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The functional and structural stabilization of trypsin by sucrose

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ABSTRACT

Docking and spectroscopic techniques were performed to probe the stabilizing effect of sucrose on the dynamics, structure and activity of trypsin. The thermodynamic folding properties, melting temperature (T_m), enthalpy change (ΔH°) and entropy change (ΔS°) were measured by thermal stability studies to understand the picture of trypsin folding. Sucrose acted as an enhancer for trypsin stability. Fluorescence spectroscopy revealed the static model of the quenching. The number of binding sites was 1. The Absorption, Fluorescence and circular dichroism spectral analysis illustrated that sucrose could protect the native structural conformation of enzyme and prevent the enzyme unfolding. Fluorescence spectroscopy and the molecular docking technique simulation displayed that the hydrogen bonding and Vander Waals forces played a main role in stabilizing the trypsin-sucrose complex, and the number of direct H-bonds between sucrose and trypsin was low; thus, the direct interactions had little contribution in the stabilizing effect and the indirect interactions caused by the preferential hydration were resulting from a molecular mechanism principally causing the stabilizing effects of sucrose.Upon sucrose conjugation, the k_{cat}/K_m value of the enzyme was increased. T_m of the trypsin-sucrose complex was increased due to the higher H-bond formation and the lower surface hydrophobicity after sucrose modification. Sucrose acted as enhancers for trypsin stability and activity. The result shows the ability of sucrose to protect the native structural conformation of trypsin. These results explicitly describe that stabilizing sucrose is preferentially excluded from the surface of trypsin, since water has a higher tendency toward favorable interactions with functional groups of trypsin than with sucrose.

1. Introduction

Protein molecules are very complex and sensitive. The protein unfolding and folding principles have been a favorite subject of study in the past decades. There are different methods for increasing stability and enzyme activity. Environmental change is one of the best ways to increase the stability and activity of enzymes. The past researches have proposed that the addition of co-solvents can increase the protein stability; these investigations have elucidated the effects of aqueous solvation, hydrogen binding and hydrophobicity of proteins. Protein stability depends on the van der Waals, hydrophobic, steric and electrostatic interactions of the molecule with the solvents and itself [1]. Organic osmolytes such as polyols act as some chemical chaperone guarding protein from denaturation by protecting the maintenance of the natural folds and functional states of protein in difficult conditions [2]. Osmolytes are a cluster of molecules that have shown considerable success in the natural conformation of proteins and hence, their functional forms.

Between osmolytes, sucrose and trehalose disaccharides have the unique ability to maintain the normal structure of proteins and other biological molecules in unfavorable conditions [3,4]. Back et al. found that sucrose and glucose strengthened pairwise hydrophobic interactions between hydrophobic groups in model systems and argued that sugars stabilized proteins to heat denaturation through their effect on the structure of water [5]. The water molecules are preferably attracted to the protein surface in solution. Due to the steric repulsion, other solutes such osmolyte are excluded from the proximity of protein. This phenomenon is called 'excluded-volume effects' [6,7]. The exclusion of osmolyte from the surface of protein leads to the molecular stabilization of proteins with no change inthe function of proteins. The osmophobic effect

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has been proposed by Bolin et al., who have described that the solvophobic thermodynamic force of osmolyte causes the protein stability [8,9]. There are two molecular interaction models of the polyol-induced protein stability: direct and indirect interaction. In the direct model, a polyol binds to protein by H-bonds and stabilizes the native protein, but in indirect one, a polyol changes the structure and dynamics of water in the solution; therefore, this increases the hydrophobic interactions between pairs of hydrophobic groups and stabilizes the native state [5].

Bovine trypsin is a serine protease that plays an important designation role in the digestion into the pancreas. It is a soluble globular protein including 223 amino acids with a molecular weight of 23.3 kDa. It has an isoelectric point, pI, of around 10.5 [10]. Trypsin has two equal domains with six antiparallel strands making some αb-sheet unite linked through a network of hydrogen bonds [10]. The active sites of trypsin are the catalytic triad – His 57, Asp 102 and Ser 195. In order to investigate the osmolyte effect on trypsin, we have chosen sucrose from the polyols. The target of this study was to investigate the effects of sucrose on the structure, stability and activity of trypsin at pH 8.0.

2. Materials and methods

2.1. Materials

Trypsin from bovine pancreas (MW: 23.3 kDa) and N_α-benzoyl-L-arginine ethyl ester (BAEE) were purchased from Sigma-Aldrich Co. Sucrose was supplied from Merck. The trypsin and N_α-benzoyl-L-arginine ethyl ester were dissolved in a Tris-HCl buffer (pH 8.0, 50 mM).

2.2. Methods

2.2.1. Fluorescence spectroscopy

Steady state fluorescence spectra were recorded on a Shimadzu RF-5301 spectrofluorometer. Fluorescence spectra were measured by an excitation wavelength of 280 nm. The slits were set to 3 and 5 nm for excitation and emission scans, respectively. The emission spectra were recorded between 290 and 450 nm in the absence and presence of different amounts of sucrose. Quenching studies were carried out by scanning changes in IF (the fluorescence intensity) at the maximum emission of 332 nm, and they were plotted as a function of sucrose concentration. The binding parameters were obtained by the Stern-Volmer equation [11–13].

2.2.2. Absorption assays

The absorption spectra of trypsin in the presence of various concentrations of sucrose were recorded over the wavelength 260–310 nm at 310 K. Trypsin concentration was fixed at 0.1 mg/ml and that of sucrose was varied between 0 and 0.5 mM. The absorption spectra were monitored at λ_{280}.

2.2.3. CD spectroscopy

Circular dichroism (Macdonald, #20) spectroscopy is a useful method to understand the interaction and structure of protein. The CD spectroscopic measurements were recorded by a spectrophotometer, model AVIV 215. The sample containing trypsin, sucrose and Tris-HCl buffer was pre-equilibrated for 15 min at the room temperature. The secondary structure of enzyme was analyzed by using far-UV CD (0.8 mg/ml trypsin concentration, 200–260 nm), and the tertiary structure of trypsin was monitored by near-UV CD (1.6 mg/ml trypsin concentration, 260–300 nm). Determination of the percentages of the secondary structures was done by using CDNN software.

2.2.4. Enzyme stability

Enzyme stability in the absence and presence of sucrose at different concentrations was analyzed at 280 nm and pH 8.0. Trypsin was incubated with sucrose for 10 min at room temperature. An Ultraviolet- Visible (UV–vis) spectrophotometer (Pharmacia 4000) was used to measure the UV–vis spectrum of trypsin. All samples were prepared in a 0.1 mg/ml trypsin concentration with different concentrations of the co-solvent. By the two-state model, the absorption data of trypsin-sucrose complex were plotted as a function of temperature [14]. The melting temperature, T_m, was determined as a midpoint of the melting curve.

2.2.5. Enzyme activity

The activity of 25 μg/ml trypsin was measured using 1 mM BAEE as a substrate in 50 mM Tris-HCl buffer at pH 8.0. The catalytic activity was determined by using a UV–vis spectrophotometer at 253 nm and 308 K. The increase in trypsin absorption per minute was pursued in solution.

2.2.6. Molecular docking studies

Molecular mechanics of trypsin and sucrose were carried out using AutoDock 4.0 software package [15]. The crystal structure of trypsin available in RCSB protein data bank (ID code: 2PTN) was used. The sucrose structure was modeled using HyperChem program [16]. Table 1 displays the structure of sucrose minimized using the MM^+ force field. Trypsin was as rigid and fully flexibleas sucrose during docking. All water molecules and hetero-atoms attached to the enzyme were removed from the original Protein file during molecular calculation studies. Furthermore, polar hydrogen atoms, Kollman united atom partial charges and atomic solvation parameters were also assigned in the trypsin [17].

Table 1
Structure of sucrose.

Ligand	Structure ligand	3D Structure ligand
Sucrose