First insights into the structure and function of the human glycogen debranching enzyme

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Glycogen is the most important short-term reserve of carbon and energy, consisting of α-1,4 linked glucose with a regular α-1,6 branch every 8-12 residues. Complete degradation of glycogen requires the action of the glycogen phosphorylase and the glycogen debranching enzyme (GDE). Human GDE is a multi-domain enzyme with dual activity, having glucanotransferase and glycosidase activity. First, GDE transfers a maltosyl/maltotriosyl group from a branch to a neighbouring linear chain via its transferase activity. Second, it cleaves the last glucose residue of the remaining branch, thus allowing the glycogen phosphorylase to hydrolyse the linear part of glycogen [1]. GDE mutations can lead to a rare genetic syndrome, characterised by liver, skeletal muscle and/or heart dysfunctions [1, 2]. Available data on structure and function are mainly derived from yeast homologs [2, 3], whereas human GDE (hGDE) is poorly understood on a molecular level.

Here, we show that 175 kDa hGDE expressed in *Escherichia coli* and purified by liquid chromatography is functional and monomeric. We aim to characterise the function of its different domains by rational design of protein constructs, combined with biochemical assays and structural analysis, to shed light on the important functional motifs for efficient catalysis. Our CryoEM structure shows that the overall architecture of hGDE agrees with the previously released crystal structure of a characterised yeast homologue [2]. Additionally, suitable crystallisation conditions were identified by biased hanging-drop crystallisation experiments, paving the way to an integrative structural analysis of this enzyme. Together, the collected results will provide insights into the catalytic mechanism and the overall interaction of the enzyme with its complex, natural substrate. Moreover, the role of disease-causing mutations will be assessed, paving the way to a deeper understanding of the clinical features observed in patients.

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