J Proteomics Bioinform 2018, Volume 11
DOI: 10.4172/0974-276X-C5-125

# Protein carbamylation: The discovery of a carbon dioxide control system

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Carbon dioxide ($CO_2$) is fundamental to life with critical roles in respiration, photosynthesis and acid-base homeostasis. Carbamates are formed rapidly but reversibly by the nucleophilic attack of an uncharged amine on $CO_2$ at physiological temperatures and pressures. The presence of this post-translational modification has been demonstrated in a small number of key proteins, such as RuBisCO and haemoglobin. These identified carbamates have clear functional roles confirming that carbamate formation is a mechanism for linking protein activity to environmental $CO_2$. The systematic identification of carbamates has been hindered due to their labile nature; therefore previous work has involved their study under non-physiological conditions. We have developed a novel technology using a chemical trapping technique to covalently modify carbamates and remove their labile nature. This is combined with downstream proteomic analysis via tryptic digest and ESI-MS, open access proteomic software X!Tandem has been used to validate the carbamate modifications identified. The developed method successfully identified the haemoglobin carbamate binding site under physiologically relevant conditions. These results were confirmed using ESI-MS combined with $^{12}C$ and $^{13}C$ isotope incorporation. Screening of *A. thaliana* leaf proteome homogenates identified several carbamylated proteins previously unknown to directly interact with $CO_2$. The carbamate binding site within peroxidase At3g49120 was investigated and has been demonstrated to directly influence the protein activity by mutation of the lysine binding site. This research has produced a method capable of removing the labile nature of carbamates and thereby providing the first description of systematic carbamate identification in a physiologically relevant environment.

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