Insulin hormone is associated with a clinical condition known as insulin injection amyloidosis, characterized by formation and tissue deposition of amyloid fibrils. The milieu within pancreatic β-cells represents a particularly favorable environment for protein glycation. Recent evidences with the animal models of diabetes demonstrated that during different stages of synthesis and storage, a substantial proportion of proinsulin and insulin become glycated. In this study, insulin has been subjected to the non-enzymatic glycation under both reducing- and non-reducing conditions. The glycated insulin sample and its non-glycated counterpart were used for the structural analysis and the stress-induced aggregation. Different spectroscopic techniques and mobility shiftelectrophoresis under reducing and non-reducing conditions were applied to assess the structural alteration and oligomerization of insulin molecules as a result of non-enzymatic glycation. While non-glycated control sample of insulin easily get aggregate under chemical stress, its corresponding glycated form resists to different extent against amorphous aggregation, and the degree of resistance was correlated to the level of insulin glycation. Also the results of gel mobility shifts suggest a significant role for disulfide cross-linkings in the oligomerization of both glycated and non-glycated insulin samples. Insulin glycation may induce different structural constrains which resist against stress-induced aggregation of this protein. The proper binding of this hormone to its membrane receptor is highly important in insulin action, insulin clearance and the development of insulin resistance. The glycated insulin molecules may demonstrate an altered ability for the receptor binding which affect different biological functions of this glucose regulating hormone.