FREE AMINO ACID SUPPLEMENTATION PREVENTS PROTEIN MODIFICATIONS ASSOCIATED WITH CHRONIC KIDNEY DISEASE

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Introduction

Chronic kidney disease (CKD) affects over 10% of the population worldwide. In CKD patients the plasma concentration of uremic toxins is increased due to reduced kidney function [1]. These increased concentrations cause numerous pathological post-translational modifications (PTMs), like carbamylation, guanidinylation or oxidation of proteins, which can lead to an alteration of their conformation, activity or function [1,2]. Specifically, PTMs of proteins like albumin are undesired, since their high mass prevents them to be cleared by the kidney or hemofiltration, ultimately leading to an accumulation of uremic modified proteins [3]. Therefore, preventing PTMs beforehand is of high interest. Here we analyse the effect of amino acid supplementation and their capability to protect proteins from PTMs.

Methods

PTMs were induced in the proteins Carboxypeptidase E and Calpain1 after incubation with different concentrations of o-methylisourea and hydrogen peroxide. Afterwards dialysis and digestion of the protein samples with trypsin was done and analysis with mass spectrometry was performed. Once finding optimal conditions to induce PTMs, amino acids were given to the sample before inducing modification.

Results

Incubation with o-methylisourea led to guanidinylation of Carboxypeptidase E. The modification was only observed at one fragment. This fragment contains the sequence ***ELLVIELSDNPGVHEPGEPEFK**** (87-108). Amino acid marked with * (Lysine) indicates position of modification. Addition of free lysine before the toxin led to prevention of the PTM in the protein.
Incubation with hydrogen peroxide led to oxidation of Calpain1. Modification was observed in multiple fragments: ***LETM*FR*** (682-687), ***M*AIESAGFK*** (642-650), and ***M*EDGEFWM*SFR*** (329-339). Amino acids marked with * indicates position of modification. In all fragments the methionine residues were oxidized. Addition of free methionine before the toxin led to prevention of the PTM in all fragments.

Discussion

Post-translational modification is an important mechanism in mediating proper protein function, as they can influence multiple aspects of proteins including: folding, binding affinity, translocation or activity. Pathological post-translational modification mediated by uremic toxins might interfere with these tightly regulated mechanism. Therefore, finding an option to prevent these uremic toxin associated PTMs and reducing uremic toxin concentration in CKD patient in general is an anticipated therapy option to improve patient outcome. Here we analyzed the induction of PTMs by incubation of proteins with two uremic toxins. Guanidinylation was successfully induced by o-methylisourea and oxidation by hydrogen peroxide, both modification are known to be more abundant in patients with chronic kidney disease [4]. Moreover, here we showed that free amino acid supplementation is able to prevent toxin induced PTMs and seems to be a promising approach to tackle the problem of PTMs in CKD patient.
The underlying mechanism will be further elucidated, as these preliminary results show first promising effects *in vitro*. The next step is to analyze if amino acid supplementation also prevents CKD associated PTMs in an *in vivo* mouse model.

References

1. Laget, J., *et al.*, *Molecular Aspects of Medicine*, *86*. (2022).
2. Schunk, S., *et al*. *Journal of the American Society of Nephrology*, *32*(12), 1–15 (2021).
3. Sánchez-Ospina, D., *et al*. *Journal of Clinical Medicine*, *13*(5). (2024)
4. Noels, H., *et al*. *Nature Reviews. Nephrology*. (2024)

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