Characterization of the C-terminal Propeptide Involved in Bacterial Wall Spanning of α-Amylase from the Psychrophile Alteromonas haloplanctis*

(Received for publication, October 22, 1997, and in revised form, March 6, 1998)

Georges Feller‡¶§, Salvino D’Amico¶, Abderrafi M. Benotmane‡, Fabian Joly‡, Jozev Van Becumen¶, and Charles Gerday‡‡

From the ¶Laboratory of Biochemistry, Institute of Chemistry B6, University of Liege, B-4000 Liege and the ‡Laboratory of Protein Biochemistry and Protein Engineering, University of Gent, B-9000 Gent, Belgium

The antarctic psychrophile Alteromonas haloplanctis secretes a Ca²⁺- and Cl⁻-dependent α-amylase. The nucleotide sequence of the amy gene and the amino acid sequences of the gene products indicate that the α-amylase precursor is a preproenzyme composed by the signal peptide (24 residues), the mature α-amylase (453 residues, 49 kDa), and a long C-terminal propeptide or secretion helper (192 residues, 21 kDa). In cultures of the wild-type strain, the 70-kDa precursor is secreted at the mid-exponential phase and is cleaved by a nonspecific protease into the mature enzyme and the propeptide. The purified C-terminal propeptide displays several features common to β-pleated transmembrane proteins. It has no intramolecular chaperone function because active α-amylase is expressed by Escherichia coli in the absence of the propeptide coding region. In E. coli, the 70-kDa precursor is directed toward the supernatant. When the α-amylase coding region is excised from the gene, the secretion helper can still promote its own membrane spanning. It can also accept a foreign passenger, as shown by the extracellular routing of a β-lactamase-propeptide fusion protein.

Most Gram-negative bacteria actively secrete proteins to the extracellular medium in amounts sometimes comparable to those achieved by Gram-positive bacteria or by yeast. Polypeptides secreted by Gram-negative bacteria include biodegradative enzymes, toxins, and pathogenicity factors that have important industrial or medical applications. As a result, there is now considerable interest in the elucidation of the molecular mechanisms that allow a polypeptide to initiate a journey in the cytoplasm and its subsequent routing to a specific cellular compartment. These studies have highlighted the remarkable diversity of the targeting processes involved in bacterial secretion (1–4).

Proteins secreted by the major secretory pathway cross the bacterial wall in a two-step mechanism via the periplasm. Exoproteins taking this two-step route possess a N-terminal signal peptide and use the general secretion system. Hemolysin-type secretory pathway cross the cell envelope by a single-step process without periplasmic intermediates. Proteins targeted through this pathway have no N-terminal signal sequence and show sec-independent translocation to the medium. However, they require the assistance of accessory proteins, encoded by genes contiguous to the exoprotein gene, which are presumed to form a “pore” or an intermembrane channel. Secretion signals essential for translocation are located in the C-terminal part of these proteins.

All proteins transported by these pathways do not contain sufficient internal information to reach the external medium without assistance. By contrast, the unusual secretion system of gonococcal IgA proteases employs a two-step route, but after sec-dependent translocation of the inner membrane, the periplasmic intermediate is directed to the outer membrane by a C-terminal propeptide, which is subsequently cleaved by autolysis of the enzyme precursor (5–7). Propeptides are not uncommon but are usually found in the N-terminal sequence of proteases; they are essential for acquisition of the final folding of the active enzyme (8, 9).

α-Amylase from the antarctic psychrophile Alteromonas haloplanctis has been extensively analyzed in the context of enzyme adaptations to catalysis at low temperatures (10–13). We found that its secretion is assisted by a C-terminal propeptide. Unlike other propeptides, the C-terminal domain of the α-amylase precursor has no intramolecular chaperone function but constitutes an autonomous secretion signal that can be purified from culture supernatant after proteolytic processing. We report here structural and functional analyses of this C-terminal secretion helper.

EXPERIMENTAL PROCEDURES

Purification of α-Amylase, Propeptide, and Protease—The antarctic bacteria A. haloplanctis A23 was grown at 4 °C for 3–5 days in 1-liter Erlenmeyer flasks containing 400 ml of broth (16 g/liter Bactotryptone, 16 g/liter yeast extract, 20 g/liter NaCl, 10 g/liter sea salts, 30 g/liter maltose, pH 7.6) run at 250 rpm. After concentration and dialfiltration (10, 11), the culture supernatant was loaded on a DEAE-agarose column (2.5 × 40 cm) equilibrated in 50 mM Tris-HCl, 1 mM CaCl₂, pH 7.5, and eluted with a NaCl linear gradient (500 ml–500 ml, 0–0.8 M NaCl). The propeptide was eluted in front of the gradient, the protease at 0.3 M NaCl, and the α-amylase at 0.5 M NaCl. The latter was further purified as described (11).

Fractions containing the propeptide were brought to 4 M NaCl and loaded on a Phenyl-Sepharose CL-4B column (1.5 × 20 cm), subsequently washed with a decreasing gradient (40 ml) from 4 to 0 M NaCl in 50 mM Tris-HCl, pH 7.5, at room temperature. Proteins were eluted with a gradient (40 ml) of 0–10% (v/v) isopropanol containing 2 mM membrane translocation. Transport across the outer membrane in the second step requires a secretory apparatus encoded by large gene clusters, which are either specific or common to several exoproteins. Polypeptides transported via the hemolysin-type secretory pathway cross the cell envelope by a single-step process without periplasmic intermediates. Proteins targeted through this pathway have no N-terminal signal sequence and show sec-independent translocation to the medium.
PMFS. The abbreviation for the then-proposed were then loaded on a DEAE-agarose column (1.5 x 40 cm) equilibrated in 20 mM Tris-HCl, 2 mM PMFS, 7.5, and eluted with a gradient of 0-0.2 M NaCl (600 ml). An Ultrogel AcA 54 column (2.5 x 100 cm) eluted with 20 mM Tris-HCl, 7.5, was used as the last chromatographic step.

The vector pEH12WT encoding for the α-amylase precursor was constructed by ligation the HpaI site located 60 nucleotides upstream from the initiation codon of the amy gene in the Sma1 site of the pUC12 polynucleotide. This construction was used as a template for subsequent PCR and inverse PCR amplifications by VentR DNA polymerase (New England Biolabs) using optimized conditions described elsewhere (14).

The vector pEH12WT encoding for the recombinant mature α-amylase was constructed by PCR amplification of the amy gene using a sensitive primer and the mutating antisense primer 5’-CCCTCATGAAGAGGGAAGAGACCTG-3’, which introduces a stop codon and a XhoI site after the mature enzyme coding sequence. The mutations were returned in the template using a PwoII-XhoI restriction fragment. Deletion of the α-amylase coding region in pEPCT was carried out by inverse PCR of pEH12 using silent primers, 24 nucleotides in length, ending at codon GCT for Ala-30 (antisense primer) and starting at codon AAT for Asn-448 (sense primer). The amplification product was purified (QiAquick PCR purification kit, Qiagen), phosphorylated by T4 polynucleotide kinase, and circularized by ligase before transformation. Deletion of the amy gene in plasmid pEPST1 was produced by PstI digestion of pEH12 and recircularization of the vector, retaining only a coding sequence ending at Ala-30.

The β-lactamase-α-amylase fusion in pBLACT was performed by inverse PCR on a construction made of a peptide coding region (EcORV-XhoI) cloned downstream from the bla gene (XhoI-SphI) of Psychrobacter immobilis (15) in the polynucleotide pSP73 (Promega). Amplification used an antisense primer ending at codon AAC for Asn-448 (sense primer). The amplification product was purified (QiAquick PCR purification kit, Qiagen), phosphorylated by T4 polynucleotide kinase, and circularized by ligase before transformation. Deletion of the amy gene in plasmid pEPST1 was produced by PstI digestion of pEH12 and recircularization of the vector, retaining only a coding sequence ending at Ala-30.

The vector pEH12WT encoding for the recombinant mature α-amylase was constructed by PCR amplification of the amy gene using a sensitive primer and the mutating antisense primer 5’-CCCTCATGAAGAGGGAAGAGACCTG-3’, which introduces a stop codon and a XhoI site after the mature enzyme coding sequence. The mutations were returned in the template using a PwoII-XhoI restriction fragment. Deletion of the α-amylase coding region in pEPCT was carried out by inverse PCR of pEH12 using silent primers, 24 nucleotides in length, ending at codon GCT for Ala-30 (antisense primer) and starting at codon AAT for Asn-448 (sense primer). The amplification product was purified (QiAquick PCR purification kit, Qiagen), phosphorylated by T4 polynucleotide kinase, and circularized by ligase before transformation. Deletion of the amy gene in plasmid pEPST1 was produced by PstI digestion of pEH12 and recircularization of the vector, retaining only a coding sequence ending at Ala-30.

The β-lactamase-α-amylase fusion in pBLACT was performed by inverse PCR on a construction made of a peptide coding region (EcORV-XhoI) cloned downstream from the bla gene (XhoI-SphI) of Psychrobacter immobilis (15) in the polynucleotide pSP73 (Promega). Amplification used an antisense primer ending at codon AAC for Asn-448 (sense primer). The amplification product was purified (QiAquick PCR purification kit, Qiagen), phosphorylated by T4 polynucleotide kinase, and circularized by ligase before transformation. Deletion of the amy gene in plasmid pEPST1 was produced by PstI digestion of pEH12 and recircularization of the vector, retaining only a coding sequence ending at Ala-30.

The β-lactamase-α-amylase fusion in pBLACT was performed by inverse PCR on a construction made of a peptide coding region (EcORV-XhoI) cloned downstream from the bla gene (XhoI-SphI) of Psychrobacter immobilis (15) in the polynucleotide pSP73 (Promega). Amplification used an antisense primer ending at codon AAC for Asn-448 (sense primer). The amplification product was purified (QiAquick PCR purification kit, Qiagen), phosphorylated by T4 polynucleotide kinase, and circularized by ligase before transformation. Deletion of the amy gene in plasmid pEPST1 was produced by PstI digestion of pEH12 and recircularization of the vector, retaining only a coding sequence ending at Ala-30.

The β-lactamase-α-amylase fusion in pBLACT was performed by inverse PCR on a construction made of a peptide coding region (EcORV-XhoI) cloned downstream from the bla gene (XhoI-SphI) of Psychrobacter immobilis (15) in the polynucleotide pSP73 (Promega). Amplification used an antisense primer ending at codon AAC for Asn-448 (sense primer). The amplification product was purified (QiAquick PCR purification kit, Qiagen), phosphorylated by T4 polynucleotide kinase, and circularized by ligase before transformation. Deletion of the amy gene in plasmid pEPST1 was produced by PstI digestion of pEH12 and recircularization of the vector, retaining only a coding sequence ending at Ala-30.
Propeptide-assisted Secretion

Fig. 2. Far-UV circular dichroism spectra of α-amylase and its propeptide. Spectra of the propeptide (long dash) and of α-amylase (line) were taken in 5 mM NaH2PO4, pH 7.0. Propeptide in the presence of 8 M urea (dashed). Data are expressed in terms of the mean residue ellipticity, θ.

Expression of the amy Gene in A. haloplanctis—A. haloplanctis is a psychrophilic bacterium efficiently growing at near-zero temperatures (generation time of 4.0 h at 4°C) and reaching cell densities as high as 5 × 10^10 cells/ml after 100 h of growth (Fig. 3). Addition of maltose up to 3% results in a 200-fold induction of α-amylase expression. Fig. 3 also shows that the amylolytic activity is sharply produced in the supernatant during the exponential growth phase. Rabbit antibodies raised against α-amylase and the propeptide were used to study expression of the amy gene in the wild-type strain. Western blot analysis of samples taken at all growth stages (Fig. 4) reveals that α-amylase is expressed in the culture supernatant as a 70-kDa precursor, which further dissociates into the mature enzyme and the free propeptide. The level of extracellular activity correlates with the production of the precursor rather than with the appearance of the mature enzyme, already suggesting that the precursor is active. Neither the precursor nor its two products have been detected in cell pellets.

Expression of the Recombinant Precursor in E. coli—In order to study the maturation mechanism, the precursor was expressed in E. coli. The amy gene was cloned downstream from the lacZ promoter of pUC12, resulting in the production of 60–100 mg of precursor/liter in the host culture supernatant. N- and C-terminal amino acid sequence determinations indicated that the signal peptide is correctly cleaved in non-denaturing conditions. It contains four cysteine residues. Sulphydryl titration by DTNB of both the native and the denatured protein in 8 M urea indicates that there is no free thiol group, demonstrating the occurrence of two disulfide linkages. There is no significant difference in the amino acid molar ratios of both native α-amylase and the cleaved C-terminal domain. A search through the GenBank/EMBL data banks failed to reveal a homology in 200 amino acid overlap of the hypothetical α-amylase from the nematode Caenorhabditis elegans (Swissprort P91982). To date, only the nucleotide sequence of C. elegans α-amylase is known, but multiple sequence alignment with the translated nucleotide sequences, except with the C-terminal region (34% identity in 200 amino acid overlap) of the hypothetical α-amylase from the nematode Caenorhabditis elegans (Swissprort P91982). To date, only the nucleotide sequence of C. elegans α-amylase is known, but multiple sequence alignment with the translated nucleotide sequences indicates that it also belongs to the Ca^2+ - and Cl^- dependent α-amylase family. Its C-terminal extension (200 residues predicted) is possibly involved in secretion or it could serve to anchor the enzyme to the eukaryote membrane.

Secondary structure prediction algorithms (GCG 9.0) suggest a high content of β-sheet-forming residues, with several possible amphipathic β-sheets as indicated by hydrophobic moment analysis. This is further emphasized by the far-UV circular dichroism spectra of the secretion helper (Fig. 2). Secondary structure analysis of CD spectra (21) correctly estimated the α-helix and the β-sheet content (20% and 30%, respectively) of the known α-amylase three-dimensional structure (22) taken as reference. CD spectra of the propeptide contrasted with those of α-amylase and were typical of a β-sheet protein (50–60%) with a low α-helical content (~10%). Addition of urea abolished the CD signals and confirms the existence of secondary structure organization of the isolated propeptide.

Fig. 3. Growth of A. haloplanctis and α-amylase production. Growth of the psychrophilic bacteria at 4 °C (●) and α-amylase activity in the culture supernatant (○).

Proteolytic Maturation of the α-Amylase Precursor—Only one proteolytic enzyme was detected in A. haloplanctis culture supernatants. This 45-kDa protease (AHP) is a metalloenzyme (60% inhibition by excess EDTA) from the serine-protease family (98% inhibition by 2 mM PMSF). AHP has a broad specificity and readily hydrolyses macromolecular substrates such as precipitated casein or azo-labeled casein. The N-terminal amino acid sequence of AHP (S-T-P-N-D-P-P-F-D-D-Q-S-Y-Y-E-Q-A-G-) shows strong homology with some other microbial Ser proteases such as those from Bacillus thuringiensis (accession no. JN0369) and Dichelobacter nodosus (no. L18984) and, notably, with the protease from a mesophilic Alteromonas strain (no. D38600).
When the purified AHP and the recombinant precursor are mixed in the in vivo ratio (≤1/10 in sterile broth), cleavage into α-amylase and the propeptide occurs at a rate similar to that recorded in A. haloplanctis cultures (Fig. 5). Furthermore, the N-terminal sequence of the in vitro processed propeptide is identical to that of the wild-type propeptide, demonstrating that AHP is the extrinsic factor required for the maturation of the precursor.

**Linker Susceptibility to Proteolysis**—The specific cleavage of the propeptide by the nonspecific AHP protease prompted us to test the action pattern of other nonspecific proteases such as Pronase, proteinase K, and subtilisin. As shown in Fig. 5, these proteases preferably cleaved the precursor in the linker region. Proteolytic cleavage by E. coli proteases was also noted during expression of the recombinant precursor. Indeed, about 5% of the produced enzyme is cleaved before starting the purification procedure. N-terminal sequence of the propeptide processed in E. coli reveals that the cleavage site is displaced between Thr-455 and Glu-456 (Fig. 1). A Mr value of 21,331 for this propeptide is predicted from the nucleotide sequence, in perfect agreement with the electrospary ionization mass spectrometric analysis (21,330 ± 4).

It is concluded that the linker region between α-amylase and the propeptide probably consists of a disordered, solvent-exposed loop, prone to various proteolytic attacks. This is also supported by the lack of defined electron density for the last five residues in the x-ray structure of A. haloplanctis α-amylase (22).

**The Propeptide Has No Foldase Activity**—The foldase activity generally associated with propeptides (8, 9) has been probed by removing the propeptide sequence from the amy gene and introducing a stop codon after Ser-453, the last residue of the mature wild-type enzyme. Properties of the wild-type and recombinant α-amylases as well as those of the recombinant precursor are compared in Table I. It is shown that both the kinetic and ion-binding parameters are identical in the three related enzymes. All the cysteine residues of the native α-amylase and of the propeptide are engaged in disulfide linkages. Thus, the lack of significant free sulphydryl groups, as detected by DTNB titration, also confirmed the absence of misfolded species in the recombinant enzymes.

**Heterologous Secretion in E. coli**—Involvement of the propeptide in the secretion pathway of A. haloplanctis α-amylase has been analyzed by genetic modification of the amy gene and its expression in E. coli. Its function of secretion helper during translocation across the outer bacterial wall is well illustrated in Fig. 6. When the amy gene (pH12) is expressed in E. coli, the extracellular targeting of the precursor follows the bacterial growth curve. At the end of the exponential growth phase (~30 h), about 80% of the total enzyme production is found in the cell-free supernatant of E. coli, 15% is found in the periplasmic space, and 5% remains cell-associated as determined by osmotic shocks (data not shown). The same results were obtained when the amy gene expression was reduced 100-fold (E. coli BL21 expressing the LacI repressor), showing that the appearance of the precursor in the medium is not the result of its overexpression. By contrast, removal of the propeptide sequence (pH12WT*) leads to periplasmic accumulation of the recombinant α-amylase (followed by its release into the medium during cell lysis). It is concluded that the propeptide efficiently assists outer membrane translocation in E. coli. The outer membrane integrity has been checked by monitoring the β-lactamase activity in the cell-free supernatants (β-lactamase is a periplasmic enzyme encoded by the plasmid vector and is responsible for the antibiotic resistance). Fig. 6 shows that heterologous secretion of the α-amylase precursor (pH12) induces outer membrane damage in E. coli as indicated by the release of β-lactamase in the medium, by the slight growth inhibition and early cell lysis.

**Self-secretion of the Propeptide**—The autonomous outer membrane insertion of the propeptide was analyzed by removing the α-amylase coding region from the amy gene (pEPCT). In order to target the modified gene product into the periplasmic space, the peptide signal and the 30 N-terminal amino acids, which can form the export initiation domain (23, 24), were not
deleted. In addition, the six last C-terminal residues of the mature α-amylase were also conserved. Indeed, sequence alignment with other chloride-dependent α-amylases from insects and vertebrates shows that the bacterial enzyme is six residues longer at the C terminus. These residues can therefore belong to the linker region with the propeptide and were not deleted.

The control construction (pEPST1) is identical but only encodes for the peptide signal and the 30 first α-amylase residues.

When the pEPCT construct is expressed in E. coli, two gene products recognized by IgG anti-propeptide already appear in the supernatant during the exponential growth phase and further accumulate in the extracellular medium (Fig. 7). As shown by Western blots, one compound corresponds to the wild-type propeptide, whereas the second has a slightly higher molecular mass (≥3.5 kDa). It is likely that the propeptide is expressed with the export initiation domain and that cleavage at the linker by E. coli proteases further occurs in the periplasm, as already noted for the complete precursor. Autonomous propeptide translocation also induces outer membrane damage as indicated by β-lactamase leakage, whereas the control vector encoding for the export initiation domain alone does not affect the host cells.

**β-Lactamase Targeting to the Extracellular Medium**—The ability of the α-amylase propeptide to export a foreign passenger was tested by the construction of a β-lactamase-propeptide protein fusion. The class C β-lactamase from the Gram-negative bacterium P. immobilis A5 (15) was selected because (i) it is also a heat-labile psychrophilic enzyme; (ii) like other β-lactamases, the recombinant enzyme accumulates in the periplasmic space of E. coli; and (iii) it is devoid of disulfide bonds that may impair outer membrane translocation in E. coli as reported previously (25), although the α-amylase contains four disulfide bonds but is secreted efficiently.

### TABLE I

| Kinetic parameters, dissociation constants, and free thiol groups for the wild-type and the recombinant α-amylases |
|---|
| α-Amylase | \( k_{\text{cat}} \) | \( K_m \) | \( k_{\text{cat}}/K_m \) | \( K_{\text{in}}^{(\mathrm{Ca})} \) | \( K_{\text{in}}^{(\mathrm{Cl}_2)} \) | Cysteines\( ^a \) | Free thiol |
|---|---|---|---|---|---|---|---|
| Wild-type | 780 ± 25 & 174 ± 8 | 4.6 | 5.9 ± 0.2 | 2.10 ± 8 | 8 | 0.03 |
| Native, recombinant (pαH12WT*) | 792 ± 34 & 168 ± 14 | 4.7 | 6.1 ± 0.2 | 2.10 ± 8 | 8 | 0.05 |
| Precursor, recombinant (pαH12) | 781 ± 39 & 171 ± 8 | 4.6 | 6.2 ± 0.2 | 2.10 ± 8 | 12 | 0.06 |

\( ^a \) Cysteines from the amino acid sequence.

**Fig. 6.** Expression of the precursor and of the recombinant native α-amylase in E. coli. Upper panel, gene constructs encoding the 70-kDa precursor (pαH12) and the native α-amylase (pαH12WT*) devoid of the propeptide coding sequence. SP, signal peptide; Ct, C-terminal propeptide. Lower panel, α-amylase and β-lactamase activity in E. coli culture supernatants (activity is expressed as percent of the maximal activity recorded in the cell-free supernatant of E. coli (pαH12)) and bacterial growth at 18 °C (A\(_{550}\)).

**Fig. 7.** Expression of the propeptide in E. coli. Upper panel, gene construct encoding the propeptide (Ct) preceded by the signal peptide (SP), the export initiation domain (Ex), and the linker (L) in pEPCT. The control construct pEPST1 only encodes signal peptide and export initiation domain. Lower panel, β-lactamase activity in E. coli culture supernatants (activity is expressed as percent of the maximal activity recorded in the cell-free supernatant of E. coli (pEPCT)) and bacterial growth at 18 °C (A\(_{550}\)). Inset, Western blot of the purified wild-type propeptide (A) and of the cell-free supernatant of E. coli (pEPCT) after 30 h (B) using IgG anti-propeptide for detection.
Propeptide-assisted Secretion

The coding sequence of the propeptide and of the six C-terminal linker residues were fused to the bla gene by inverse PCR and cloned in a kanamycin-resistant vector (pBLACT). The control vector pBLAC4 is identical but only carries the PCR and cloned in a kanamycin-resistant vector (pBLACT). Lower panel, β-lactamase activity in E. coli culture supernatants (activity is expressed as percent of the maximal activity recorded in a cell-free supernatant of E. coli (pBLACT)) and bacterial growth at 18 °C [(A)500].

The following two-step secretion pathway of A. haloplanctis α-amylase can be proposed. The 200-fold induction of α-amylase production suggests the occurrence of a maltose-regulated promoter as already reported for some other microbial α-amylases (27). After gene induction and initiation of the translation, the nascent α-amylase precursor undergoes the classical sec-dependent inner membrane translocation, as evidenced by the occurrence of a cleavable signal peptide. The periplasmic intermediate then enters into the outer wall via its C-terminal propeptide in a way probably similar to other bacterial outer membrane proteins. However, the next specific events involve translocation of the α-amylase domain across the outer wall and the extracellular release of the uncleaved precursor in a native conformation. The last step requires the assistance of the nonspecific AHP protease in order to remove the C-terminal secretion helper by cleavage at the easily accessible linker region. As the precursor is not detected in A. haloplanctis cell pellets, the post-transcriptional events leading to secretion seem very fast, without accumulation of detectable cell-associated intermediates. The propeptide-assisted mechanism of membrane spanning (through or beside a possible β-barrel) and the translocation driving force remain unknown.

To our knowledge, few other bacterial exoenzymes possessing a C-terminal propeptide have been reported: Neisseria IgA proteases (5–7), Thermus aquaticus aqaulysin I protease (28), Serratia marcescens SSP protease (29), Helicobacter pylori VacA cytotoxin (30), Lysobacter enzymogenes alkaline phosphatase (31), E. coli and Shigella virulence proteins EspC (32) and VirG (33), and Bordetella pertussis pertactin (34). According to the available data, the extracellular routing of these enzymes and of α-amylase should follow the same main steps of the above mentioned secretion pathway. However, the propeptide from A. haloplanctis α-amylase precursor is unusual because it remains associated to the precursor in the external medium, it requires external proteolytic assistance for cleavage, it can be recovered from supernatants, and it has no intramolecular chaperone function.

Acknowledgments—We are grateful to the Institut Français de Recherche et de Technologie Polaire for the support and facilities offered at the Antarctic station Dumont d’Urville during earlier stages of this work. We also thank B. Samyn for expertise in the C-terminal amino acid analysis, as well as N. Gerardin-Otthiers and R. Marchand for expert technical assistance.

REFERENCES
1. Pugsley, A. P. (1993) Microbiol. Rev. 57, 50–108
2. Salmond, G. P. C., and Reeves, P. J. (1993) Trends Biochem. Sci. 18, 7–12
3. Gennis, R. A., and Inouye, M. (1991) Curr. Opin. Biotechnol. 2, 661–667
4. Hirst, T. R., and Welch, R. A. (1988) Trends Biochem. Sci. 13, 265–268
5. Pohliner, J., Halter, R., Beyreuther, K., and Meyer, T. F. (1987) Nature 325, 458–462
6. Klau, T., Krämer, J., Otzelberger, K., Pohliner, J., and Meyer, T. F. (1993) J. Mol. Biol. 234, 579–593
7. Klau, T., Pohliner, J., and Meyer, T. F. (1993) BioEssays 15, 799–805
8. Shinde, U., and Inouye, M. (1993) Trends Biochem. Sci. 18, 442–446
Propeptide-assisted Secretion

9. Eder, J., and Fersht, A. R. (1995) *Mol. Microbiol.* 16, 609–614
10. Feller, G., Lonhienne, T., Deroanne, C., Libioulle, C., Van Beeumen, J., and Gerday, C. (1992) *J. Biol. Chem.* 267, 5217–5221
11. Feller, G., Payan, P., Theys, F., Qian, M., Haser, R., and Gerday, C. (1994) *Eur. J. Biochem.* 222, 441–447
12. Feller, G., le Bussy, O., Houssier, C., and Gerday, C. (1996) *J. Biol. Chem.* 271, 23836–23841
13. Aghajari, N., Feller, G., Gerday, C., and Haser, R. (1996) *Protein Sci.* 5, 2128–2129
14. Cease, K. B., Potcova, C. A., Lohff, C. J., and Zeigler, M. E. (1994) *PCR Methods Appl.* 3, 298–300
15. Feller, G., Zekhnini, Z., Lamotte-Brasseur, J., and Gerday, C. (1997) *Eur. J. Biochem.* 244, 186–191
16. Spratt, B. G., Hedge, P. J., te Heesen, S., Edelman, A., and Broom-Smith, J. K. (1986) *Gene (Amst.)* 41, 337–342
17. Rauscher, E., Neumann, U., Schäich, E., von Bulow, S., and Wahlefeld, A. W. (1985) *Clin. Chem.* 31, 14–19
18. O'Callaghan, C., Morris, A., Kirby, S., and Shingler, A. (1972) *Antimicrob. Agents Chemother.* 1, 283–288
19. Darvill, S., Feller, G., Narinx, E., and Gerday, C. (1994) *J. Biol. Chem.* 269, 17448–17453
20. Habeeb, A. (1973) *Anal. Biochem.* 56, 60–65
21. Provencher, S. W., and Glückner, J. (1981) *Biochemistry* 20, 33–37
22. Aghajari, N., Feller, G., Gerday, C., and Haser, R. (1997) *Protein Sci.* 7, 564–572
23. Rasmussen, B. A., and Silhavy, T. J. (1987) *Genes Dev.* 1, 185–196
24. Anderson, H., and van Heijne, G. (1991) *Proc. Natl. Acad. Sci. U. S. A.* 88, 9751–9754
25. Klauser, T., Pohlner, J., and Meyer, T. F. (1992) *EMBO J.* 11, 2327–2335
26. Struyve, M., Moons, M., and Tommassen, J. (1991) *J. Mol. Biol.* 218, 141–148
27. Virolle, M. J., Long, C. M., Chang, S., and Bibb, M. J. (1988) *Gene (Amst.)* 74, 321–334
28. Kurosaka, K., Ohta, T., and Matsuzawa, H. (1996) *Mol. Microbiol.* 20, 385–389
29. Ohnishi, Y., Nishiyama, M., Horinouchi, S., and Beppu, T. (1994) *J. Biol. Chem.* 269, 32600–32606
30. Schmitt, W., and Haas, R. (1994) *Mol. Microbiol.* 12, 307–319
31. Au, S., Roy, K. L., and von Tigerstrom, R. G. (1991) *J. Bacteriol.* 173, 4551–4557
32. Stein, M., Kenny, B., Stein, M. A., and Finlay, B. B. (1996) *J. Bacteriol.* 178, 6546–6554
33. Suzuki, T., Lett, M.-C., and Sasakawa, C. (1995) *J. Biol. Chem.* 270, 30874–30880
34. Charles, I., Fairweather, N., Pickard, D., Beesley, J., Anderson, R. Dougan, G., and Roberts, M. (1994) *Microbiology* 140, 3301–3308