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Quantifying the co-solvent effects on trypsin from the digestive system of carp *Catla catla* by biophysical techniques and molecular dynamics simulations†

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Here, circular dichroism (CD) spectroscopy, fluorescence spectroscopy, UV-Vis spectroscopy, SDS-PAGE, substrate SDS-PAGE, and molecular dynamics (MD) simulations techniques have been employed to understand the structural behavioral changes of trypsin (MW: 19.72 kDa, source: digestive system of adult Indian major carp, Catla *C. catla*) in the presence of various chemical environments. The stability of the trypsin can be increased by stabilizers, including trimethylamine *N*-oxide (TMAO), proline, and betaine, without affecting its native structure. Trypsin has shown unusual high thermal stability in the presence of betaine. Further, these experimental results were confirmed by means of MD simulations. The present results explicitly elucidated that the behavior of a co-solvent may vary depending upon the type of the protein.

1. Introduction

Fish viscera have been known as a potential source of different enzymes, especially proteases.[1] Proteases are used for a plethora of industrial applications, *e.g.*, in the detergent, food, pharmaceutical, leather and silk industries.[2] One of the main digestive proteases detected in the pyloric caeca and intestine of fish is trypsin (EC 3.4.21.4). Trypsin, an important enzyme belonging to the large and diverse family of serine proteases, has been found in numerous higher mammals, fish, plants, insects and bacteria.[3] These proteins are also used to recover proteins from fish carcasses that would otherwise go to waste after filleting.[4] Among all trypsins, fish trypsins are of immense interest because they exhibit higher catalytic activity than their mammalian counterparts, making them more suitable for a number of biotechnological and food processing applications.[5–7] The crystal structure of trypsin is shown in Fig. 1.

Protein stability has elicited extensive studies in many scientific disciplines. Most of the proteins lie in their collective properties and the sequences in amino acids of the polypeptide chains that fold in their compact native structure. The native structure of proteins is only marginally stable, and achieves stability/destability only within narrow ranges of conditions of co-solvent environment which can dramatically affect the

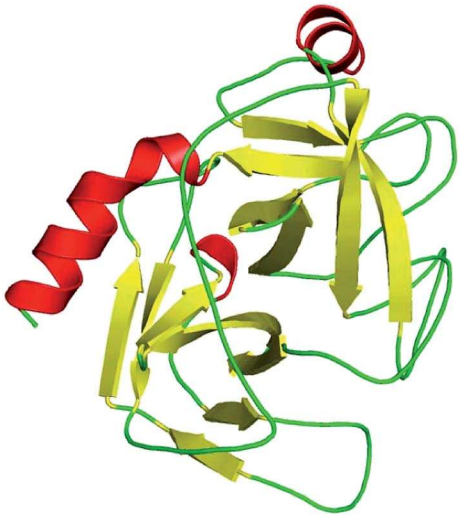

Fig. 1 Schematic diagram of the crystal structure of trypsin, which was downloaded from protein data bank and processed with PyMOL viewer software.

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