Solution SAXS Reveals Glycation-Dependent Structural Expansion and Early Aggregation in Human Serum Albumin

Y. Yeh [1], K. Liao [1], O. Shih [1], J. Chang [1], and U. Jeng [1,2]*

[1] National Synchrotron Radiation Research Center, Hsinchu 30076, Taiwan
[2] Department of Chemical Engineering, National Tsing Hua University, Hsinchu 30013, Taiwan

pennyyq3@gmail.com

Human serum albumin (HSA) is a major transport protein and an important short-term glycemic marker. Under hyperglycemic conditions, reducing sugars can non-enzymatically glycated HSA, altering its conformation, microenvironment, and aggregation behavior. To clarify these glycation-induced changes, we established an integrated analytical platform to separate and characterize fructose-glycated HSA prepared over 1-5 weeks.
An integrated BAC-SEC (Boronate Affinity Column-Size Exclusion Column) platform coupled with MALS(Multi-Angle Light Scattering), DLS(Dynamic Light Scattering), RI(refractive index), UV-Vis(Ultraviolet–visible spectroscopy), and synchrotron SAXS(Small-Angle X-ray Scattering) was used to characterize fructose-glycated HSA. SEC/MALS-DLS/RI revealed progressive formation of dimers, oligomers, and aggregates, with increased hydrodynamic radius and molecular weight during glycation. BAC-SEC separated monomeric glycated HSA into low-, medium-, and high-glycation populations. HPLC-UV showed glycation-dependent microenvironmental changes, including greater Trp exposure and tertiary loosening. SAXS further demonstrated glycation-induced structural expansion, with Rg increasing by about 2% in LGA and 10% in MGA within 3 weeks, together with subtle conformational heterogeneity.
Overall, the BAC-SEC/MALS-DLS/RI and SAXS-UV/RI platforms successfully differentiate glycated HSA by glycation degree and aggregation state, while revealing progressive size expansion and structural perturbation caused by fructose-induced glycation. These results provide a solution-based framework for evaluating how glycation alters albumin structure and support future studies of ligand or drug-binding behavior in diabetic conditions.

Keywords:
glycated HSA, SAXS, Boronate Affinity Column

References:

(1) Clinica Chimica Acta 542 (2023) 117272
(2) Diabetes 69 (2020) 760–770

Acknowledgements:
13A biological small- and wide-angle X-ray scattering (SWAXS) beamline at the Taiwan Photon Source (TPS)