Structural properties of human D-3-phosphoglycerate dehydrogenase

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L-Serine (L-Ser) plays a pivotal role in the central nervous system and alterations of its level have been linked to severe neurological disorders (previously reported in: Tabatabaie L et al. (2011) J Inherit Metab Dis., 34(1):181-184). D-3-phosphoglycerate dehydrogenase (PHGDH) catalyses the rate-limiting step in the synthesis of L-Ser via the phosphorylated pathway. Structurally, the human PHGDH is an homotetramer with an N-terminal region involved in the dimerization and formation of the substrate and nucleotide binding sites, and a C-terminal region including two regulatory domains: the ACT (aspartate kinase-chorismate mutase-tyrA prephenate dehydrogenase) and ASB (allosteric substrate binding) domains. The crystal structure of human PHGDH was solved only for a truncated dimeric form consisting of the substrate and cofactor binding domains. In this work, in silico analysis using AlphaFold and focusing on the inter-subunit interactions at the tetrameric interface, allowed to produce a model of the tetrameric PHGDH and the identification of essential residues involved in the oligomerization, which were eventually analysed via alanine-scanning mutagenesis. Interestingly, R454A, L478A, P479A, and Y495A variants within the ACT domain appear crucial for preserving a stable tetrameric assembly. These substitutions lead to significant structural alterations, resulting in hampered activity, decreased stability and misfolding. Conversely, the F418A variant in the putative ASB domain, halved the activity, slightly modified the tetrameric structure and the protein stability. This latter position appears to be mainly involved in the recognition of dimers leading to the formation of the tetramer. In conclusion, the predicted residues appear to be crucial for the tetramer formation and the proper folding of human PHGDH. This research was funded by a grant from the Ministero Università e Ricerca Scientifica PRIN 2017.