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Title of the Thesis	Effects of Non-methylamine Osmolytes in Counteracting the Deleterious Effects of Urea on Structure, Function and Stability of Lysozyme

Organisms adapt to stressful environment by accumulating organic osmolytes. These osmolytes are acquired most economically by exploiting the metabolic end products. Urea can diffuse across cell membranes and contributes to balance the intracellular and extracellular osmotic equilibrium. Mammalian renal cells are found to accumulate urea in the concentration range of 500-600 mM due to its osmoregulatory mechanisms. However, urea is a potent denaturant and has been observed to perturb enzyme catalysis and protein-protein interactions. It is believed that, in order to counteract the deleterious effects of urea, organisms use and accumulate another class of osmolytes – the methyl ammonium compounds. *In vitro*-studies have shown that methyl ammonium compounds such as TMAO, sarcosine and betaine stabilize proteins and also have the ability to counteract the denaturing effects of urea. The generally held belief is that the urea-methylamine counteraction works at a specific ratio (2: 1 molar urea: methylamine) as observed in many elasmobranches tissues and cells. Earlier studies have demonstrated that the counteraction phenomenon at 2: 1 (urea: methylamines) is largely protein specific. In some enzymes, counteraction fails to work. In many cases the counteraction is partial and therefore, the ratio of counteraction varies from protein to protein.

In addition to these methyl ammonium compounds, urea-rich cells build up certain non-methylamine osmolytes, namely myo-inositol, sorbitol, taurine, β-alanine. A question arises: Do the stabilizing osmolytes, non-methylamines present in the urea-rich cells also counteract the deleterious effects of urea? We have also tried to investigate, although, methyl ammonium compounds namely, sarcosine and betaine, the methylated derivatives of glycine are part of osmoticum of urea-rich cells, glycine has been excluded, why is it so?

To answer these questions, we have measured structure, thermodynamic stability (ΔG_D^o) and functional activity parameters (K_m and k_{cat}) of lysozyme in the presence of various concentrations of urea and each non-methylamine osmolyte alone and in combination. We observed that (i) myo-inositol, and β-alanine provide perfect counteraction at the predicted ratio, (ii) any concentration of sorbitol and glycine fails to refold denatured proteins in the presence of urea at concentration $\geq$ 0.6 M, and (iii) taurine counteracts urea only partially.

Our study indicates that all of the osmotically active solutes in the urea-rich cells are not counteractants for urea's effect on proteins. Inside the cells the urea-counteraction system is not confined only to methylamine osmolytes. There exist multiple urea-counteracting systems. The most efficient (based on this study) are the urea-myo-inositol and urea-β-alanine systems and others include urea-taurine and urea-methylamine systems. The most probable reason for the absence of a stabilizing osmolyte, glycine in the urea-rich cells is due to the fact that this osmolyte is non-protective to macromolecules against the hostile effects of urea; however, its methylated derivatives (sarcosine and glycine betaine) are the major counteractants of the deleterious effects of urea on proteins. It is, therefore, very likely that incorporation of methyl groups to glycine potentiates the compatible osmolyte, glycine to have urea-counteractive property on proteins.