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HETEROLOGOUS PRODUCTION AND CHARACTERISATION OF
A YEAST PEPTIDE:N-GLYCANASE

A thesis presented in partial fulfillment of the requirements for
the degree of Master of Science
in Biochemistry
at Massey University, New Zealand

Kun Hong
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Abstract

Peptide: N-glycanases (PNGases) remove N-linked glycans from glycoproteins. Three distinct families of PNGases have been characterised, although all of them not completely. Some of these PNGases are cytosolic, others are secreted. Cytoplastic PNGases (Png1p) are implicated in the proteasomal degradation of newly synthesized misfolded or unfolded glycoproteins that are exported from the endoplasmic reticulum (ER). Cytoplastic PNGases are encoded by the PNG1 gene and have been classified as members of transglutaminase-like superfamily based on the sequence analyses. There has, however, been no report of transglutaminase activity in any PNGase. The three-dimensional structures of recombinant PNGases from yeast (S. cerevisiae) and mouse have been determined in complex with the XPCB domain of Rad23 and mHR23B respectively. These PNGases were both produced as insoluble proteins, and could only be refolded and crystallised in the presence of their physiological binding partners.

In this study, the gene encoding for S. pombe PNGase has been cloned and heterologously expressed as a soluble thioredoxin-fused protein. The proteolytic cleaved recombinant protein (rPNGase Sp) remained soluble as a monomer and retained its deglycosylating activity. It did not have, however any transglutaminase activity despite its homology to the transglutaminase family of proteins. The activity of rPNGase Sp in vitro is both reductant and Zn\(^{2+}\) dependent. rPNGase Sp showed apparent heterogeneity on SDS-PAGE, which was characterised by the appearance of two bands differing in their molecular weights by an ~ 2.3 kDa. This heterogeneity was eventually shown to be the result of two different local conformations that were dependent on disulfide bond and/or Zn\(^{2+}\). The enzyme was shown to only deglycosylate the denatured glycoproteins, not their native counterparts. Moreover, it preferred to deglycosylate glycoproteins with high mannose-type glycan chains, both of which are consistent with the biological function of cytoplasmic PNGases.

Compared to bacterial PNGase F, rPNGase Sp is not very active, at least on the substrate used in this study. A higher $K_m$ (186 µM) determined for rPNGase Sp using a FITC-labelled glycopeptide which carries a complex-type glycan as the substrate also suggests that complex glycans are not favoured substrates for these PNGases. rPNGaseSp has similar characteristics to the yeast (S. cerevisiae) and mouse PNGases; it has a neutral pH optimum and is strongly inhibited by Cu\(^{2+}\), Cd\(^{2+}\) and Ni\(^{2+}\). EDTA treatment deactivates it, and the addition of Zn\(^{2+}\) could not restore its activity. Interestingly, addition of exogenous Zn\(^{2+}\) was found to strongly inhibit rPNGase Sp.
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