# Cellular studies of the two main isoforms of human D-aspartate oxidase

P-02.2-08

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Human D-aspartate oxidase (hDASPO, EC 1.4.3.1) is a peroxisomal flavoenzyme that selectively degrades the D-enantiomers of acidic amino acids and is the only enzyme known to degrade D-aspartate (D-Asp). In mammals, D-Asp is present in the central nervous system, where it acts as a signaling molecule and is involved in neural development, brain morphology and behavior [1]. Studies performed in animal models (DASPO$^{-/-}$ knock out mice) demonstrated that, by regulating D-Asp concentration in the brain, DASPO impacts on glutamatergic neurotransmission, thus preventing precocious age-related deterioration processes [2]. The UniProtKB database reports three hDASPO isoforms, constituted by 369, 341 and 282 amino acids. To date the different isoforms have only been partially characterized and notably the properties of the longest putative isoform have never been studied. Here, we identified the additional N-terminal peptide of the hDASPO_369 isoform only in the hippocampus of female Alzheimer's disease (AD) patients, while peptides common to hDASPO_369 and hDASPO_341 isoforms were present in samples from both male and female healthy controls and AD patients. Unfortunately, the hDASPO_369 isoform was largely produced in *E. coli* as inclusion bodies, thus hampering its biochemical characterization. However, the functional properties, the degradation kinetics and the mechanisms involved in cellular turnover of hDASPO_341 and hDASPO_369 were investigated by ectopically expressing these isoforms in the U87 human glioblastoma cell line. This study demonstrated that both protein isoforms are active (showing similar kinetic properties), localize to the peroxisomes, are very stable (with an estimated half-life of approximately 100 hours) and are primarily degraded through the ubiquitin-proteasome system [3].

[1] Usiello A et al. (2020) Int J Mol Sci 21, 8718.
[2] Cristino L et al. (2015) Neurobiol Aging 36, 1890-1902.
[3] Rabattoni V et al. (2021) FEBS J, in press.