THESIS ABSTRACT

The blood glucose is not directly related to the microvascular diabetic complication as previously thought, but highly reactive molecules including 3-DG, methyl glyoxal and AGEs (Advanced Glycation End Products). These heterogeneous molecules are implicated in the pathogenesis of diabetic complications leading to acute health problems. Further, AGEs covalently interact with proteins causing structural and functional alterations. The primary targets of such alterations are plasma proteins due to prolonged exposure to elevated glucose levels. Therefore, association of AGEs in regulation of diabetes and diabetic complications was mechanistically studied by identifying and characterizing glycated proteins using a combination of 2DE, Western blot and Mass spectrometric approaches.

‘Factors influencing glycation reaction in vitro’

Glycation, a non-enzymatic reaction between glucose and protein is the primary cause of diabetic complications. Albumin, the most abundant plasma protein undergoes glycation both in vivo and in vitro. The influence of albumin on glycation of less abundant proteins has not been addressed. For the first time, we show that albumin competitively inhibits the glycation of less abundant proteins, suggesting that at least in the initial stages of diabetes, albumin may protect other proteins from glycation. Also, glycation is known to be restricted to certain proteins. Previous studies report that glycation is dependent mainly on protein structure and its turnover. The role of molecular mass of protein and protein abundance in determining glycation was addressed in this study. Large molecular mass proteins such as IgG, HSA, and BSA, upon glycation showed higher increase in mass compared to small molecular mass proteins such as papain, apomyoglobin and insulin. Also, the extent of glycation was found to be more in the HSA, BSA and IgG compared to papain, apomyoglobin and insulin. This study combined with previous study on albumin glycation suggests that, in addition to the protein structure and turnover, the molecular mass of protein as well as protein abundance determines the glycation.
‘Association of albumin levels with plasma protein glycation and HbA1c in diabetes’

Albumin is one of the most abundant plasma proteins and heavily glycated in diabetes. In this study we have addressed whether variation in the albumin levels influences glycation of plasma proteins and HbA1c. The study was performed in three systems (1) streptozotocin (STZ) induced diabetic mice plasma (2) diabetic clinical plasma (3) in vitro glycated plasma. Diabetic mice and clinical plasma samples were categorized as diabetic high albumin plasma (DHAP) and diabetic low albumin plasma (DLAP) based on their albumin levels. While for in vitro experiment, two albumin levels, high albumin plasma (HAP) and low albumin plasma (LAP) were created by differential depletion of plasma albumin. Protein glycation was studied by using a combination of two dimensional electrophoresis (2DE), western blotting and LC-MS\(^E\). Identification of glycation modification was achieved by using “zoom in” approach by performing targeted database search. In both mice and clinical experiments, increased plasma protein glycation was observed in DLAP than DHAP. Additionally, plasma albumin levels were negatively correlated with HbA1c. In vitro experiments with differential depletion of albumin mechanistically showed that the low albumin levels are associated with increased plasma protein glycation, and albumin competes for glycation with other plasma protein.

‘Proteomic study reveals down regulation of Apolipoprotein A1 in plasma of poorly controlled diabetes’

Differential protein expression in diabetic plasma sample was studied by a combination of proteomic and western blot approaches. Plasma samples were categorized depending on HbA1c levels as non diabetic (ND) with HbA1c >5.8%, controlled diabetic (CD) with HbA1c 7-8 % and poorly controlled diabetic (PCD) with HbA1c > 8%. Ten plasma samples from each group were used for proteomic studies involving 2DE and LCMS\(^E\). Amongst six differentially expressed proteins in diabetes, the down-regulation of apolipoprotein A1 was more prominent in poorly controlled diabetes. Down regulation of apolipoprotein A1 could be a potential early marker of diabetic complications.