Assembly of Lamb protein into the outer membrane of Escherichia coli

Vos-Scheperkeuter, Grietje Henny

9

Summary

Assembly of outer membrane proteins is a very complicated process. It involves protein synthesis in the cytoplasm, transport of the newly synthesized protein across the cytoplasmic membrane and the periplasmic space, folding of the polypeptide chain into its proper conformation and - in some cases - conversion of the folded molecules into larger complexes, for example oligomers. In this thesis, several aspects of the synthesis and assembly of LamB protein, an outer membrane protein of *Escherichia coli*, are described.

LamB protein is a part of the *E. coli* maltose system; the protein is assembled in the outer membrane as a trimer and it functions as a pore facilitating diffusion of maltose sugars (maltose or larger maltodextrins). In addition, the maltose system comprises a number of other transport proteins and metabolic enzymes. Maltose activates the regulatory *malT* gene product which, in turn, stimulates expression of the three operons of the maltose system (see Fig.1, Ch.1). The maltose system is also regulated by catabolite repression.

Following induction, LamB protein is synthesized and transported to the outer membrane. These processes most likely occur synchronously, i.e. growing polypeptide chains are transported to the outer membrane in a vectorial way, thereby guided by the transient N-terminal signal peptide. The implications of this mode of insertion, called co-translational insertion, are described in Chapter 2. An important notion is that there must be insertion regions, defined as envelope areas lying above polysomes synthesizing an outer membrane protein.

In Chapters 3 and 4, the localization of LamB insertion regions on the intact cell is described. The analysis was performed with two types of cells: wild type cells in which the LamB protein is induced by cyclic AMP and maltose (Ch.4) and

operon-fusion cells in which the *lamB* gene is expressed under *lac* promoter control (Ch.3). In the latter case, induction was achieved by IPTG, the inert inducer of the lactose system. One and a half minute after the first, newly induced, LamB molecules appeared at the cell surface, cells were fixed and the position of the LamB molecules was determined with the aid of specific antibodies and an electron dense probe. The probe consisted of gold particles (with an average diameter of 16 nm) which were coated with protein A; since the latter protein binds specifically to IgG, the gold particles thus revealed the position of the LamB molecules. By analysing large numbers of cells, it was established that the newly induced protein was distributed homogeneously all over the (wild type or operon-fusion) cell; areas of local enrichment could not be observed. Since lateral diffusion - most likely - can not account for such quick homogeneous distribution (see Fig.8, Ch.3), it is concluded that LamB protein is inserted into the outer membrane at sites distributed all over the cell. A remarkable feature was observed with respect to the number of LamB molecules that was synthesized during the short period of induction (i.e. the rate of LamB synthesis): this rate was constant and independent of cell size for non-dividing cells, whereas the rate of synthesis was found to increase two-fold during the division process.

The events preceding cell surface exposure of the newly synthesized LamB molecules could largely be resolved by immunoprecipitation with LamB-specific antisera (Chapter 5). Two antisera with different specificity ranges were used: one directed against the monomeric form of LamB protein and the other one directed against LamB trimers. The number of antigenic determinants recognized by each of the antisera and their location on the protein sequence could partly be determined (Chapter 6).

In whole cell lysates, newly synthesized LamB protein was detected as a number of different species (Ch.5): (1) nascent polypeptide chains of various sizes, (2) polypeptides - smaller than LamB protein - of discrete sizes, (3) precursor LamB (LamB protein which still contains the signal peptide), and (4) mature LamB protein, either in monomeric form or as trimers. Most LamB species were recognized by both types of specific antiserum. There were, however, two exceptions: anti-monomer antibodies recognized a larger fraction of nascent LamB, whereas LamB trimers were recognized by anti-trimer antibodies, exclusively. Correspondingly, anti-trimer antibodies were the only ones to recognize cell surface exposed parts of LamB protein.

Using the different specificities of the two antisera (see also Ch.8), the assembly pathway of newly induced LamB protein could be determined. Following

induction, the fi
at least 60 secon
nascent polypepti
polypeptides of d
lation that was t
on the mRNA corre
pletion of transl
15 seconds, monom
are metastable: t
LamB protein. Sub
lying peptidoglyc
they are re-arran
seconds after syn
structures follow

Samenvatting

Gram-negatieve
hun cel-inhoud (cy
mantel bestaat uit
(1) het cytoplasma
(2) een waterige l
(3) het buiten-mem
In de periplasmati
glycanlaag: deze b

In dit proefsch
bepaald buitenmemb
genoemd naar het b
wit bevat: het *lam*
geaktiveerd wordt
nen) in het omring
kers als energiebr
transport-systemen
moeten enzymen kor
deze noodzakelijke

induction, the first antigenic determinants were detected after a time lag of at least 60 seconds. These first determinants were found to be located on nascent polypeptide chains. Sometimes, translation appeared to be abortive: polypeptides of discrete sizes were observed that possibly originated from translation that was terminated prematurely. Interruptions might occur at positions on the mRNA corresponding to minor codons (see Supplement to Ch.6). Upon completion of translation, the LamB protein is found in a monomeric form. Within 15 seconds, monomers are assembled into trimers. These newly assembled trimers are metastable: they dissociate at much lower temperatures than fully matured LamB protein. Subsequently, the new trimers start an interaction with the underlying peptidoglycan-layer (about 30 seconds after completion of translation) and they are re-arranged such as to become exposed at the cell surface (within 46 seconds after synthesis). Finally, maturation of the new trimers into heat-stable structures follows synthesis after at least 3 minutes.

Samenvatting

Gram-negatieve bacteriën zoals *Escherichia coli* hebben, ter bescherming van hun cel-inhoud (cytoplasma), een nogal ingewikkelde mantel (cel-envelop). Deze mantel bestaat uit een drietal lagen; van binnen naar buiten zijn dat (1) het cytoplasmatische of binnen-membraan dat de cel-inhoud direkt omgeeft, (2) een waterige laag tussen twee membranen in (de periplasmatische ruimte) en (3) het buiten-membraan dat in direkt kontakt staat met het omringende milieu. In de periplasmatische ruimte bevindt zich een soort skelet, de zgn. peptidoglycanlaag: deze bepaalt in hoge mate de (staaf)vorm van de bacterie.

In dit proefschrift worden verschillende aspekten van de synthese van één bepaald buitenmembraan-eiwit beschreven. Het betreffende eiwit is het LamB eiwit, genoemd naar het brokstukje erfelijke informatie (DNA) dat de code voor dit eiwit bevat: het *lamB* gen. Het eiwit vormt een deel van het maltose-systeem, dat geaktiveerd wordt als er maltose (of langere, soortgelijke suikers: maltodextrinen) in het omringende milieu aanwezig zijn. Wil de bacterie deze speciale suikers als energiebron kunnen gebruiken, dan moet het zich aanpassen: er moeten transport-systemen komen zodat het maltose in de cel kan worden opgenomen en er moeten enzymen komen die voor de verdere verwerking van het voedsel zorgen. Al deze noodzakelijke componenten liggen besloten in het maltose-systeem; het wordt