PRELIMINARY STRUCTURE OF THE *E. coli* GlnD BY ELECTRON MYCROSCOPY

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Escherichia coli GlnD protein is a bifunctional uridylyltransferase/uridylyl-removing enzyme, which senses the intracellular nitrogen levels via glutamine. This signal is transduced to GlnB and GlnK (PII-like proteins) that are uridylylated or deuridylylated by GlnD, under nitrogen limitation or excess, respectively. The covalent modification of PII proteins controls the activity of the NTR system and glutamine synthetase (GS). NTR is a two component system: NtrB/NtrC which controls the transcription of nitrogen-regulated genes. Under nitrogen excess, PII proteins interact with NtrB and catalyses the NtrC dephosphorylation, inactivating it. Under nitrogen limitation, NtrB does not interact with PII-UMP and in this form NtrB catalyses the NtrC phosphorylation, activating it. GS catalyses glutamine synthesis and its activity is controlled in several ways including covalent modification by GlnE. PII proteins interact with GlnE under nitrogen limitation and excess, leading to GS deadenylylation (activation) or adenylylation (inactivation), respectively. GlnD plays a pivotal role in sensing the cytoplasmic nitrogen level, however until now there is no structural information available. The objective of this study is to determine the GlnD structure by electron microscopy and 3D reconstruction. GlnD was overexpressed in *E. coli* YMC10 from the plasmid pDOP1 and purified by ion exchange and size exclusion chromatography. Single particle EM data was collected of purified GlnD under negative stain conditions, using uranyl-acetate. Under low salt conditions (50mM KCl), the images showed that the protein mostly formed unspecific aggregates of higher oligomeric nature. However, under high salt conditions (200mM KCl) GlnD seems to mostly form hexameric, ring. This observation is consistent with the molecular weight of 614kDa as determined by size exclusion chromatography.

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