

# Usefulness of spectroscopy for biomedical engineering

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Modifications of phenylalanine amino acid after its exposure to near-infrared (NIR) radiation have been investigated using ATR-FTIR (Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy). The process of amino acid aggregation after its exposure to NIR has been observed. A possible mechanism of amino acid dimer formation has been proposed with the help of theoretical calculations of quantum mechanics (MP2 and B3LYP/6–31 G\* level) using the GAUSSIAN 03 package. The usefulness of spectroscopy for biomedical engineering is discussed. ATR-FTIR appears to be a powerful tool for measuring tissue damage in aqueous environments.

*Key words:* ATR-FTIR, quantum mechanical calculations, NIR, L-phenylalanine, amino acid, conformational structure, aggregation, biomedical engineering

## 1. Introduction

The biological functions of amino acids are often dependent upon the conformation that the molecule can adopt. Conformational studies of amino acids can help to understand the reactivity of biological molecules and the mechanisms of processes taking place at the molecular level. Thus, there have been a lot of experimental and theoretical studies on biomolecular conformation of amino acids at the molecular level in various physical states [1]–[4].

Conformational structures seem to be important in the photophysical and photochemical effects of light that influences significantly our life. Light is indispensable for all organisms living in the soil, water and air. It influences many different molecular processes, causing changes in biological systems. The aim of our global study is to explain the mechanism of Near Infrared (NIR) light action, the character of changes of

amino acid and protein molecules at the molecular level.

NIR radiation is used both in medical therapy and diagnostics. Studies of the electromagnetic (i.e. infrared) radiation influence on biological systems are performed now in many scientific laboratories all over the world. NIR light is known to accelerate wound healing processes, to reduce inflammations, to support central nervous system regeneration, to promote vascular and lymphatic microcirculation, to stimulate the immune system, to reduce and control pain [5]–[7].

Between 600 and 1200 nm there is the so-called “therapeutic window” which is used in phototherapy. In this region, optical parameters of tissues are most “visible”. Infrared radiation is weakly scattered by spatial structures, which allows penetration of light in tissues. 600–1200 nm radiation is absorbed mainly by such tissue components as: water, glucose, haemoglobin, melanin, lipids, amino acids, proteins, and nucleic acids. It should be noted that the radiation

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influence on tissues and whole organisms is a result of reactions at the cellular and molecular level. Therefore, the research into radiation effects at the molecular level is so important [8]. In spite of many advanced studies in scientific laboratories and clinics, the molecular mechanisms caused by NIR have not yet been established. Thus, further studies are advisable.

It can be concluded from our previous results that the primary effect of NIR upon biological objects (from single molecules to whole tissues) is dehydration. This hypothesis is supported by our studies on whole cells (erythrocytes and liposomes) and single molecules or macromolecules [9]–[13]. Therefore, it is suggested that every biological structure undergoes the process of dehydration. Simultaneously modifications of water structure lead to protonation and secondary processes: aggregation and conformational changes [10], [14]. These effects induce physiological response of living organisms. For example, water activity plays a crucial role in metabolic processes, kinetical mechanism of allosteric regulation in proteins, the photochemical dissociation of oxyhaemoglobin to deoxyhaemoglobin [15], intramolecular proton transfer and isomerization process [16], which is confirmed by our experimental and theoretical studies [8], [10]–[13].

In order to explain in detail the structural processes taking place after the exposure to NIR, a simpler compound, i.e. phenylalanine amino acid, has been studied. A specially constructed system of NIR radiation of 700–2000 nm has been used in the experiments. ATR-FTIR spectroscopy has been chosen as an experimental technique, because it is one of the most promising methods for diagnostic studies at the molecular level, simultaneously allowing us to understand global processes. At the same time, quantum mechanical calculations have been performed. The application of different methods guarantees the complementarity of the results obtained and allows us to confirm the changes occurring in model biological systems.

## 2. Materials and methods

### 2.1. ATR-FTIR spectroscopy

All the ATR-FTIR spectra of L-phe aqueous solutions were measured between 700 and 4000  $\text{cm}^{-1}$  with a triangular apodization function using the Perkin-

Elmer 2000 spectrometer. The spectrometer was supplied with an ATR accessory [17]. Samples were analyzed by ATR using a diamond crystal prism (the ATR crystal has a triangular geometry with mirrored angle faces). The sample cell was purged with a current of dry air and decarbonated air provided by an appropriate compressor. All spectroscopic measurements were performed at  $21 \pm 1$  °C with 4  $\text{cm}^{-1}$  spectral resolution and 20 scan averaging. All experiments were repeated at least three times before validating the results.

### 2.2. NIR procedure

The ATR-FTIR spectra of L-phe aqueous solutions were recorded before and after NIR radiation. To investigate the effect of NIR, L-phe aqueous solutions of different pH values were exposed to the radiation of a halogen lamp equipped with a 700–2000 nm filter. Samples were kept in a glass-closed cell and light was focused on a flat glass tube containing the solution. The power density of the incident light was 6.9  $\text{mW cm}^{-2}$ . During the exposure the solution was gently stirred at 1000 r.p.m. by a magnetic mixer, model BMM 21. The irradiation temperature of  $21 \pm 1$  °C was kept constant by means of an additional water-cooling system. The samples were irradiated for 15 minutes. The effects of NIR radiation were measured. The effects of NIR radiation increased with irradiation time. After 15 minutes the effects measured were stable. When the lamp emitting irradiation was switched off, during the first 5 minutes the infrared spectra of L-phe were collected.

### 2.3. MP2 and B3LYP calculations

The calculations were performed using the Gaussian 03 software package [18]. Several basic sets have been selected for this study. Some of them contain both polarization and diffusion functions, as we have recognized the need to include these functions in the basic set for calculations on hydrogen-bonded systems [19].

### 2.4. Data treatment

All the spectra were smoothed using the Savitzky–Golay algorithm with 17 points [20] and were baseline corrected by using the scattering correction method of the OPUS Bruker software.

### 3. Results and discussion

Aqueous solutions of phenylalanine amino acid were irradiated by Near Infrared Radiation (NIR). The ATR-

FTIR spectra of phe after NIR radiation demonstrate modifications due to intermolecular interactions and water solvation (figure 1). The results of our research show clearly that modifications of water structures as a result of their exposure to NIR weaken interactions be-

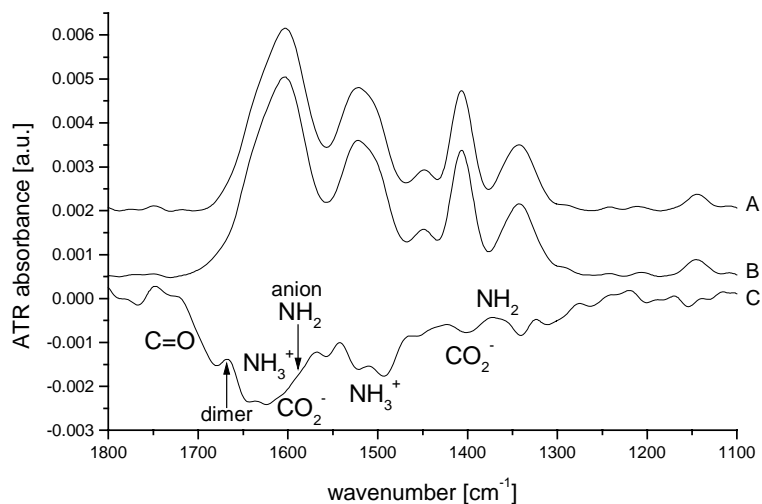


Fig. 1. ATR-FTIR absorption spectra of L-phenylalanine in aqueous solution: after NIR radiation (A) and before NIR radiation (B) in the 1,100–1,800  $\text{cm}^{-1}$  region. Difference spectrum (A – B) (C); the spectrum was multiplied by 5.5

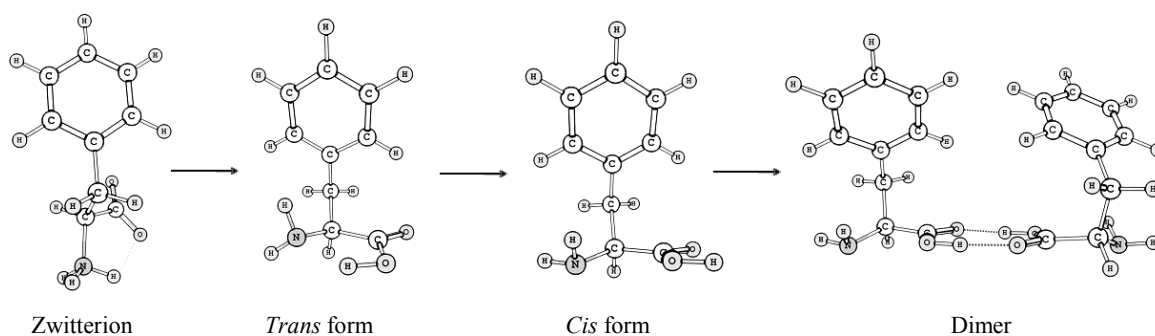


Fig. 2. Possible mechanism of dimer forming due to exposure to NIR, exemplified by phenylalanine amino acid

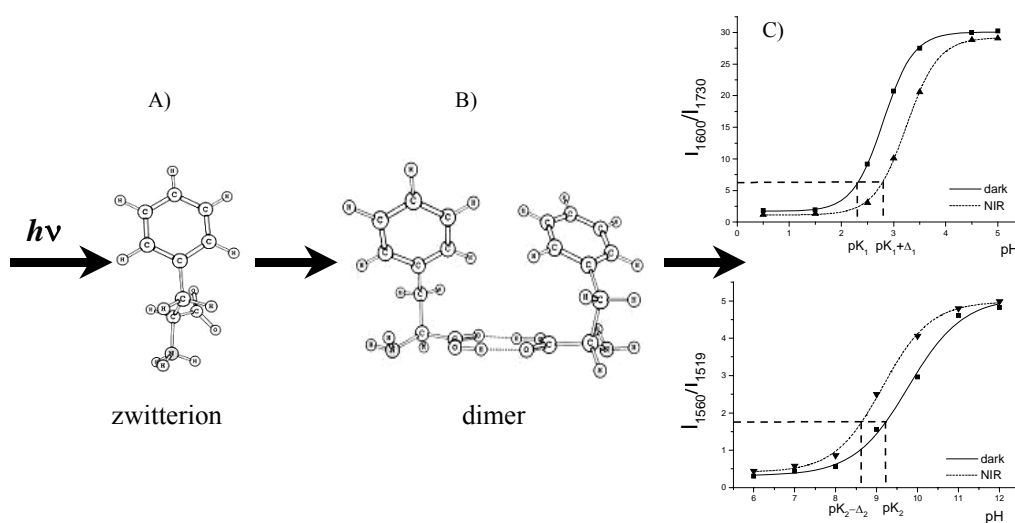


Fig. 3. Effect of propagation of waves from NIR range on phenylalanine (A), aggregation of amino acid (B), and shifts of curves representing acid–basic equilibrium (C)

tween polar groups of phenylalanine amino acid, which is also confirmed by our previous studies [8]–[11]. After NIR irradiation an intramolecular proton transfer reaction occurs and the concentration of neutral groups increases, resulting in the formation of strong hydrogen bond between the carboxylic (–COOH) and carboxylate (–CO<sub>2</sub><sup>–</sup>) groups. In these conditions, dimerization can appear (figure 1). Additionally, characteristic bands of amino acid obtained in the ATR-FTIR spectra were highlighted and pK<sub>a</sub> values were determined from IR measurements by using the intensity ratio method [1].

The process of phenylalanine aggregation due to its exposure to NIR has been observed using ATR-FTIR spectroscopy. Additional quantum chemical calculations allowed us to propose the possible mechanism of dimer formation using the example of phenylalanine (figure 2). The interaction between two identical molecules of the amino acid involves hydrogen bonding –C=O···HOOC–, forming cyclic dimers (figures 2 and 3B), analogous to dimers formed by carboxylic acids. However, before dimer formation conformational changes of the amino acid should probably occur (figure 2). Based on the literature *trans/cis* isomerization is most possible. The *trans* form is energetically more stable than the *cis* one, but due to the entropy-effect of association, the *cis* form can be dominating as it results in two hydrogen bonds.

As a consequence of the aggregation process pK<sub>a</sub> values of the amino acid are evidently shifted (figure 3C). [21] The presence of hydrophobic groups can influence these effects. The increase of hydrophobic interactions after NIR radiation favours the aggregation in biological systems, which can result in metabolic processes at the molecular level.

## 4. Conclusions

The results of our research evidently show that Near Infrared Radiation can really be effective in light therapy or medical diagnostics. It is possible to monitor processes in biological systems using spectroscopic methods, namely infrared spectroscopy induced by NIR can play an important role in many processes taking place in tissues and at the molecular level (i.e. transport of hydrophilic and hydrophobic pharmaceuticals by liposomes; interactions of these systems with cancer cells; metabolic processes – influence on enzymes activity, condensation of amino acids etc.). Therefore, the processes induced by NIR can lead to serious consequences both in medicine and biomedical engineering.

Spectroscopic methods, especially vibrational spectroscopy (infrared, Raman), are spectroscopic techniques highly specialized to investigate systems, not only biological. They have versatile applications, e.g. in physics of solid states, studies of biophysical and mechanical properties of tissues (i.e. skin), and advanced diagnostic methods in medicine and biomedical engineering. They allow us to investigate crystal structures, vibrational states of various systems in different physical states, activity of surface states, a very sensitive analysis of composition (including monitoring) and very modern diagnostic methods. Based on the changes in spectral parameters of biological systems it is possible to know the processes taking place in tissues at molecular level. It should be stressed that spectroscopic methods are designed for *in vivo* and *in vitro* studies. ATR-FTIR spectroscopy appears to be a powerful tool for measuring changes in tissues, single cells and small molecules.

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