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Effects of Osmolytes on The Structural Stability of Bovine Trypsin: A Brief Review

Shivani Jain[1], Sarvesh Seechurn[1], Parth Gupta[2], Gaurika Garg[2], Bhavuk Dhamija[2], N. Latha[2], and Kameshwar Sharma YVR[2*]
[1]Biological Sciences, Sri Venkateswara College, University of Delhi, Delhi-110021
[2]Department of Biochemistry, Sri Venkateswara College, University of Delhi, Delhi-110021

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ABSTRACT

Proteases represent a formidable class of industrial enzymes, accounting for about half of the total sale of the enzymes in the world. Trypsin, a protease that has strong specificity, acts as a digestive enzyme and plays a pivotal role in digestive physiology. Proteins have evolved to function within the cellular milieu, where macromolecule and small molecule solutes are present at high concentrations. It has long been known that osmolytes and other solutes transmute protein stability in vitro while certain osmolytes stabilize proteins in vivo to thermal and chemical denaturation arising in stress conditions. The role of various osmolytes and denaturants in the conformational properties and stability of the enzyme, trypsin has been scarcely investigated. In this study an attempt has been made to probe the effects of osmolytes, Glucose, Glycine, Proline, Sucrose, Trehalose and denaturants Guanidine Hydrochloride and Urea along with their concentration effects on the activity and stability of bovine trypsin. Detailed circular dichroism, steady state and temperature dependent fluorescence spectra, UV-Vis spectra and enzyme assay have been studied to scrutinise osmolyte induced stability and denaturant-induced unfolding properties of trypsin. The specific activity peak was found to be highest in sucrose and lowest in urea and GnHCl. The UV-Vis spectroscopic data showed highest absorbance peak for Proline depicting it as the strongest stabilizer, supported by low absorbance peak in fluorescence spectra. On the other hand, Urea was found to be strong denaturant with its lowest absorbance peak and highest intensity peak in UV-Vis and fluorescence spectra respectively. High osmolyte concentration demonstrated an increase in stability in case of Proline and significant rise in denaturing capacity of urea. CD analysis depicted highest positive peak in Proline, concluding it as the strongest stabilizer followed by Sucrose and Trehalose. Alpha helix and beta sheet content was estimated by K2D2 software which supported the trend in stability concordant with the rest of the data.

KEYWORDS: Trypsin, protease, osmolytes, protein folding, denaturants, spectroscopy, enzyme kinetics

INTRODUCTION

Proteins are polymers formed of smaller units known as amino acid residues held together by peptide bonds between the carboxylic and amide groups. In order to perform their biological functions, these proteins fold into specific spatial conformations, propelled by a number of non-covalent interactions such as hydrogen bonding, ionic interactions, Van der Waals forces, and hydrophobic packing[1]. These three dimensional structures discern their function in the cellular milieu to a great extent. They have evolved here to work concomitantly with other macromolecules and small molecule solutes present at high concentrations. Most cells in vivo are majorly dominated by the presence of water, electrolytes, metabolites and osmolytes as medium[2]. A protein may undergo reversible changes in its structure in order to perform its biological function. The stability studies of these transition states increasingly form a major focus of research.

***Corresponding author.**
Dr. Kameshwar Sharma YVR
Assistant Professor
Department of Biochemistry
Sri Venkateswara College
University of Delhi
Delhi-110021,India.

A protein structure is highly complicated comprising of different structural units like domains, motifs and folds. The repeating sequence of atoms along the core of the polypeptide chain is referred to as the polypeptide backbone and attached to these chains are side chains which confer upon amino acids their unique properties, setting them apart. Some of these side chains are hydrophobic or non-polar, others are charged positively or negatively[1]. The hydrophobic side chains of amino acids such as Phenylalanine, Leucine, Valine, and Tryptophan tend to cluster in the interior of the molecule enabling them to avoid contact with water surrounding them. On the other hand, the polar side chains like those of Arginine, Glutamine, and Histidine have a tendency to arrange themselves outside the molecule where