Supporting Information

Exploring the Effect of Choline-Based Ionic Liquids on the Stability and Activity of Stem Bromelain

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1. EXPERIMENTAL SECTION

1.1. Materials

Choline acetate (≥98.0%), cholinium bitartrate (99%), choline hydroxide (46 wt% in H_2O) and bromelain (BM) E.C. 3.4.22.32 lot No. B4882 from Ananas Comosus (molecular weight: 23.8 kDa), were purchased from sigma Aldrich. Choline chloride (≥99%) and choline iodide (＞98%) were obtained from alfa aesar. Choline dihydrogen phosphate (＞98%) was purchased from Iolitech Company. Sodium phosphate dibasic dehydrate, sodium phosphate monobasic, trichloroacetic acid (TCA), casein (Hammarsten) for the study of activity were parched from Sisco Research lab (SRL), India.

1.2. Sample Preparation

All the BM samples were prepared 0.5 mg/mL concentration in 0.10 M sodium phosphate buffer at pH 7 for all measurements. For DLS measurement, protein concentration was kept at 2 mg/mL. Water was removed from choline hydroxide (46 wt% in H_2O) by using rotary evaporator and it was kept 24 h under nitrogen atmosphere for moisture removal. All the samples were prepared gravimetrically using a Mettler Toledo balance with a precision of ±0.0001 g. The BM solution was filtered with a 0.45 μm disposal filter (Millipore, Millex-GS) through a syringe

prior to measurements. We used 0.01, 0.05, 0.10, 0.50, 1.0, and 1.5 M of all choline-based ILs for all the measurements.

1.3. Experimental Methods

UV-visible absorption spectra of BM in the presence as well as in absence of ILs were measured by means of a double beam UV- visible spectrophotometer (Shimadzu UV- 1800, japan) with the highest resolution (1 nm) at room temperature. Steady state fluorescence emission spectra measurements were conducted at room temperature by Cary Eclipse fluorescence spectrofluo-rimeter (Varian optical spectroscopy instruments, Mulgrave, Victoria, Australia). The hydrodynamic diameter (d_H) of all samples were measured by dynamic light scattering (DLS), Zetasizer Nano ZS90 (Malvern Instruments Ltd., UK). Circular dichroism (CD) spectra performed by using a Jasco-815 spectrophotometer equipped with a peltier system for temperature control around the cell with an accuracy of ±0.1 ^{0}C. The secondary and tertiary structures of BM were obtained using far-UV (190−240 nm; 0.1 cm path length cuvette cell) and near-UV (250−300 nm; 1.0 cm path length cuvette cell) spectra, respectively. The enzymatic activity of BM was assayed by using casein as the substrate with a UV-visible spectrophotometer. The aqueous denatured casein solution (0.5 mL of 0.5% at pH 7) was kept for 10 min at room temperature with BM in the buffer (0.5 mg/mL) and various concentrations of choline based-ILs. The undigested casein precipitate was removed by the centrifugation, which were formed after addition of 1 mL of 110 mM trichloroacetic acid (TCA) to stop the reaction. The specifications and complete details of experimental methods used in the current investigation have discussed in our previous reports.[1-3]

REFERENCES

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