



Comparison of modification sites in glycated crystallin in vitro and in vivo.

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Streszczenie

Glycation of α -crystallin is responsible for age- and diabetic-related cataracts, which are the main cause of blindness worldwide. We optimized the method of identification of lysine residues prone to glycation using the combination of LC-MS, isotopic labeling, and modified synthetic peptide standards with the glycated lysine derivative (Fmoc-Lys(i,iFru,Boc)-OH). The in vitro glycation of bovine lens α crystallin was conducted by optimized method with the equimolar mixture of [12C6]- and [13C6]D-glucose. The in vivo glycation was studied on human lens crystallin. The glycated protein was subjected to proteolysis and analyzed using LCMS. The results of in vitro and in vivo glycation of α crystallin reveal a different distribution of the modified lysine residues. More Amadori products were detected as a result of the in vitro reaction due to forced glycation conditions. The developed method allowed us to identify the glycation sites in crystallin from eye lenses obtained from patients suffering from the cataract. We identified K166 in the A chain and K166 in the B chain of α -crystallin as major glycation sites during the in vitro reaction. We found also two in vivo glycated lysine residues: K92 in the B chain and K166 in the A chain, which are known as locations for Amadori products. These modification sites were confirmed by the LC-MS experiment using two synthetic standards. This study demonstrates the applicability of the LC-MS methods combined with the isotopic labeling and synthetic peptide standards for analysis of post-translational modifications in the biological material.

Słowa kluczowe

Crystallin, Mass spectrometry, Solid phase synthesis, Amadori products, Non-enzymatic glycation

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