ANALYTICAL STUDIES OF RADICALAR ACETYLATION OF AMINO ACIDS BY BIACETYL/PEROXYNITRITE USING CE-MS

Assunção, N. A., Massari, J. F., Tokikawa, R., Bechara, E. J. H.

Departamento de Bioquímica - Instituto de Química – Universidade de São Paulo São Paulo, Brasil

Recently we hypothesized that acetyl radical produced by the biacetyl/peroxynitrite system might modify proteins and DNA, threatening cell functions experiencing nitrosative and carbonyl stress. This work aims the development of methods to analyze acetylated free amino acids via capillary electrophoresis-mass spectrometry (CE-MS). Analyte separation and detection was achieved with a Beckman, model MDQ™ capillary system and a Thermo Finningan, model LCD ION MAX ADVANCED mass spectrometer with ESI source. Seven amino acids (1.0 mM) were studied under conditions favorable to radicalar acetylation: L-arginine, L-histidine, L-lysine, L-phenylalanine, L-threonine, L-tryptophan and L-tyrosine. The reaction mixtures contained peroxynitrite (1.0 mM)/biacetyl (1.0 mM) in 200 M phosphate buffer, pH 7.2 at 25°C. All spent reaction mixtures, analysed by CE-MS, revealed the presence of the correspondent acetylated amino acid: *m*/*z* = 217, 198, 189, 208, 162, 247 and 224, respectively. All experiments presented satisfactory reproducibility of peak areas and time of migration. These results support our hypothesis of radicalar acetylation of biomolecules such as protein and DNA leading to possible cell injury and pave the way to unveil new biomarkers for cell damage. Support: FAPESP, CNPq, Milênio Redoxoma.