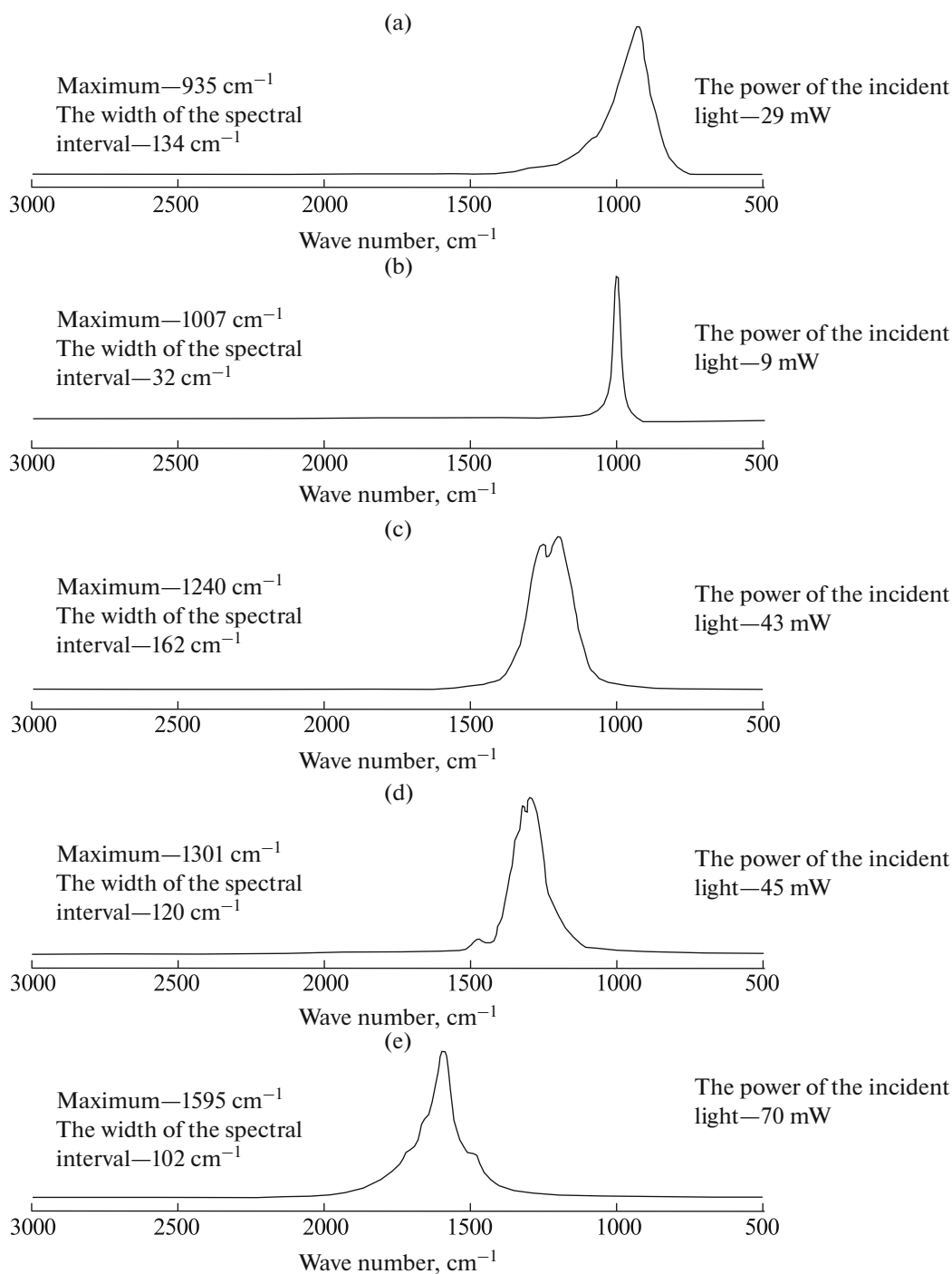
The linear structure of the lysine molecule showed a noticeable sensitivity to the frequencies of excitation light. The resulting excess of vibrational energy in lysine was not directly reradiated by the accepting mode, but was redistributed between the modes. The nature of the redistribution of the obtained excess of vibrational energy and the value of the IR emission signal depended not so much on the magnitude of the received energy excess, but on the specifics of the accepting mode itself (see, for example, Fig. 5), which, apparently, is determined by the nature of the intramolecular interaction between the modes.

Thus, when using a bandpass filter that maximally transmits at  $936\text{ cm}^{-1}$  in the spectral interval of  $134\text{ cm}^{-1}$  wide in the IR emission spectrum (Fig. 5a), a number of bands are observed, the most intensive of which are near  $669\text{ cm}^{-1}$  ( $\text{COO}^-$ )def.,  $794\text{ cm}^{-1}$  ( $\text{COO}^-$ )def.,  $1221\text{ cm}^{-1}$  ( $\text{C-OH}$ )def.,  $1336\text{ cm}^{-1}$

( $\text{CH}_2$ )def. and  $2826\text{ cm}^{-1}$  (stretching ( $\text{CH}$ )CH vibrations). At the same time, when using a bandpass filter that transmits at the maximum of  $1007\text{ cm}^{-1}$  in the spectral range of  $32\text{ cm}^{-1}$  wide, where stretching vibrations of the C–C group are the accepting mode, one can observe bands in the IR radiation spectrum (Fig. 5b), the most intensive of which are near  $674\text{ cm}^{-1}$  ( $\text{COO}^-$ )def.,  $1600\text{ cm}^{-1}$  ( $\text{NH}_3^+$ )<sub>as</sub>def.,  $1884$ ,  $2280$ , and  $2882\text{ cm}^{-1}$ . The high frequency bands of  $1884$ ,  $2280$ , and  $2882\text{ cm}^{-1}$  fall into the region of combination frequencies and overtones of stretching vibrations of C–H groups. Although in the second case the incident light power was almost three times lower, the intensity of the IR spectra (Figs. 5a and 5b) was almost the same.

However, when using a bandpass filter that transmits that maximum radiation at  $1240\text{ cm}^{-1}$ , in the  $162^{-1}$  wide spectral range, the IR spectrum intensity (Fig. 5c) was lower at a higher incident light power. In this case, the probe beam fell into the region of torsional  $\text{CH}_2$  groups. In the IR spectrum, bands that have a different relative intensity distribution compared with the spectra (Figs. 5a and 5b) were observed. Among these, the most intensive are the bands near  $674\text{ cm}^{-1}$  ( $\text{COO}^-$ )def,  $821\text{ cm}^{-1}$  (not assigned),  $911\text{ cm}^{-1}$  (not assigned),  $1186\text{ cm}^{-1}$  ( $\text{CH}_2$ )def,  $1401\text{ cm}^{-1}$  ( $\text{COO}^-$ ), a wide band with a maximum near  $1860\text{ cm}^{-1}$  and a narrow band  $2123\text{ cm}^{-1}$ . These two high frequency bands fall into the region of combination frequencies and overtones of stretching vibrations of C–H groups. Similarly, the spectra in Figs. 5d and 5e show a large set of bands for which the relative intensity distribution was different from what was observed above. It is interesting that in all the cases



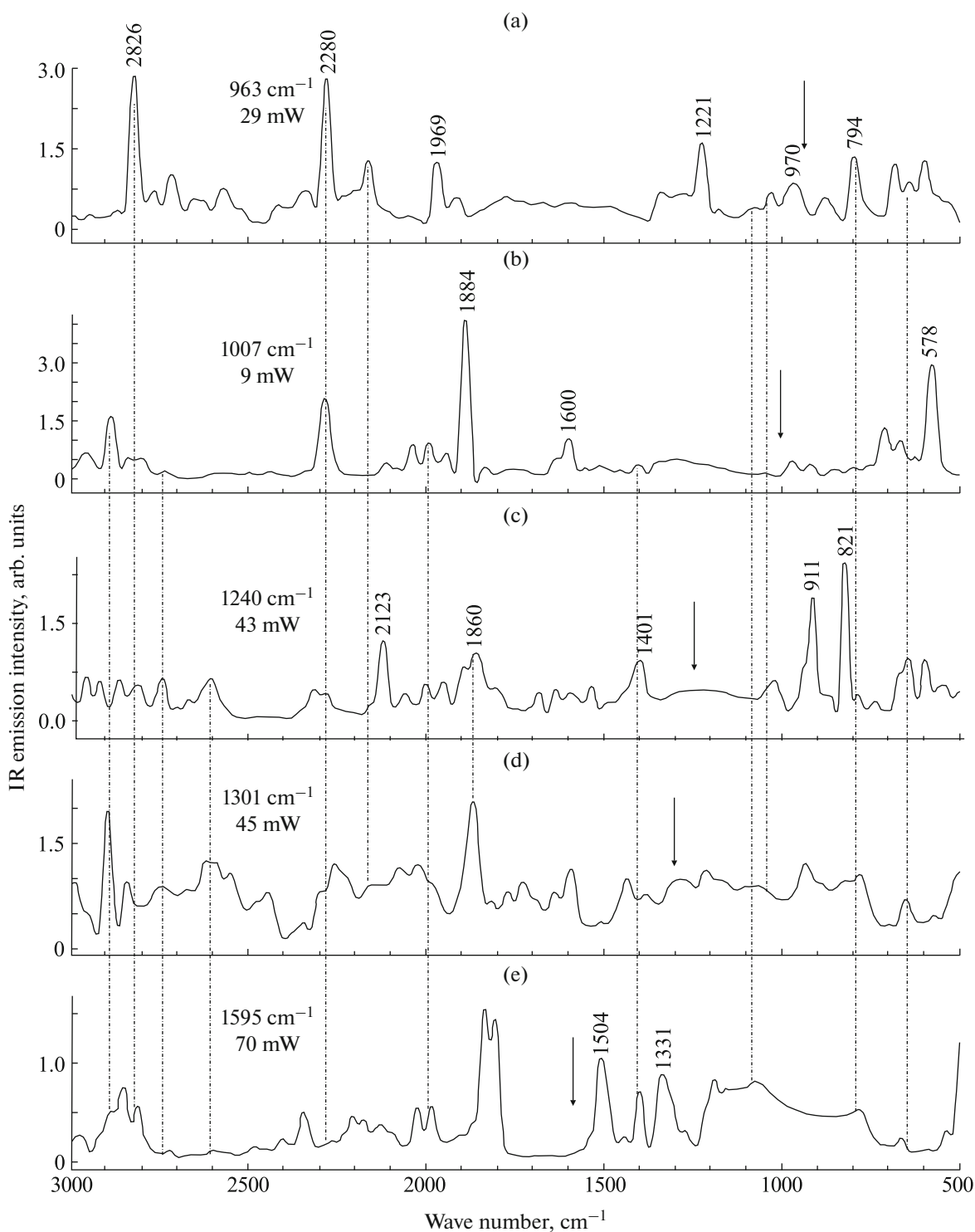


**Fig. 4.** The characteristics of IR filters used to excite lysine molecule at different regions of the IR spectrum.

considered, the scattering of excess of vibrational energy occurred predominantly with the participation of CH and COO groups, rather than  $\text{NH}_3^+$ . In this case, one can see in the spectra that energy transfer occurs not only in the direction from high-frequency higher energy vibrations to lower frequency low-frequency vibrations, while in contrast, energy is transferred from low-frequency vibrations to higher-energy

high-frequency vibrations. It is interesting that in all the considered cases, scattering of vibrational energy occurred predominantly with the participation of CH and COO groups, rather than  $\text{NH}_3^+$ . At the same time, one can see in the spectra that energy transfer occurs not only in the direction from high frequency higher energy vibrations to low frequency vibrations that are lower in energy, while in contrast the energy is trans-





**Fig. 5.** The IR emission spectrum of L-lysine hydrochloride in the range of 2000–700  $\text{cm}^{-1}$ , recorded under illumination in narrow regions of the mid-IR range. The assignment of bands in the spectrum was carried out based on the data of [5, 31, 84–86].

ferred from low frequency vibrations to high frequency vibrations that are higher in energy. In fact, in these experiments, the movement of energy was observed not only downstream in the lysin molecule, as with the stepwise mechanism, but also upstream, which is pos-

sible only if there is a close anharmonic interaction between the modes [87–89]. Due to this, excitation of any local oscillation was able to activate a sufficiently large number of vibrational modes, whose frequencies are found in a wide spectral range, including the

region of fingerprints, the low frequency region, and the region of overtones. This result appears to be especially important, since it shows that a vibrational excitation of the equilibrium structure of the molecule of small energy is quite sufficient to change the mobile geometry of lysine, involving it in many different vibrational movements, many of which could occur with a large amplitude. The participation of a large number of vibrations in the relaxation process is a reflection of the ease of overcoming energy barriers in the energy distribution and the high mobility of the structure of the lysine molecule in the vibrationally excited state. Lysine is present in almost all of the active centers of proteins and enzymes and such lability of its structure is of great importance for the manifestation of the biological activity of lysine.

### CONCLUSIONS

The results of this study showed that Fourier transform IR techniques, which have the ability to observe weak IR emissions, provide a simple and at the same time sensitive tool for obtaining chemical and structural information about biological molecules. Fourier transform infrared emission spectroscopy as a biophysical method can be especially useful in studying the mechanisms of photophysical and biochemical reactions.

### COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that they have no conflict of interest. This article does not contain any studies involving animals or human participants performed by any of the authors.

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