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FT-IR Study of Effect of Chromium on Tissue Protein of an Edible Fish *Cirrhinus mrigala*

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Fourier transform infrared spectroscopy (FT-IR) is a powerful technique for molecular conformations of proteins, lipids and nuclei acids as well an interaction of the complex materials comprising biological tissues. The present study is aimed to investigate the effect of chromium on tissue protein of an edible fish by FT-IR Technique. The spectra revealed significant differences in band intensities and intensity ratios between treated and control groups. A significant decrease in the protein content was observed in treated samples compared to the control. The amide I band observed at *ca.* 1657 cm^{-1} indicate that the protein is dominated by α-helical structure.

Key Words: Fish tissue, Chromium, Protein, FT-IR.

INTRODUCTION

Chromium is an essential element to life at low concentration, but toxic to many systems at higher concentrations. It is considered as a potent human carcinogen by IARC[1]. Of the various forms of chromium, the hexavalent is considered to be more toxic than the trivalent form because of its high oxidizing potential, high solubility and its ability to cross biological membranes[2]. It is released into aquatic systems from industrial sources such as the tanning industry[3], electroplating process[4] and burning of fossile fuels[5]. The main sources of chromium to the marine environment are wastes of metal finishing industries, dumping of solid wastes and municipal wastes[6]. This discharge of industrial effluents into the natural water bodies causes severe water pollution and affects the aquatic environment. Fish, a common source of protein contains greater quantity of protein than any other living organisms. Hence determination of protein and amino acid levels in various tissues of fish is considered to be paramount importance since the food value of fish is directly dependent on their protein concentration[7,8].

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The determination and understanding of the relationship between the structure and function of protein play an important role in the field of biochemistry. Substantial progress has been made in identifying the functional properties of proteins, while progress in determining structural information has been very limited. Fourier transform infrared spectroscopy is gaining recognition as a promising method in diagnostic medicine and biological studies and is used for studying molecular structure of protein[9]. It has been extensively employed in the study of biological systems, in order to obtain indicators about structural and chemical/physical properties at the molecular level[10]. The present paper has been aimed to study the effect of chromium on tissue protein of an edible fish, *Cirrhinus mrigala*, exposed to different concentration of chromium for a period of 28 d by FT-IR technique.

EXPERIMENTAL

Cirrhinus mrigala fingerlings of length (6 ± 1 cm and weight 8 ± 2 g) were collected form the freshwater bodies near local fish farm, Puthur, Tamil Nadu and acclimatized under laboratory conditions (29 ± 1)°C for 7 d. Boiled eggs, rice bran and earthworm pieces were used as feed on every alternate days. The LC_{50} for chromium (as $K_2Cr_2O_7$) for 96 h was determined by using Probit method[11]. The 1/10th and 1/3rd of LC_{50} were taken as lower (1.82 ppm) and higher (6.06 ppm) concentrations, respectively. The experimental animals were divided into the following five groups and each group consisted of 20 animals. (Group 1) control fish reared in pure water without any treatment, (Group 2) animals reared in water treated with chromium (1.82 ppm) for 28 d, (Group 3) animals reared in water treated with chromium (6.06 ppm) for 28 d and (Group 4 and 5) 10 animals were randomly selected from group 2 and 3, separately and kept in a pure water for further 28 d to assess any possible reversibility of chromium effects taken as recovery.

At the end of the specific experimental periods, fishes were sacrificed and muscle tissues were taken and kept in lyophilizer to remove water from tissues. The dried samples were powdered (approximately 5 mg) using agate mortar along with KBr and made pellet of diameter 13 mm. Nicolet Avartar 360 FT-IR spectrometer equipped with KBr beamsplitter and a DTGS detector at centralized Instrumentation and services laboratory (CISL), Annamalai university was employed in the present work for recording the spectra of the tissue samples. The spectrometer was continuously passed with dry nitrogen. For each spectrum 100 scans were loaded to give a spectral resolution of 4 cm^{-1}. The absorption intensity was calculated using base line method.

RESULTS AND DISCUSSION

FT-IR spectra recorded in the range 4000-400 cm^{-1} for control, lower and higher chromium treated samples along with their corresponding recovery. The spectra of tissues are consistent with the general features observed by Chiriboga *et al.*[12] and are dominated by the amide I band at *ca.* 1657 cm^{-1}, the amide II band, at *ca.* 1545 cm^{-1} and weaker amide III band at *ca.* 1240 cm^{-1}. In addition, a broad band centred around 3300 cm^{-1} due to contributions from amide II proteins and C-H stretching region which includes the CH_3 asymmetric stretching (*ca.* 2960 cm^{-1}), CH_2 asymmetric stretching (*ca.* 2926 cm^{-1}), CH_2 symmetric stretching (*ca.* 2873 cm^{-1}) and the CH_3 scissoring (1446 cm^{-1}) modes are also observed. These results are consistent with the observation made by Feride Severcan *et al*[13].

The amide I band observed at 1657 cm^{-1} is principally associated with the stretching motion of the (C=O) group. This band is sensitive to the environment of the peptide linkage and also depends on the protein's overall secondary structure. In the present study (Table-1), the amide I bands occur at 1657, 1655, 1652 and 1655 cm^{-1} for control, lower, higher and its recovery, respectively. The amide II band, mainly arises due to C-N stretching and N-H bending vibrations and are found at *ca.* 1545 cm^{-1}. The weaker proteins vibration of amide III bands occurs at *ca.* 1240 cm^{-1} and is normally due to C-H stretching and N-H bending motions. In the present study the amide II and amide III bands are observed in the region 1545-1538 cm^{-1} and 1240-1236 cm^{-1}, respectively.

TABLE-1
TENTATIVE IR BANDS (cm^{-1}) ASSIGNMENTS FOR CONTROL AND CHROMIUM TREATED TISSUES OF *Cirrhinus mrigala*

Control	Chromium Treatment		Recovery		Frequency assignment
	Lower	Higher	Lower	Higher	
3291 s	3295 m	3290 m	3294 m	3287 w	N-H amines and amide
2960 s	2961 m	2960 m	2961 m	2959 w	CH_3 asymmetric stretching lipid, protein.
2926 s	2928 m	2929 m	2928 m	2926 w	CH_2 asymmetric stretching mainly lipid, low signal from protein.
2873 m	2874 m	2875 w	2874 w	2872 w	CH_2 symmetric stretching
1657 s	1655 s	1652 s	1655 s	1655 m	C=O stretching (Amide I)
1545 s	1544 s	1541 s	1538 m	1538 w	C-N stretching/N-H bending (Amide II)
1446 m	1448 w	1449 w	1449 w	1451 vw	CH_3 scissoring, lipids
1240 w	1239 vw	1240 vw	1236 w	1237 vw	C-H stretching/N-H bending (Amide III)

The ratio of the intensities of the bands at 1540 and 1650 cm^{-1} due to amide II and amide I vibrations of protein components was suggested by Benedetti *et al.*[14] to analyse pure proteins in order to evaluate their denaturation state. Since the amide absorptions are sensitive to protein conformations, an increase or a decrease in this ratio could be attributed to changes in the composition of the whole protein pattern. In addition to amide ratio the peak intensities of the bands at 1550 and 3300 cm^{-1} (I_{1550}/I_{3300}) has been used as an indicator of the relative concentrations of proteins to water included near the surface of the biological tissues. The results of the present study have shown that the ratio of amide II to amide I band intensities decreases from 0.82 to 0.69 for lower chromium treated samples and to 0.56 for higher chromium treated samples compared to control, which corresponds to 16 and 32% depletion of protein for lower and higher treatment, respectively. Also, the peak intensity ratio I_{1550}/I_{3300} decreases from 0.72 to 0.70 for lower concentration and to 0.62 for higher concentration with the decrease of 3 and 14% of protein compenents, respectively. This decrease in the protein content may be due to the diversification of energy through gluconeogenis to meet the impending energy demands when the animals were under toxic stress[15]. A similar trend of depletion in protein content was reported by Thatheyus *et al.*[16], for *Cyprinus carpio* exposed to copper, Ragothaman[17] for *Cirrhinus mrigala* exposed to cadmium and Vincent *et al.*[18] for *Catla catla* exposed to chrromium using biochemical methods.

It has also been observed that during the period of recovery the ratio I_{1550}/I_{1650} due to protein content was decreased from 0.69 to 0.45 for lower recovery and 0.56 to 0.45 for higher recovery samples compared with the treated samples. Similar observations was made by the toxic effect of nickel on tissue protein of edible fish *Cirrhinus mrigala*[19]. Also the ratio I_{1500}/I_{3300} decreases from 0.70 to 0.58 for lower recovery and 0.62 to 0.59 for higher recovery with respect to treated samples. The depletion in protein content also suggest an increased proteolysis and possible utilization of the products of their degradation for metabolic purpose. They may be fed into TCA cycle through aminotransferase system to cope up with excess demand of energy during the elimination of toxicants from the body[20].

All spectroscopic investigators to date have demonstrated high amide frequency (1657-1650 cm^{-1}) for α-helical secondary structure. In the present study amide I maximum occurs at *ca.* 1657 indicating that the protein is dominated by α-helical structure[21].

In conclusion, FT-IR spectroscopy has given a valuable information about the functional groups which might have diagnostic value of biological systems. Further, it can be used to detect the changes in the composition between normal and chromium treated tissues.

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