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Title of the Thesis : **SURVIVAL OF PROTEINS IN UREA ACCUMULATING CELLS OF THE MAMMALIAN KIDNEY: In Vitro Studies of the Effects of Kidney Osmolytes and Urea Individually and in Combination on the Structure and Function of Proteins**

ABSTRACT

The cells in the renal medulla of mammalian kidney are under constant urea stress due to its urine concentrating mechanism. As a result of this, the cells in renal medulla are exposed to high urea concentration in the range of 3.0- 4.0 M. 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, the renal cells accumulate low molecular weight organic compounds known as osmolytes. *In vitro*-studies have shown that methyl ammonium compounds such as TMAO, sarcosine and glycine 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 and sorbitol. We wanted to know whether non-methylamine osmolytes present in mammalian kidney (sorbitol and myo-inositol) are involved in counteracting the effects of urea on kidney proteins. To answer this question, we

have purified sheep serum albumin (SSA), calreticulin (CRT), H2AX and CRT-DNA complex from renal medulla of sheep kidneys.

Then we measured structure and thermodynamic stability (ΔG_D^0) parameters of SSA in the presence of various concentrations of urea and each mammalian kidney osmolyte alone and in combination. We observed that the urea- and GdmCl-induced denaturation of SSA follows a biphasic transition; Native state (N) Intermediate state (X) ↔ Denatured state (D). However we have seen that all the kidney osmolytes (i) transform this biphasic transition into a co-operative, two-state transition and (ii) increase the stability of the protein in terms of midpoint of denaturation (C_m) and Gibbs free energy change in the absence of both denaturants (ΔG_D^0). The relative effectiveness of different kidney osmolytes on the stability of SSA follows the order: glycine betaine > myo-inositol > sorbitol.We also observed that (i) for each protein glycine betaine and myo-inositol provides perfect counteraction at 1:2 ([glycine betaine]:[urea]) and at ([myo-inositol]:[urea]) ratio (ii) and any sorbitol concentration fails to refold urea denatured proteins. Since sorbitol is not used by kidney cells as the counteractant against the deleterious effect of urea, however, it may be used to maintain osmotic balance of the kidney cells under high osmotic stress conditions.

A similar study was also planned for other kidney proteins, namely, CRT, H2AX and CRT-DNA which we had isolated and purified to homogeneity. Since thermal and urea-induced and GdmCl-induced denaturation of each protein in the absence and presence of each kidney osmolyte were irreversible, thermodynamic stability parameters of these proteins could not be measured.