



Characterization of a bovine serum albumin model system for *in vitro* fortification of N^ε-carboxymethyl-lysine

MT Nogueira Silva Lima¹, M Howsam¹, C Delayre-Orthez², P Jacolot², S Jaisson³, J Criquet⁴, M Billamboz^{1,5}, A Ghinet^{1,5}, C Fradin¹, E Boulanger¹, F Bray⁶, S Flament⁶, C Rolando⁶, P Gillery³, C Niquet-Léridon², FJ Tessier¹

U1167—RID—AGE—Facteurs de Risque et Déterminants Moléculaires des Maladies Liées au Vieillissement, Institut Pasteur de Lille, University Lille, Inserm, CHU Lille, F-59000 Lille, France; ²Institut Polytechnique UniLaSalle, Université d'Artois, ULR 7519, Equipe PETALES, 60000 Beauvais, France; ³University of Reims Champagne-Ardenne, Laboratory of Biochemistry and Molecular Biology, CNRS/URCA UMR 7369 MEDyC, Faculté de Médecine, 51095 Reims, France; University Hospital of Reims, Laboratory of Biochemistry-Pharmacology-Toxicology, 51092 Reims, France ⁴ Univ. Lille, Laboratory of Advanced Spectroscopy for Interactions, Reactivity and Environment, CNRS, UMR 8516 – LASIRE, Lille F-59000, France; ⁵Junia, Health and Environment, Laboratory of Sustainable Chemistry and Health, 59000 Lille, France; ⁶Miniaturization for Synthesis, Analysis & Proteomics, UAR 3290, CNRS, University of Lille, 59655 Villeneuve d'Ascq Cedex, France.

Various approaches are employed to explore the physiological impacts of dietary advanced glycation end-products (dAGEs). One commonly examined dAGE is N^ε-carboxymethyl-lysine (CML) both in its free or protein-bound CML forms. The latter is usually created by subjecting a protein to a combination of glyoxylic acid (GA) and NaBH₃CN. However, this procedure generates harmful hydrogen cyanide (HCN), which might not be entirely removed through dialysis. This has led us to explore alternative methods for producing protein enriched with CML. Our goal was to explore various approaches for the production of CML-enriched bovine serum albumin (BSA), as substitutes for the conventional method employing glyoxylic acid (GA) and sodium cyanoborohydride (NaBH₃CN). The initial experiment involved a comparison of the effectiveness of three different reducing agents, namely NaBH₄, NaNH₃CN, and NaBH(OAc)₃, when introduced to a mixture containing BSA and GA. In a separate experiment, the quantity of CML generated within BSA was assessed using varying concentrations of ribose and glyoxal (GO), all without the use of a reducing agent. These preparations were made in a phosphate buffer (0.2M, pH 8.0) and incubated at 37°C for different lengths of time. Subsequently, each sample underwent dialysis and was subjected to analysis through western blot and LC-MS/MS following acid hydrolysis. Finally, the most promising samples underwent proteomic characterization. The reaction involving GA (6 mmol/L) and NaBH₃CN (21 mmol/L) remained the most efficient in yielding CML at rates ranging from 24-35%. Following this, the use of 300 mmol/L GO resulted in CML yields of 13-24%. GA exhibited a preference for specific lysine modification into CML, causing fewer structural changes to the BSA molecule compared to GO. This distinction was confirmed through fluorescence and proteomic analyses. Conversely, GO promoted a higher degree of arginine modification in comparison to GA (76% vs. 23%). Despite the structural alterations to BSA caused by GO, the clearance of CML in murine feces was consistent with previously reported values. Thus, the glycation of BSA with 300 mmol/L GO represents a viable alternative to GA and NaBH₃CN for generating CML-enriched protein without the generation of HCN. However, a model focusing solely on CML fortification remains to be developed.