SUMMARY

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Knowledge of molecular properties and structure of heat-stable enzymes is important for an understanding of the basic principles governing thermostability of proteins and evolution of life at high temperatures. Information on functional characteristics of thermostable enzymes is necessary for improving existing biotechnologies and developing new ones. Because of these reasons enzymes from thermophilic organisms are being exploited. Thermophilic xylose (glucose) isomerase enzyme isoforms were identified in the eukaryotic xerophytic species of *Cereus pterogonus* and *Opuntia vulgaris*. The thermophilic isoforms were capable of converting glucose to fructose at 53 and 54% efficiency and xylose to xylulose at 50 and 61% efficiency. The fructose forming ability of these isomerase isoforms will find greater utility in the production of HFCS. The ability to produce these isoforms in quantity will find industrial and commercial use for these enzymes and will augment the economics of the enzyme industry. Isolation purification and characterization of the thermophilic xylose isomerase from the plant species studied has already established a method of choice for the production of these enzymes employing conventional techniques for protein precipitation and protein separation through column chromatographies. Enzyme activity in relation to molecular size was determined using gel permeation and SDS-PAGE methods. Immunological studies included generation of polyclonal anti xylose isomerase antibodies in rabbit and its use for determining immune complex formation with the xylose isomerases from the different species, as also for cross reactivity studies between them. Structure and functional studies involve employing denaturation kinetic methods, Fluorescent spectroscopy and Differential Scanning Calorimetry. Conformational changes in the enzyme structure and phase differences during denaturation of the enzyme species were detected through these techniques.

Thermodynamic contributions during denaturation were calculated using denaturation rate constants, estimation of activation energy by Arrhenius plot and using the DSC technique. Attempts was made to determine the thermophilic xylose isomerase gene size and sequence using PCR technique but were met with difficulties. Primer designing directed to conserve sequence domains of selected xylose isomerase genes in selected species was carried out using Bioinformatics tools. The generation of an 800 and 450bp for CPXI and 600 and 200bp for OVXI PCR amplified product obtained employing the pair of degenerative primers designed for the purpose is under further investigation. Results of these studies are presented in the thesis.