FOURIER TRANSFORM INFRARED SPECTROSCOPIC STUDY ON LIVER OF FRESHWATER FISH *OREOCHROMIS MOSSAMBICUS*

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Abstract. This investigation was aimed to determine and investigate the bimolecular composition of the liver of freshwater fish *Oreochromis mossambicus* by using Fourier transform infrared spectroscopy. The spectrum of liver tissue is quite complex and contains several bands arising from the contribution of different functional groups belonging to proteins, lipids and other biomolecules. The spectral analysis showed variations in composition of bio molecules of the liver samples at a wave number region of 4000–400 cm^{-1}. These are observed in different liver samples of *O. mossambicus.* The total protein content of the liver samples was found to lie between 12.42 μg/μL and 18.5 μg/μL.

Key words: FTIR, *Oreochromis mossambicus,* AAS, UV spectroscopy, liver, biochemical content.

INTRODUCTION

Infrared spectroscopy is a powerful method for studying molecular structure and intra molecular interaction in biological tissues and cells [18]. Several authors have studied infrared spectroscopy on biological substances like muscle, liver, etc.: studies in primary, secondary and tertiary structure of nucleic acid and RNA in rats exposed to Gamma radiation through FTIR spectroscopic studies [9], FTIR study of the influence of *Tribulus terrestris* on mercury intoxicated mice [10], study the effect of streptozotocin (STZ) induced diabetes on rat liver and heart tissue using FTIR spectroscopy [7]. The biological macromolecules provide us the most sensitive expression of the relationship between molecular structure and chemical and physical properties of a substance [21]. Fish tissue, especially the liver and kidney, is endowed with an antioxidant defense system consisting of CAT, a superoxide dismutase to protect them from oxidative stress caused by metals [19]. IR spectroscopy is a promising technique both to define the biochemical basis of

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cell viability more clearly with quantitative information about chemical functional groups in cells and to identify those characteristics specific to viable cells [10]. FTIR allows measurement of the entire spectrum simultaneously, providing a means to collect spectral information accurately and rapidly [12].

The aquatic environments are particularly sensitive to the toxic contaminants, because a considerable amount of the chemicals used in industry, urbanization, and agriculture enters the marine and other aquatic environments [3]. The discharge of potentially toxic race metals into the marine and fresh water environments has become a global problem. Continuous exposure of fresh water organisms to a low concentration of heavy metals may result in bioaccumulation, causing changes in several liver enzymes [20]. Fish, as living bioindicator species, play an increasingly important role in the monitoring of water pollution, because they respond with great sensitivity to changes in the aquatic environment [1, 16].

Fourier transform infrared spectroscopy has been used extensively to probe structural changes in proteins and lipids [2, 4]. In the present paper, attempts have been made to study the bio molecular composition in liver of the freshwater fish *Oreochromis mossambicus* by using Fourier Transform Infrared Spectroscopy.

MATERIALS AND METHODS

FISH SAMPLE COLLECTION

Adult freshwater fish *O. mossambicus* [length 16±1 cm, weighing about 200±1 g] were procured from the fish habitat with the help of fishermen by using a dip net located near the Nanjangud industrial area in Kapila River, Mysore district, Karnataka, India.

SAMPLE PREPARATION

After collecting the fish it was brought to the laboratory and the liver of the fish was dissected. The separated liver was cut into small pieces. All these steps were carried out on ice. The separated liver tissue is homogenized and quickly subjected to protein analysis followed by the UV method at 280 nm.

TOTAL PROTIEN ESTIMATION

Total protein was estimated according to the UV method, 280 nm [21]. Collected samples were stored in the cold saline before analysis, after chopping the liver a sample was transferred into the homogenizer for homogenization (30 min) after homogenization samples were transferred into the micro tubes for centrifugation at 10,000 rpm for 20 min at 2°C. Lytic buffer is used for chopping and homogenization. The absorbance of the complex was measured at 280 nm.

IR SPECTROSCOPIC ANALYSIS

The whole liver tissue samples of each group were isolated. The isolated whole liver tissue samples were lyophilized and made into fine powder. The tissue powder samples and Kbr (all dry solid state) were again lyophilized in order to remove most bound water, which might interfere with the measurement of amide I, band. 5 mg of liver tissue sample was mixed with 100 mg of dried Kbr and subjected to a pressure of 5×10^6 Pa and made into a clear pellet of 13 mm diameter and 1 mm thickness. The spectrometer was continuously purged with dry nitrogen. The absorption intensity of the peak was calculated using the base line method. Each observation was confirmed by taking at least three replicates. The spectra were recorded in the range of 4000–400 cm^{-1} using FTIR 460 plus Jasco. In the present study is possible to directly relate the intensities of the absorption bands of the corresponding functional groups.

RESULTS AND DISCUSSION

The present study was carried out to analyze the molecular structure and molecular composition of the liver of the freshwater fish *Oreochrmis mossambicus* using FTIR spectroscopy. The intensity and/ or more accurately the areas of the absorption bands in FTIR spectrum are directly related to the concentration of the molecules [6]. Figures 1–6 show the FTIR spectra of the fish liver tissue of *O. mossambicus* in the 4000–400 cm^{-1} range. The spectrum was normalized with respect to the amide A (3293 cm^{-1}) band as seen in Fig. 1.The spectrum is quite complex and contains several bands arising from the contribution of different functional groups belonging to protein, lipids and carbohydrates. The absorption band and assignment were shown in Table 1.

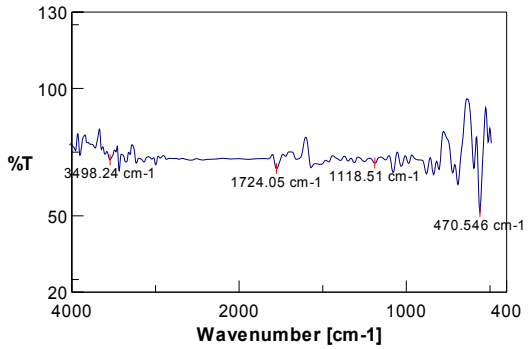

Fig. 1. FTIR spectra of liver tissue of *O. mossambicus* (sample 1) in the region of 4000–400 cm^{-1}.

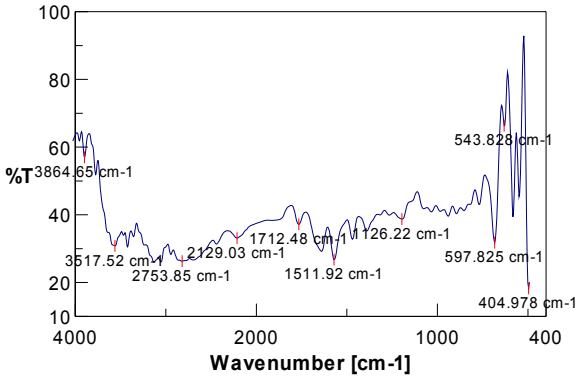

Fig. 2. FTIR spectra of liver tissue of *O. mossambicus* (sample 2) in the region of 4000–400 cm^{-1}.

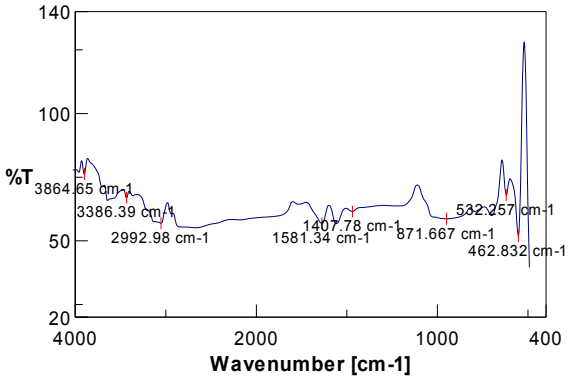

Fig. 3. FTIR spectra of liver tissue of *O. mossambicus* (sample 3) in the region of 4000–400 cm^{-1}.

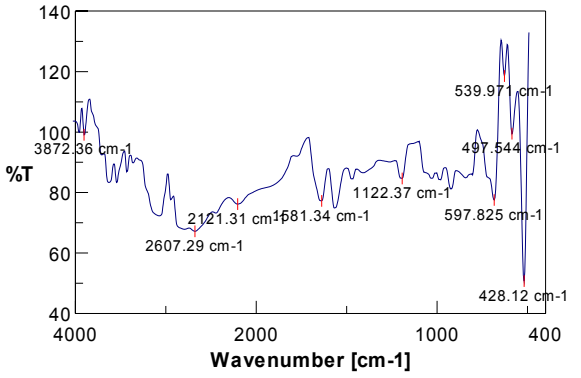

Fig. 4. FTIR spectra of liver tissue of *O. mossambicus* (sample 4) in the region of 4000–400 cm^{-1}.

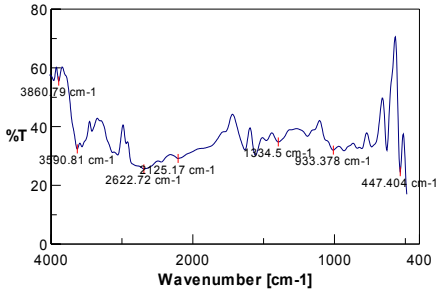

Fig. 5. FTIR spectra of liver tissue of *O. mossambicus* (sample 5) in the region of 4000–400 cm^{-1}.

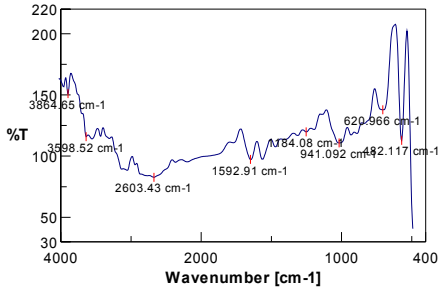

Fig. 6. FTIR spectra of liver tissue of *O. mossambicus* (sample 6) in the region of 4000–400 cm^{-1}.

Table 1

General band assignments of the FTIR spectra of liver tissues of freshwater fish *O. mossambicus* based on literature [4, 6]

S. No.	Wave number(cm^{-1})	Definition of the spectral assignments
1.	3293	Amide A: mainly N–H stretching of proteins
2.	3081	Amide B: N–H stretching of proteins
3.	2958	CH_3 asymmetric stretch: mainly lipids
4.	2926	CH_2 asymmetric stretch: mainly lipids
5.	2872	CH_3 symmetric stretch: mainly proteins
6.	2854	CH_2 symmetric stretch: mainly lipids
7.	1659	Amide I: mainly C═O stretching of proteins
8.	1534	Amide II: N–H bending and C–N stretching of proteins
9.	1451	CH_2 bending: mainly lipids
10.	1388	COO^-/ symmetric stretch: fatty acids and amino acids
11.	1230	PO_2^-/asymmetric stretch: mainly nucleic acids
12.	1170	CO–O–C asymmetric stretching: glycogen and nucleic acids
13.	1081	PO_2^- /symmetric stretch: mainly nucleic acids
14.	1044	C–O stretching: polysaccharides

The nutritional value of the different organisms depends on their biochemical constituents like proteins, carbohydrates, lipids, amino acids and minerals [10]. It is known that tissue proteins, carbohydrates and lipids play a major role as energy provider for organisms exposed to stress conditions [3, 11]. The infrared of the protein is characterized by a set of absorption regions known as the amide region and the C–H region. The most widely used modes regions are amide I, amide II and amide III. Amide I band arises principally from C=O stretching vibration of the peptide group. Amide II band is primarily N–H bending with a contribution from C–N stretching vibrations. The amide III absorption is normally weak and arises primarily from N–H bonding and C–N stretching vibrations. The amide absorption is considered sensitive to protein conformation; hence an increase or decrease in the ratio of the intensities of the band at 1541 cm^{-1} amide (II) and 1653 cm^{-1} (amide I) could be attributed to a change in the composition of the whole protein pattern. The ratio of the peak intensities of the bands observed 1541 cm^{-1} and 3297 cm^{-1} due to N–H bending and O–H stretching respectively could be used as indicators of the relative concentration of the protein to water of biological tissues [10, 22].

The overall spectral profile is similar except for the variation in intensities of the bands. The most widely used modes in protein structural studies are amide I, II and III. The broad band at 3297 cm^{-1} has been assigned in the present study to O–H stretching, the bands of proteins have made a small contribution to it. The bands observed at 2923 cm^{-1} and at 2853 cm^{-1} are due to the asymmetric and symmetric starching modes of the methylene chain in the membrane lipids. The sharp bands observed at 1653 cm^{-1} are assigned to the in plane C=O stretching vibration (amide) and to the C–N stretching/N–H bending vibration (amide II) of the protein respectively [17, 20]. The amide I band primarily associated with the stretching motion of the C=O group. This C=O band is sensitive to the environments of the peptide linkage and also depends on the proteins overall secondary structure [14]. And 1396 cm^{-1} are mainly due to asymmetric and symmetric CH_3 bending modes respectively of the methyl groups of protein. The medium intensity band observed at 1235 cm^{-1} is that of the PO_2^- asymmetric stretching modes of the phosphodiester indication of phospholipids and amide III/CH_2 wagging vibration from the glycine backbone and protein side chain [14, 12]. The depletion of protein profile induces diversification of energy to meet the impending energy demands during the toxic stress [8, 15]. Generally, the liver tissue stores the energy rich molecules, glycogen which is glucose polymer and glycogen exhibits absorption due to C–O and C–C stretching and C–O–H deformation motions with peak at 1080 cm^{-1} [12]. The bands between 1700 cm^{-1} and 1600 cm^{-1} in the spectra of liver tissues mainly stem from the amide I vibrational modes of tissue proteins and exhibit a high sensitivity to conformational changes in the secondary structure. The strong band observed at 1659 cm^{-1} can be assigned to α–helical structure. This amide I band is due to in-plane stretching of the C=O bond, weakly coupled with C–N stretching and in-plane N–H bending [6].

In the present study the total protein content of the fish liver was determined in all the samples. The tissue level protein represents the immediately available energy sources at the time of stress. It has been found that amount of protein in these samples ranged between 12.42 μg/μL and 18.5 μg/μL. In this study occurrence of such variations were obvious, maybe because of pollution stress in the fish habitat.

CONCLUSIONS

FTIR collects a rapid molecular fingerprint of tissue that consists of detailed infrared absorption bands. The band areas of symmetric and asymmetric CH_2 stretching modes observed in the tissues suggested the presence of lipids. The band areas and intensities of amide bands in tissue indicate the protein quantity of the system in the liver. The result further suggests that the major biochemical constituents such as lipids, proteins and nucleic acids can be easily evidenced by FTIR spectroscopy.

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