University of Fribourg / Faculty of Science and Medicine / Department of Chemistry

Synthesis of Nerve Agent- and Pesticide-Protein Bioadducts as Reference Materials for Retrospective Verification of Exposure

Andreas Bielmann

Cholinesterase inhibiting organophosphorus compounds are highly toxic and are used as pesticides or nerve agents. As most chemical warfare agents, nerve agents are prohibited by the Chemical Weapons Convention. To enforce the Convention, the Organisation for the Prohibition of Chemical Weapons maintains a network of laboratories, which are designated to analyse samples after alleged incidents involving chemical warfare agents. The gold standard for the retrospective verification of exposure to nerve agents in biomedical samples is the LC-MS analysis of a specific nonapeptide with the sequence FGES*AGAAS obtained after pepsin digestion of butyrylcholinesterase. Organophosphorus inhibitors will leave a specific marker on the serine indicated by *. Reference materials for this analysis are often produced by spiking of blood with agents. However, this method only gives reference peptides of inadequate purity. For the development of analytical methods and for quantitative analysis highly pure synthetic peptides are required.

In this work, the synthesis of phosphylated peptides via a building block approach was investigated. Synthesis protocols were established and the limitations of the method were investigated. Phosphylated amino acid precursors and the resulting peptides have some intrinsic labilities, therefore an optimized SPPS protocol was developed to overcome them. Using this protocol, two pathways to synthesize the aged-nonapeptide adduct were investigated and compared. Further, the bioadducts of the nerve agents sarin, cyclosarin, VX, CVX and RVX, as well as the methyl methylphosphono- and isopropyl isopropylphosphono nonapeptides were synthesized to determine the scope of the method. The method was further extended to synthesize the soman adduct and two biomarkers of tabun exposure. The synthesis of a third tabun adduct was investigated intensively. The BuChE-bioadducts of the pesticide fonofos and diethoxy type pesticides were successfully synthesized. Work towards the synthesis of thiophosphate adducts remained unfinished, but promising first results were achieved. Further, three newly published bioadducts of albumin containing a phosphorylated tyrosine site were produced. Peptides carrying different isotope labels were synthesized and evaluated for their usefulness as reference materials for LC-MS analysis. The stability and storability of synthetic peptides in different media was investigated over several months.

As an alternative to the building block approach, the global phosphonylation of the nonapeptide with sarin, chlorosarin, chlorosoman and chloro-VX was investigated. Most promising results were obtained when semi-protected peptidyl-resins were treated with phosphonochloridates.

Jury:
Prof. Dr. Christian G. Bochet (thesis supervisor)
Prof. Dr. Katharina Fromm (internal co-examiner)
Dr. Fabien Cougnon (external co-examiner)
Dr. Christophe Curty (external co-examiner)
Prof. Dr. Marco Lattuada (president of the jury)