Identification and properties of unconventional advanced glycation end-products present in human serum

mgr inż. Kinga Gostomska-Pampuch

Advanced glycation end-products (AGEs) are formed in result of a multistep non-enzymatic Maillard reactions leading to accumulation of highly cross-linked, stable aggregates disturbing tissue homeostasis. Glycation occurs between the carbonyl groups of reducing sugars or low molecular weight aldehydes and basic groups of proteins, lipids, and nucleic acids. AGEs can be formed either endogenously by the reaction of various metabolites or can be obtained exogenously during thermal food processing. Due to the large variety of substrates and the conditions in which glycation process occurs, the resulting products constitute a heterogeneous group of compounds with diverse biological and physicochemical properties. Glycation is exacerbated in metabolic diseases accompanied by oxidative stress, such as diabetes and in other including atherosclerosis, Alzheimer's disease or cancer. Understanding of the detailed mechanisms leading to these pathologies is the key to proper diagnosis, monitoring disease progression, and developing effective treatments. AGEs may be the diagnostic markers for specific pathological conditions, however, there is still too little information about the structures and biological properties of glycation products that accumulate in an organism.

Previous studies of our group revealed the presence of the new AGE antigen in the human body that the model structural analog is formed during glycation of proteins with melibiose in anhydrous conditions (MAGE, Melibiose-derived AGE). The aim of this thesis was to isolate the naturally occurring MAGE from human blood, identify the type of carrier protein and characterize it using spectrometric and immunochemical methods.

Several model protein glycation products (including MAGE) were synthesized by glycation of selected protein (myoglobin) with different sugars and aldehydes using either previously optimized method in dry conditions or the conventional glycation reaction in solution. The analysis of the absorption and fluorescence spectra of individual AGEs allowed for physicochemical characterization and indication of changes between structures formed with different methods. The results confirmed that MAGE is unique in comparison to the conventional AGEs described previously in literature. Moreover, the obtained low molecular weight MAGE product (LMW-MAGE) showed fluorescence and structure corresponding to the modifications present on the high molecular weight MAGE (HMW-MAGE), as confirmed by mass spectrometry analysis.

The studies consisted a selection of the single clone of the mouse hybridoma cells producing the anti-MAGE antibody using a serial dilution method. Immunochemical analysis confirmed the specificity of the obtained antibody toward the MAGE epitope on LMW-MAGE and HMW-MAGE and no reactivity with any of conventional AGEs. The natural analog of MAGE present in human blood was isolated using affinity chromatography on the resin with immobilized anti-MAGE monoclonal antibody. Using mass spectrometry and immunochemistry it has been established that MAGE epitope is present on several blood proteins including albumin, IgG and IgA. In serum of diabetic patients MAGE was identified mainly on albumin and IgG, while in healthy subjects it was found specifically on IgG and IgA.

Identification of serum proteins bearing the MAGE antigen provides new knowledge about the glycation process in the human body. In addition, the obtained specific anti-MAGE antibodies can be used in the future as a tool for determination of MAGE in tissues, while using LMW-MAGE a standard in the immunoassay will allow for absolute quantification of MAGE in biological samples.