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POSTER ABSTRACT PRESENTATIONS

SESSION TITLE: APPLICATIONS OF CRYO-EM IN MEDICINE

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Abstract P-35: Unusual Spiral Structures, Formed by Glycated Casein in the Presence of Thioflavin T

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Background: Information about the stimulation by glycation of the amyloid transformation of proteins, including beta-casein, is not well defined. On the one hand, it is possible to detect signs characteristic of amyloid structures (primarily changes in the fluorescence of thioflavin T (ThT) by various indirect methods, and on the other hand, using direct methods, primarily electron microscopy, it is not possible to reveal characteristic beta-casein amyloid fibrils. In the present work, we investigated the thermal aggregation of glycated beta-casein, paying particular attention to the effect of ThT on the formation of various aggregates, including unusual spiral structures, first discovered in this work.

Methods: Glycated beta-casein transformation was studied by the use of ThT fluorescence, fluorescence microscopy and transmission electron microscopy.

Results: This study reports an appearance of unusual spiral-like structures in aggregates formed by glycated beta-casein in the presence of ThT. Different incubation conditions – glycation agent, temperature, pH, incubation time and concentration of protein and modifier were characterized, but only glycation by 0.2 M glucose for 2 days at 37°C leads to formation of spiral structures. The beta-casein content of these structures was confirmed by ninhydrin reaction, as well as immunohistochemical staining of spiral aggregates. Interaction of glycated beta-casein with ThT leads to a 3.5-fold increase in the intensity of its fluorescence, which is about 2 times less than for dye, bound to beta-sheet structures of amyloid proteins. ThT affects the size of the particles formed by glycated beta-casein and also stimulates heat-induced aggregation not only for glucose modification but also with a relatively rapid modification by methylglyoxal. Reduction with sodium cyanoborohydride has no significant effect on the ability of ThT to interact with beta-casein and alter its properties.

Conclusion: The results suggest that such interactions of ThT molecule with glycated proteins should be taken into account while using it as an indicator for amyloid structures and as a potential agent for prevention and treatment of amyloidosis.

Key Words: beta-casein • glycation • thioflavin T • amyloid structure

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