# ROLE OF CHEMICAL CHAPERONE METHYLAMINE IN COUNTERACTING THE DELETERIOUS EFFECTS OF UREA ON THE PROTEIN: THERMODYNAMIC, FUNCTIONAL AND STRUCTURAL ASPECTS

**AN ABSTRACT SUBMITTED TO JAMIA MILLIA ISLAMIA IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE**

**OF**

**DOCTOR OF PHILOSOPHY**

**IN**

***BIOSCIENCES***

***BY***

TANVEER ALI DAR

**CENTRE FOR INTERDISCIPLINARY RESEARCH IN BASIC SCIENCES**

**JAMIA MILLIA ISLAMIA**

**JAMIA NAGAR, NEW DELHI-110025**

***INDIA***

***MAY, 2008***

**Abstract**

Methylamines such as TMAO, sarcosine and betaine have been found to stabilize proteins and also have the ability to counteract the denaturing effects of urea. It is believed that a 2:1 molar ratio of urea and methylamine works as a magic ratio, giving compensation of proteins and enzymes in terms of structure, function and thermodynamics. We first focus on what makes methylamines equipped to circumvent the deleterious effects of urea. Structurally most of the methylamines are methylated derivatives of an amino acid osmolyte, glycine, which has been found to stabilize proteins. It has already been shown that methylation affects the stabilizing effect of methylated derivatives of glycine (methylamines) and their stabilizing effect varies greatly and depends on the nature of the protein. However, no systematic study has been done so far to elucidate the effect of methylation of glycine on the counteraction of urea effects by methylamines. So it will be interesting to see the possible role of methylation in the counteraction of perturbing effects of urea by methylamines.

The effect of glycine methylation on the protein stability was evaluated by normalizing the effect of methylamines (both $\Delta T_m$ and $\Delta\Delta G_D^0$) against the effect caused by glycine on protein stability (See Figure 1A). It can be seen in this figure that all methylated glycine derivatives (sarcosine, DMG and betaine) have lower stabilizing effect than glycine. Glycine was found to be the best stabilizer with the stability rank order of glycine > sarcosine ≥ TMAO > DMG > betaine. Therefore, higher the degree of methylation the lesser is the stabilizing effect. The stabilizing effect was found to be pH dependent with the stabilizing effect increasing as the pH decreases from 6.0 to 2.0. Increased methylation leads to the greater affinity for non-polar substances than that of the glycine. Also it leads to the increase in the degree of preferential exclusion from the hydrophilic protein surface, resulting in an increased preferential hydration of the native protein. However, the increase in hydrophobicity also decreases their preferential exclusion from the denatured protein, i.e., increased preferential binding to denatured state, shifting the N ↔ D equilibrium toward D state.

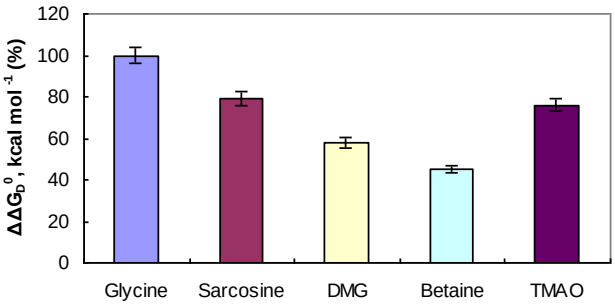


**Figure 1A: Effect of methylation on stabilizing effect of methylamines on RNase-A at pH 6.**

We then attempted to investigate whether the same observation is true for the counteraction of urea's effects by these methylamines. For this, thermal denaturation curves of RNase-A were measured in the presence and absence of different concentrations of methylamines, urea and their 2 : 1 [urea] : [methylamine] combinations. It was found that glycine fails to counteract the denaturing effects of urea at any pH tested while as all other methylamines counteract the denaturing effect of urea. Based on these results, we concluded that glycine is methylated to have a counterbalancing effect of the effect of urea. Extent of compensation (in terms of % $\Delta\Delta G_D^0$) versus the methylamines was plotted as shown in Figure 2A. It was found that

with the increase in methylation the degree of compensation decreases with the rank order as sarcosine ≥ TMAO > dimethylglycine > betaine. MDS studies have shown that methyl groups help in ordering and strengthening water structure via an increase in water-water hydrogen bonds, stronger hydrogen bonds and greater spatial ordering of the water. It was hypothesized that methylation to glycine is an inevitable phenomenon in the counteraction of urea's effects.

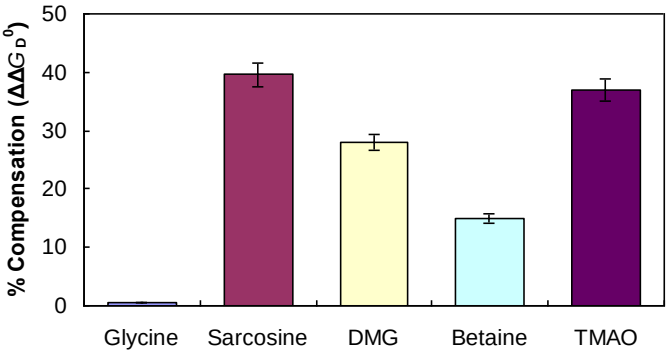


**Figure 2A: Role of methylation in urea-methylamine compensation**

As reported earlier the extent of the counteraction is not found to be perfect. Several kcal of free energy change is still required to bring about a complete compensation of the RNase-A stability. This again makes us to search for a plausible way that nature has devised for the perfect compensation to occur. Having obtained enough evidence that cellular salt (NaCl) may play an additional role in the counteraction mechanism, we thought to investigate the possible role of cellular salt in the urea-methylamine compensation. In order to elucidate the role of salt in the urea-methylamine compensation, measurement of $T_m$ and $\Delta G_D^0$ were done in the presence of urea and different concentrations of NaCl. It was observed that at a fixed concentration of urea, when increasing amount of NaCl is added, there is tremendous increase in the stability (in terms of $T_m$ and $\Delta G_D^0$) of the protein. Required salt concentration for perfect compensation at 2 : 1 (urea : methylamine) was estimated. In order to test whether our prediction for complete compensation is correct, thermal transition curves in the presence of urea-methylamine mixture at the respective estimated amount of NaCl were measured. It was found that all the transition curves are, within experimental errors, identical to that in their absence. In other words, $T_m$ values were found to be same as that of control. It was found that higher the methylation of a methylamine, greater is the amount of salt required for perfect compensation. Thus we hypothesize that salts may play a crucial role in the urea-methylamine compensation.

The observations on thermodynamic measurements needs to be validated since these are all physical parameters of the protein. In order to do so, activity studies of RNase-A with c>p as substrate were carried out in the presence and absence of urea, glycine, methylamine and 2 : 1 urea : methylamine mixture at pH 6.0 and 25 $^0$C. Glycine was found to have no effect on the kinetic parameters, $K_m$ and $k_{cat}$. It was found that urea increases $K_m$ and decreases $k_{cat}$ while as all other methylamines decrease $K_m$ and increase $k_{cat}$ of RNase-A. Glycine was not found to counteract the effects of urea on the activity of RNase-A. It was also found that 2 : 1 ratio of urea : methylamine mixtures have the partial compensatory effect. However, in the presence of NaCl (as predicted from the thermodynamic measurements), the measured $K_m$ and $k_{cat}$ values for the urea-methylamine mixture were, within experimental errors, identical to that of the control.