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Title of Study	**Role of Ionic Liquids in the Stability and Activity of Proteins**

ABSTRACT

In this advanced era, proteins are highly studied for their application in the different field of the science such as biochemical research, chemical, food and pharmaceutical industries. One of the major problems that limit their application is to keep them in their native folded and functional form. As protein is marginally stable, a slight change in their native environment can alter both structure and function of the protein. Most of the commonly used protein stabilizing techniques are unable to prevent the irreversible thermal denaturation and liquid formulation of the protein.

Ionic liquids (ILs) increase the activity, stability and refolding yield of the protein by preventing their irreversible aggregation. ILs act as tunable additives, therefore their stabilizing/refolding effect can be tailored according to the target protein. The tunability in properties of ILs can be easily obtained by varying the cationic and anionic component of ILs. Therefore, in this study ILs were utilized to see their effect on the stability and activity of the protein.

The behavior of three different model proteins (human serum albumin (HSA), bovine serum albumin (BSA) and hen egg white lysozyme (HEWL)) have been studied in aqueous solution of different ILs. The effect of ILs on stability and activity of proteins have been studied using fluorescence, time resolved fluorescence, UV-Vis,

circular dichroism (CD), FTIR spectroscopic techniques, tensiomatry, conductivity, molecular docking and molecular dynamic (MD) simulation methods.

The effect of N-butyl-N-methyl-2-oxopyrrolidinium bromide (BMOP) (newly synthesized) and N-butyl-N-methyl-morpholinium bromide ([Mor1,4][Br]) on HSA have been studied. It was found that [Mor1,4][Br] stabilize the HSA at its lower concentration while BMOP destabilizes the HSA. The contradictory effect was due to differential interaction behavior of different cation of ILs with HSA because anions in both ILs are same i.e. Br^- anion.

Further, the effect of same IL on closely related proteins was studied by analyzing the effect of BMOP on BSA (homologous to HSA). It was found that BMOP shows comparatively greater binding affinity with BSA however like HSA, it destabilize the BSA.

The effect of 1-methyl-3-octylimidozolium chloride ([OMIM][Cl]), 1-Butyl-1-methylpyrrolidinium tetrafluoroborate ([BMP][BF_4]) and 1-Butyl-1-methylpyrrolidinium bromide ([BMP][Br]) ILs on stability and activity of (HEWL have been studies. It was found that [OMIM][Cl] and [BMP][BF_4] induces some local conformational changes and increases the antibacterial activity of the HEWL. However, [BMP][Br] maintain native folded form of HEWL but decreases the antibacterial activity of the HEWL. Further, when comparing the effect of [BMP][Br] and [BMP][BF_4] IL it was found that contradictory effect of these ILs was due the differential interaction between cationic and anionic moiety of the IL.

From the results it was found that [BMP][BF_4] IL increase both stability and activity of HEWL thus acts as good co-solvent for HEWL. Likewise, [Mor1,4][Br] increases the stability of HSA therefore acts as good co-solvent for HSA.