Mechanism of Protein Folding: Effect of Macromolecular Crowding on the Stability Curves of Chemically Induced Denatured Proteins

ABSTRACT

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The intracellular environment is highly crowded because of the presence of high concentration of biomolecules such as proteins, DNA, RNA, cytoskeleton etc and the concentration of these biomolecules in the cytoplasm is in the range of 80-400 mg ml^{-1} which accounts for nearly 5-40% of the total cellular volume.

Various studies have shown that macromolecular crowding stabilizes the protein but few studies have also shown that crowding can destabilize protein. There have been various studies in order to study the effect of crowding on simple proteins and very few studies have been done in order to find out the effects of crowding on conjugated proteins. Keeping this point in mind we chose myoglobin in order to study the effect of crowding.

All pieces of structural evidence imply that ficoll 70 at a concentration of 300 mg ml^{-1} is leading to the formation of molten globule. In order to test the generality of our previous results we performed similar experiments using dextran 70 as the crowder. Our result suggest that ficoll 70 perturbs the native tertiary structure of myoglobin leaving the secondary structure intact while dextran 70 showed no effect on the native tertiary as well as secondary structure of myoglobin. We have also examined the effect of crowding (ficoll 70 and dextran 70) on denatured myoglobin. Thus, similar experiments were performed with denatured myoglobin in order to study the effect of crowding on the tertiary and secondary structural content of denatured myoglobin. In our experiment we observed that both ficoll 70 and dextran 70 shows insignificant effect either on the tertiary structure or the secondary structure of denatured myoglobin.

Since the structure of myoglobin is perturbed in the presence of ficoll 70, we wanted to test if the structural effects observed on the native states correlate with the stability of myoglobin in the presence of ficoll 70. Although there was no effect of dextran 70 on the structure of myoglobin, we wanted to know whether it has any effect on the stability of myoglobin or not. We observed that the values of $\Delta G_D°$ and C_m of myoglobin decreased with increasing the concentration of each crowding agent. The decrease in the value of $\Delta G_D°$ and C_m signifies destabilization of myoglobin in the presence of different concentrations of each crowding agent. It was observed that the degree of destabilization was greater in case of ficoll 70 as compared to that of dextran 70. It is assumed that dextran 70 behaves as rod like structure while ficoll 70 has a spherical structure. It has been reported that dextran 70 owing to its rod like structure excludes greater volume than that of ficoll 70. But the difference in their structure could not be the reason behind their different degree of destabilization. We can hypothesize that because ficoll 70 is disrupting the tertiary structure of myoglobin, it is leading to greater destabilization than that of dextran 70. Thus we suggested that the nature of crowding agent could be supposed to play a major role in the degree of destabilization of proteins.

We observed that both ficoll 70 as well as dextran 70 compacts the tertiary structure of apomyoglobin but neither ficoll 70 nor dextran 70 have any effect on the secondary structure of apomyoglobin. It was also observed that none of the crowding agent showed any effect either on the tertiary structure or the secondary structure of denatured apomyoglobin.