The Role of Glutamine Synthetase in the Pathogenesis of Neisseria meningitidis

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Background and Aims

- Glutamine synthetase (GlnA) is an enzyme which catalyses the ATP dependent and reversible formation of glutamine, ADP and Pi from ammonia and glutamate.
- In addition to its main roles in glutamine synthesis and nitrogen assimilation for the cell during low nitrogen conditions, GlnA has also been implicated in carrying out additional 'moonlighting' functions in several different bacteria.
- Moonlighting functions of GlnA identified to date include virulence (Chandra et al., 2010), colonization of host cells (Si et al., 2009) and binding of host proteins (Kainulainen et al., 2012).
- The role of GlnA in the bacterial pathogen Neisseria meningitidis is still poorly understood.

The aim of this study is to determine if meningococcal GlnA is a moonlighting protein and identify possible moonlighting functions.

Methods

Plasmids: the gene encoding meningococcal GlnA (NMB0359 of strain MC58) was cloned into the IPTG-inducible pQE-30 vector encoding an N-terminal His-tag.

Protein Purification: IMAC was used to purify His-tagged rGlnA under native conditions using an AKTA prime plus analyzer.

Mutagenesis: A complemented strain was created by inserting a second copy of glnA at an ectopic site in the MC58 WT strain.

ELISA: Whole cell ELISA was performed to determine if GlnA is surface localized.

Results (1) Purification of recombinant GlnA

SDS-PAGE (A) analysis of the purification process of rGlnA under native conditions, (B) SDS-PAGE analysis of desalted rGlnA, (C) Immunoblot analysis of desalted rGlnA. Lane M: pre-stained protein marker, Lane 1: whole cell lysate, Lane 2: pellet, Lane 3: supernatant lysate, Lane 4: flow through, Lane 5: wash, Lane 6-10: elution fractions, Lane 11: desalted and concentrated protein.

Results (2) Creation of complemented strain

glnA PCR amplified (A) and ligated into the unique BglII site of the complementation vector (B) and transformed into E. coli. Clones selected by restriction digestion with BglII and sequencing (C). Natural transformation of WT N. meningitidis. Correct clone selected by erythromycin resistance as well as colony PCR using primers that hybridise to NMB0102 and NMB0103 (D). Strain designated as WT-GlnA<sup>ext</sup>.

Results (2) ELISA to investigate GlnA surface localization

Data representation of three independent ELISA experiments run in triplicate. Unpaired t-test analysis between either WT or WT-GlnA<sup>ext</sup> and the control wells (BSA) showed significant difference ****p<0.0001. Unpaired t-test analysis between WT and WT-GlnA<sup>ext</sup> showed no significant difference.

Conclusions

- MC58 glnA successfully cloned and rGlnA expressed in E. coli
- rGlnA purified and rabbit polyclonal antisera (RoGlnA) generated
- glnA successfully complemented in N. meningitidis. (WT-GlnA<sup>ext</sup>)
- GlnA is surface-exposed in N. meningitidis.

References

