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Unanticipated behaviour of sorbitol towards the stability and activity of stem bromelain: An outlook through biophysical techniques

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ABSTRACT

As far as the increased risk of the diseases attributed to the misfolded/unfolded/aggregated proteins are concerned, one may not deny the importance of the various methods (chemical modifications of enzyme, protein engineering techniques and additives) which maintain the protein in its three dimensional functional native form. Out of these methods, the interest in the additives has been grown rapidly in last few decades. Among these additives, polyols are well known compatible co-solvents for the protein. We have investigated the influences of a series of structurally related polyols (ethylene glycol, glycerol, erythritol, xylitol and sorbitol) on the stability and activity of stem bromelain (BM) by using fluorescence spectroscopy, circular dichroism (CD), UV–vis, Fourier transform infrared (FTIR) spectroscopy and dynamic light scattering (DLS). Our results revealed the increased conformational stability of BM with increase in the size of polyols and the proteolytic activity of BM in reverse order. However, sorbitol was found to be the weakest stabilizer for BM among all polyols whereas BM possessed highest activity in the same. To the best of our knowledge, our results represent first detailed unambiguous proof of unusual effect of sorbitol on the interactions governing stability of BM.

1. Introduction

Compatible osmolytes have been implicated to withstand various stresses such as water deficit, salt and temperature and accumulate to high concentrations without disturbing the protein structure [1]. The prospects of increasing stress tolerance, improving the efficiency of the enzyme catalyzed reactions importantly used in various industrial applications and improving the quality of the processed food have fuelled the interest in these osmolytes [1,2]. A number of other methods can also be applied to stabilize the enzyme such as chemical modifications of enzyme, protein engineering techniques and enzyme immobilization [3]. However, the interest in the additives has been grown rapidly in last few decades [1–4]. These additives need not to be removed from the solutions after its applications in refolding and food processing. The interest in the enzyme stabilization by the use of the soluble additives stems not only from the stability against various types of stresses; salt, heat, cold, pH, water deficiency, pressure etc, but also from convenient and economic point of view which is a most important industrial impact [3].

Polyhydric alcohols (compatible osmolytes) are among the most ubiquitous compounds which the nature has been using to protect against the various stresses in several algae, certain plants and many insects exposing to freezing or high salt concentrations and found to be effective at higher concentrations (≥1M) [1]. From empirical to theoretical points of view, researchers from multiple disciplines have provided the insights into the protein stabilization by the polyols. A number of researches declared that polyols are good stabilizers of the proteins [5–11].

However, these researches are explored with different explanations about the increase in the thermal stability. According to Fujita et al., [5] the structural stability and dynamics of the globular proteins are extensively controlled by the interaction of the protein with water. Polyols added in protein aqueous solution affect these interactions and consequently modify the structural stability of the protein. Gerlsma and Sturr [6] have proposed the decrease in the hydrogen bond rupturing capacity of medium by polyols as a reason for the stabilizing effect of polyhydric alcohol. Also, according to Back et al., [7] change in the structure of water is playing the central role in the stabilization of the protein. Gekko and Timasheff have put stress on increase in the free energy of denaturation of the protein [9] whereas Lozano et al. [11] proposed that an overall protective effect results from the influence of the hydrogen bond interactions on the enzyme-water structure which

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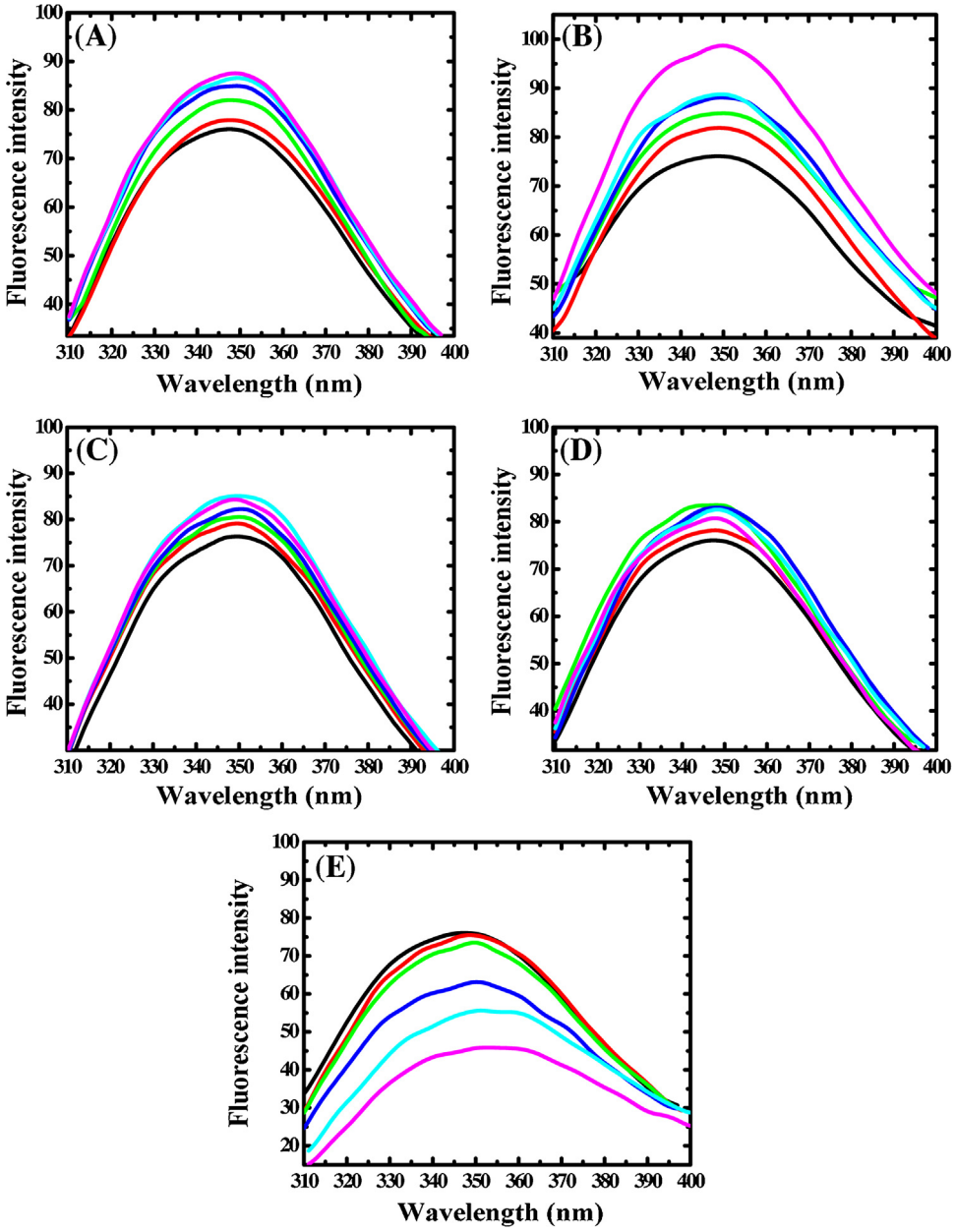

Fig. 1. Fluorescence spectra analysis of the BM conformation in the presence of polyols at 25 °C: BM in presence of buffer (black) and (A) ethylene glycol, (B) glycerol, (C) erythritol, (D) xylitol and (E) sorbitol as a function of concentrations, 0.1 M (red), 0.5 M (green), 1.0 M (blue), 1.5 M (cyan) and 2.0 M (pink). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

induces an increase in free energy of denaturation. Combes et al. [10] focussed on direct interaction (specific or not specific) between the polyhydric alcohol molecule and the protein.

Although evidence for the protein stabilization by these compatible co-solvents has been thoroughly demonstrated, [1–12] a gap in our understanding persists regarding the mode of action which remains a subject to several controversial interpretations. The precise nature of the interactions among the protein and these co-solvents is not clear yet. It is likely to be dependent on the properties of both protein and co-solvent. A co-solvent may behave as a stabilizer or destabilizer depending on its relative affinity to the protein as compared to water [13]. Therefore, stability study of each protein in presence of various co-solvents has its own significance.

In last decade, proteases have received growing attention due to their potential ability to catalyze non-conventional synthetic reactions [14]. In this context, a large number of enzymes are still unexplored. Stem bromelain (BM), a proteolytic enzyme, has a wide range of industrial and pharmaceutical applications [15–17]. An increased storage and operational stability of an enzyme is an important consideration for the practical applications of the enzymes. Nevertheless, a detailed knowledge of BM in presence of different additives is essential for the efficient operation in many industrial biochemical processes. In this regard, polyols are useful as preservatives as well as stabilizing agent in the food and pharmacological industries. Interestingly, numerous studies show that polyols not only increase the thermal stability and activity of the enzyme, but as per recent studies, also rectify misfolded proteins and disassemble disease related aggregated protein i.e, amyloids [18]. Moreover, there has been no systematic study of the influence of polyols on the BM stability and activity.

According to our previous reports, trehalose and sucrose behave as a destabilizer at lower concentrations [19] and TMAO act as