Chemical Physics Letters 689 (2017) 156–161

Contents lists available at ScienceDirect

Chemical Physics Letters

journal homepage: www.elsevier.com/locate/cplett

Research paper

α-Chymotrypsin in water-ethanol mixtures: Effect of preferential interactions

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ARTICLE INFO

Article history:
Received 11 September 2017
In final form 10 October 2017
Available online 12 October 2017

Keywords:
Protein hydration
Preferential solvation and hydration
Protein stabilization
Bovine pancreatic α-chymotrypsin
Ethanol

ABSTRACT

We investigated preferential interactions of α-chymotrypsin with water–ethanol mixtures at 25 °C. Our approach is based on the analysis of residual enzyme activity and water/alcohol sorption. There are three concentration regimes. α-Chymotrypsin is preferentially hydrated at high water content. The residual enzyme activity is close to 100%. α-Chymotrypsin has a higher affinity for alcohol than for water at intermediate water content. Residual enzyme activity is close to zero in this concentration range. At low water content, ethanol is preferentially excluded from the protein surface. This results in preferential hydration of α-chymotrypsin and significant residual catalytic activity (~50%) in water-poor ethanol.

1. Introduction

It is well-known that the stability, structure, and functions of proteins are governed by interactions of the protein macromolecules with water [1–8]. Small monohydric alcohols (for example, ethanol) are widely utilized in biophysical, biomedical, and biotechnological investigations to change the protein-water interactions and consequently modulate the protein stability. In particular, there are many advantages in employing water-poor alcohols, including the suppression of undesirable side reactions caused by water, the biocatalysis of reversed hydrolytic reactions (transesterification, esterification, and peptide synthesis), and increased thermostability [9–13]. Distinct intermediate protein states induced by alcohols may be responsible for numerous neurodegenerative diseases (Alzheimer's disease, Parkinson's disease, and Huntington's disease) [14–17]. However, the manner in which alcohols increase/decrease the thermal stability, induce/reduce the extent of denaturation, and stabilize/destabilize the partially folded conformations of proteins (amyloid fibrils and molten globules) is an intricate function of water content in alcohols.

There are numerous investigations which demonstrate the bilateral action of monohydric alcohols on the protein properties [18–23]. For example, the temperature of protein denaturation in monohydric alcohols decreases gradually with increasing organic solvent concentration [18–21]. On the other hand, the denaturation enthalpy passes through a maximum with augmenting alcohol content [19–23]. At low alcohol content and temperatures around 0–25 °C, monohydric alcohols can slightly stabilize proteins [21–23].

Understanding the bilateral impact of monohydric alcohols on the protein stability requires effective techniques that reveal biophysical information regarding protein–alcohol and protein–water interactions. Preferential solvation/hydration may be an effective and informative approach for elucidating the dual effect of water-alcohol mixtures on the protein stability. Preferential solvation is a thermodynamic quantity that describes the protein occupancy by the alcohol/water molecules [24–33]. Alcohol and water exist preferentially in the solvation layer of the protein. When a protein is placed into a water-alcohol mixture, its properties are altered as a function of the solvent composition. The preferential solvation/hydration process accounts for the augmentation or depletion of the alcohol/water molecules at the protein surface. Preferential binding is the excess of alcohol at the protein surface relative to the alcohol content in the bulk solvent. The preferential binding depends markedly on the chemical nature of the protein surface. For example, protein unfolding may be induced by the preferential binding to specific regions on the protein (peptide groups in the case of urea and guanidinium hydrochloride or hydrophobic regions in the case of alcohols) [24,25,28–33].

Preferential interactions (solvation and hydration) can be characterized by a number of techniques: by light scattering [33], by differential refractometry and densitometry [24–26,30–32], and by equilibrium dialysis [30]. These investigations have been made at high and intermediate water content. However, no attempt has

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https://doi.org/10.1016/j.cplett.2017.10.023
0009-2614/