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**Independently Expressed N-Terminal Pro-Domain of Aqualysin I Precursor Complements the Folding of Its Mature Domain to Active Form in *Escherichia coli***

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Aqualysin I is a subtilisin-type serine protease secreted into the culture medium by *Thermus aquaticus* YT-1. It is first produced as a large precursor that consists of a signal peptide, an N-terminal pro-domain, the mature protease domain and a C-terminal pro-domain. This precursor is autocatalytically processed to the mature protease. Previous our studies have shown that the N-terminal pro-domain is essential for the production of active aqualysin I and may function either in cis as a part of the precursor or in trans as a separate polypeptide. To investigate whether the N-terminal pro-domain supplied in trans as an independent peptide plays an important role in the folding and secretion of the protease, we have expressed the N-terminal pro-domain in *E. coli* independent of the mature domain with or without the C-terminal pro-domain using expression system with separate promoters and signal peptides. Protease assay and SDS-PAGE clearly showed that the N-terminal pro-domain plays an essential role in guiding the proper folding in trans of the enzymatically active conformation of aqualysin I. The N-terminal amino acid sequences of the purified enzymes were identical and had not signal peptides. These results indicate that independently expressed domains are secreted into the periplasmic space before the N-terminal pro-domain-assisted folding of mature domain.