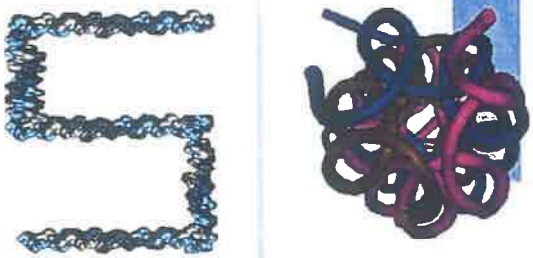
Ramachandran Manifestation:

Peptide to Proteome

Commemorating



Years of the Ramamchandran plot

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Jointly organized by

Department of Biochemistry, Sri Venkateswara College

Department of Biochemistry, Delhi University, South Campus

Prof. G.N.R. - His life in a nutshell

1922	• Born on 8th Oct, Ernakulum, Kerala
1942	• BSc(Hons) Physic, St. Joseph's College, Trichy
1944	• MSc Physics, India Institute, of Science (IISc), Bangalore
1947	• DSc Physics, HSc (Under the supervision of Prof. C.V. Raman)
1949	• PhD Crystallography, Cavendish laboratory,University of Cambridge.
1952	• Assistant Professor of Physics, IISc Bangalore
1954	• proposed and published the triple helical structure of collagen, University of Madras.
1963	• Ramachandran plot - was published in the Journal of Molecular Biology in 1963
1970	• Founded the Molecular Biophysics Unit at the Indian Institute of Science.
1971	• Developed of the theory of image reconstruction using the Convolution Technique.
1999	• Honored with the Ewald Prize for his 'outstanding contributions to crystallography'.
2001	• Died at the age of 78

ISOLATION, PURIFICATION AND CHARACTERIZATION OF SECONDARY STRUCTURE ANDKINETIC STUDY OF LIPASE FROM INDIAN MAJOR CARP, *Catla catla* (CATLA)

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Abstract

Lipases are ubiquitous enzymes which catalyse the hydrolysis of fats into fatty acids and glycerol at the water-lipid interface and reversing the reaction in non-aqueous media. Lipases occupy a place of prominence among biocatalysts owing to their novel and multifold applications in oleochemistry, organic synthesis, detergent formulation and nutrition.

Lipase was extracted and isolated from the alimentary canal and digestive gut of *CatlaCatla*(catla). The tissue was homogenized in a ratio of 1:3 with starting buffer (0.01M TrisHCl, pH 7.2). The crude extract thus obtained was precipitated using ammonium sulphate (20-80%). Excess salt was removed by Dialysis and the resultant dialysate (Desalted Enzyme) was subjected to DEAE-Cellulose column for Ion Exchange Chromatography at a flow rate of 0.5ml/min. Elution was carried out by a step gradient of NaCl (100-800 mM) in the starting buffer. Active fractions were pooled as Purified Fraction (PF) and were used for physical characterization of pH, temperature and effect of calcium on the enzyme activity, structural characterization, molecular characterization and for kinetic studies.

The Purified Fraction (PF) showed final specific activity of 1438.72 U/mg. The optimum pH was 7.8 and the optimum temperature was found to be 20° C. Melting Temperature (T_m) value was 42° C and the activation energy of purified lipase was 34.82 KJ/mol/K. Thermal stability of lipase was found to be at 20° C. Lipase activity retained upto 3 h of incubation with 10 mM and 20 mM of $CaCl_2$ in starting buffer. This shows that Calcium has enhancing property from the denaturation of enzyme. Michaelis-Menten constant (K_M) of lipase from Indian major carp, catla for the hydrolysis of pNPP was 6.695 mM. Turnover number (k_{cat}) of lipase (catla) was 0.0022 s^{-1}. Catalytic efficiency (k_{cat}/K_M) of lipase was 0.0003412s^{-1} mM^{-1}. SDS-PAGE of purified lipase (PF) revealed a homogenous single band with molecular mass of 70 KDa. Secondary structural arrangement of α helices and β strands of purified lipase results 48.51 % and 9.74 %, respectively using Circular Dichroism. Mass Spectrometric studies (MS and MALDI TOF) of purified fraction of lipase were under process for further characterization.

ENZYMATIC DETERMINATION OF CATECHOL OXIDASE AND PROTEASE FROM FRUITS (ORANGE, APPLE) AND VEGETABLES (CARROT, TOMATO)

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Abstract

The metabolic changes observed in any physical characters of fruits such as softness or colour may be a result of changes in the activity of enzymes. The activity of plant polyphenoloxidases can cause enzymatic browning of fruits and vegetables and render food unfit for consumption due to formation of benzoquinone. Proteases degrade polypeptides and facilitate seed germination, leaf senescence, storage and release of amino acids for transport to actively growing regions. Kinetic activity is significant in understanding the process of fruit and vegetable ripening. It is evident from the literature that these enzymes (Catechol oxidase and Protease) have a prominent role in the immunity and defence mechanism.

The present study has been focused on the enzymatic profiles of Catechol oxidase and Protease in fruits (Orange and Apple) and vegetables (Tomato and Carrot) which can be helpful in isolating and characterizing the enzymes from the above sources. Catechol oxidase and Protease were assayed using catechol and egg albumin as substrates, respectively. The Catechol oxidase activity was determined by measuring the change in colour of solution comprising of substrate and fruit/ vegetable extract due to formation of chromophoric substance, benzoquinone. Protease was assayed by measuring the rate at which the solution of egg albumin and fruit/vegetable extract got digested by the enzyme activity.

An increase in the absorbance due to formation of benzoquinone was observed in tomato, orange and apple juices due to increased activity on enzyme Catechol oxidase implying to more ripening of fruits/ vegetables as compared to carrot juice where the absorbance value was found to decrease indicating less enzyme activity. Protease enzyme activity in tomato juice was found to decrease due to increase in absorbance as lesser amount of egg albumin was digested by the enzyme. However Protease activity was found to increase in orange juice sample. From these results it is evident that, protease has a significant and prominent role in ripening and softening of the fruit (orange) as compared to vegetable (tomato).

Key Words: Catechol oxidase, protease, benzoquinone, orange, apple, carrot, tomato