Name of Ph.D. Scholar: Wahiduzzaman

Name of supervisor: Prof. Faizan Ahmad

Name of co-supervisor: Dr. Md. Imtaiyaz Hassan

Faculty/Centre: Centre for Interdisciplinary Research in Basic Sciences

Title of study: Adaptation of Renal Cells to High Osmolality: A Molecular Basis of Protection of Renal Medullary Proteins by Organic Osmolytes

ABSTRACT

In mammalian kidney cells, the urine concentrating mechanism of urea is very high which may denature many kidney proteins.Cells of the renal medulla are exposed under normal physiological conditions to widely fluctuating extracellular solute concentrations, and respond to hypertonic stress by accumulating the organic osmolytes. The mechanism of adaptation in the inner medulla depends on the accumulation of high concentration of certain low molecular weight organic substances, called osmoprotectants. The renal cells accumulate low molecular weight organic compounds known as osmolytes in order to counteract the deleterious effects of urea. Many studies demonstrated that methyl ammonium compounds such as trimethyl amine N-oxide (TMAO), sarcosine and glycine betaine stabilize proteins and also have the ability to counteract the denaturing effects of urea. Usually the urea-methylamine counteraction works at a specific ratio (2: 1 molar urea: methylamine) as observed in many elasmobranches tissues and many cells. Earlier studies have shows that the counteraction event at 2: 1 (urea: methylamines) is for the most part of protein specific; whereas in some proteins, counteraction fails to work. However, 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. Our study provides answer to the question: Do methyl amine and non-methylamine osmolytes present in mammalian kidney counteract the deleterious effects of urea on kidney proteins? To answer these questions we purified two kidney proteins, TIM-β-globin and HCAII.

After successful purification, we measured structural properties and thermodynamic stability (ΔG_D^0) of both proteins in the presence of various concentrations of GdmCl, urea and each mammalian kidney osmolyte alone and in combination. We observed that the and GdmCl- and urea- induced denaturation of HCAII follows a triphasic transition, i.e., native state (N) ↔ intermediate state (X_I) ↔ intermediate state (X_{II}) ↔ denatured state (D) whereas TIM-β-globin protein shows a biphasic transition, i.e., N state ↔ D state.

GdmCl-induced denaturation of proteins in the presence of urea and each kidney osmolyte alone was studied. Osmolytes increase the thermodynamic stability of folded proteins without perturbing other cellular processes. In terms of preferential binding and preferential exclusion of the co-solutes, osmolytes stabilize proteins by shifting the denaturation equilibrium, N state ↔ D state toward the left, while urea destabilizes proteins by shifting the denaturation equilibrium toward the right, and urea-osmolyte mixture brings about compensatory effect. Thus, what effects co-solutes will have on the denaturation equilibrium, N state ↔ D state under the native condition will be known only by measuring ΔG_D^0 (value of Gibbs free energy change in the absence of GdmCl (ΔG_D) at 25 °C) in different solvent conditions. To achieve this goal, we performed GdmCl-induced denaturation of proteins in the presence of the kidney osmolytes and urea alone and measured $\Delta G_{D.}^0$The thermodynamic stability ($\Delta G^0_{N\leftrightarrow D}$, the Gibbs free energy change in absence of GdmCl associated with the equilibrium, native (N) state ↔ denatured (D) state) was measured from the GdmCl-induced denaturation curves in the presence of different concentrations of urea and each kidney osmolyte individually and in combination. It was observed that glycine betaine and myo-inositol provide perfect counteraction at 2:1 molar ratio of urea to osmolyte, i.e., denaturing effect of 2 M urea is completely neutralized by 1 M of glycine betaine or myo-inositol, and sorbitol fails to refold urea denatured proteins.