Half way to hypusine. Structural characterization of human deoxyhypusine synthase.

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Deoxyhypusine synthase (DHS) is a transferase catalysing the formation of deoxyhypusine, which is the first, rate-limiting step of unique post-translational modification: hypusination. DHS catalyzes the transfer of 4-aminobutyl moiety of spermidine to a specific lysine of eIF5A precursor in an NAD-dependent manner. This modification occurs exclusively on only one protein: eukaryotic translation initiation factor 5A (eIF5A) and it is essential for cell proliferation [1]. Malfunctions of the hypusination pathway, including those caused by mutations within the DHS encoding gene, are associated with such conditions as cancer or neurodegeneration [2].

The presented study aimed to investigate substrate specificity of the first step of hypusination using macromolecular crystallography as the main tool and additionally to assess the impact of newly recognized pathological mutations in DHS coding gene on protein stability, activity and structure.

Human DHS wild type and its two mutants were expressed, purified and crystallized. Our attempts lead to six high-resolution crystal structures of DHS wt in apo form and complexes with natural substrates. Based on crystal structures and activity tests it was shown that despite almost identical binding of spermidine and spermine, probably only spermidine can serve as a proper substrate of deoxyhypusine formation. Furthermore, it was shown that against the previous studies, no conformational changes occur in the DHS structure upon spermidine-binding [3].

Availability of high-quality structural data will aid the design of novel DHS inhibitors for potential applications in cancer therapy and can significantly advance our understanding of newly recognized genetic DHS disorder.

[1] Park MH, Wolff EC. Hypusine, a polyamine-derived amino acid critical for eukaryotic translation. *J Biol Chem.* 2018;**293**(48):18710-18718.

[2]. Ganapathi M, Padgett LR, Yamada K, et al. Recessive Rare Variants in Deoxyhypusine Synthase, an Enzyme Involved in the Synthesis of Hypusine, Are Associated with a Neurodevelopmental Disorder. *Am J Hum Genet.* 2019;**104**(2):287-298.

[3]. Wątor E, Wilk P, Grudnik P. Half Way to Hypusine-Structural Basis for Substrate Recognition by Human Deoxyhypusine Synthase. Biomolecules. 2020;10(4):522.

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