Volume 12 Number 13 1984 Nucleic Acids Research

Length and structural effect of signal peptides derived from *Bacillus subtilis* α-amylase on secretion of *Escherichia coli* β-lactamase in *B. subtilis* cells

Kazutaka Ohmura, Kouji Nakamura, Hisato Yamazaki, Teruaki Shiroza, Kunio Yamane*, Yoshifumi Jigami[1], Hideaki Tanaka[1], Koji Yoda[2], Makari Yamasaki[2] and Gakuzo Tamura[2]

Institute of Biological Sciences, University of Tsukuba, Sakura, Ibaraki-ken 305, [1]National Chemical Laboratory, Yatabe, Ibaraki-ken 305, and [2]Department of Agricultural Chemistry, Faculty of Agriculture, University of Tokyo, Bunkyo-ku, Tokyo 113, Japan

Received 18 April 1984; Revised and Accepted 7 June 1984

ABSTRACT

The precursor of Bacillus subtilis α-amylase contains an NH_2-terminal extension of 41 amino acid residues as the signal sequence. The E. coli β-lactamase structural gene was fused with the DNA for the promoter and signal sequence regions. Activity of β-lactamase was expressed and more than 95 % of the activity was secreted into the culture medium. DNA fragments coding for short signal sequences 28, 31, and 33 amino acids from the initiator Met were prepared and fused with the β-lactamase structural gene. The sequences of 31 and 33 amino acid residues with Ala COOH-terminal amino acid were able to secrete active β-lactamase from B. subtilis cells. However β-lactamase was not secreted into the culture medium by the shorter signal sequence of 28 amino acid residues, which was not cleaved. Molecular weight analysis of the extracellular and cell-bound β-lactamase suggested that the signal peptide of B. subtilis α-amylase was the first 31 amino acids from the initiator Met. The significance of these results was discussed in relation to the predicted secondary structure of the signal sequences.

INTRODUCTION

Secreted proteins are synthesized as precursors with an NH_2-terminal extension "signal peptide", which is removed by a specific enzyme (1, 2). The signal peptide and signal peptidase mechanism for secretion of extracellular proteins is similar in both prokaryotic and eukaryotic cells. The signal peptides are similar in length and contain a highly hydrophobic central sequence (2 - 5). Predictions of the secondary structure of a number of these regions have demonstrated a degree of conformational similarity among the sequences (6, 7, 8), and the secondary structure is important for protein secretion (9).

B. subtilis is known to secrete a large number of soluble proteins into the culture medium. However few studies of the protein secretion mechanism in B. subtilis cells have been made. We cloned the α-amylase structural gene, which produces a saccharifying type α-amylase, and its regulatory gene from an α-amylase hyperproducing strain, B. subtilis NA64, using the temperate

phage ρ11 and the plasmid pUB110 (10). The nucleotide sequence analysis of the α-amylase genes (11, 12) and amino acid sequence analysis of the NH_2-terminal region of B. subtilis extracellular α-amylase (13), which was expressed by the same α-amylase genes (14), indicate that the B. subtilis α-amylase precursor contains 41 amino acid residues in its signal sequence (11, 12, 15). If this sequence is the socalled signal peptide, it is longer than the signal peptides reported in other exported proteins. Furthermore, most signal sequences in the precursor proteins have been cleaved between Ala and X amino acid to form mature proteins, but the α-amylase precursor is cleaved between Glu (position 41 from the initiator Met) and Leu (position 42) to form the extracellular α-amylase. It is therefore possible that a part of the 41 amino acid residues in the signal sequence of the α-amylase precursor forms the signal peptide. When E. coli β-lactamase structural gene was fused downstream of the α-amylase signal sequence region, the β-lactamase gene was expressed in B. subtilis cells and active β-lactamase was secreted into the culature medium (16).

Palva et al (17) cloned another α-amylase gene of Bacillus amyloliquefaciens, which produces a liquefying type α-amylase, in the B. subtilis plasmid pUB110. The promoter and the signal peptide coding regions of the α-amylase gene functioned actively in B. subtilis cells. They were also able to express and secrete E. coli β-lactamase (18) and human interferon α (19). The amino acid sequence of the signal peptide of B. amyloliquefaciens α-amylase, which contained 31 amino acid residues and which was cleaved between Ala and Val, was quite different from that of the signal sequence of B. subtilis α-amylase.

In order to characterize the secretion mechanism of B. subtilis α-amylase, we prepared DNA fragments coding different lengths of the signal sequence and fused them with the E. coli β-lactamase structural gene downstream of the fragments using HindIII linker.

In this paper, we have described the short signal sequences which have 31 and 33 amino acids from the initiator Met secrete the β-lactamase more efficiently than the original sequence of 41 amino acids in B. subtilis cells. But a shorter signal sequence of 28 amino acids did not secrete the enzyme. The signal peptide of B. subtilis α-amylase seemed to consist of 31 amino acid residues from the initiator Met. These results are compared with the predicted secondary structure of the signal sequences.

MATERIALS AND METHODS

Bacterial strain and plasmids

Bacillus subtilis 207-25 (m^-_{168} *hsrM recE4 amyE07 aroI906 lys21 leuA8*) is a derivative of *B.subtilis* Marburg 168. *B. subtilis* plasmid pTUB4 has an insertion of a 2.3 kilobase pair (kb) fragment which contains the α-amylase regulatory gene, *amyR2*, and the α-amylase structural gene, *amyE*$^+$, derived from an α-amylase hyperproducing strain, *B. subtilis* NA64, in the *B. subtilis* plasmid pUB110 (10). *Escherichia coli* plasmid pKTH74, provided by Dr. I.Palva (Central Public Health Lab., Mannerheimintie 166, SF-00280 Helsinki 28, Finland), contains the structural gene of *E. coli* β-lactamase (penicillin amide-β-lactamhydrolase, EC 3.5.2.6) from pBR322, which lacked the region of the signal peptide but contained *Hin*dIII termini (19). The plasmid pTUB218, which contained the promoter and signal sequence coding region of *amyE*$^+$ derived from pTUB4, is a secretion vector in *B. subtilis* (16). The plasmid pTUB228 was constructed from pTUB218 and the *E. coli* β-lactamase structural gene which was derived from the *Hin*dIII-digest of pKTH74. More than 95% of the β-lactamase synthesized in *B. subtilis* containing pTUB228 was secreted into the culture medium (16).

Medium and culture conditions

The composition of LG-medium containing 10 μg/ml of kanamycin and culture conditions were described previously (20).

DNA synthesis

Oligodeoxyribonucleic acids, 13mer (5'CGGCGGCTGCGCA3'), 15mer (5'AGCTT GCGCAGCCGC3'), 19mer (5'CGGCGGCTGCGAGTGCTCA3') and 21mer (5'AGCTTGAGCACTCGC AGCCGC3') were synthesized by the modified solid phase method of phospho-triester (21).

Antiserum

Rabbit antiserum against *E. coli* β-lactamase was provided by Dr. M.Inoue (School of Medicine, Gunma University, Maehashi, Japan).

Western blotting

After electrophoresis in SDS 7.5 % polyacrylamide gel (22), β-lactamase was visualized by the Western blotting method (23) using rabbit antiserum against *E. coli* β-lactamase and the [^{125}I]-labeled F(ab) fraction of donkey antiserum against rabbit IgG (RCC Amersham, England).

Preparation of plasmids and DNA fragments

Plasmids were prepared by the rapid alkaline method of Birnboim and Dolly (24) and purified by CsCl equilibrium centrifugation in the presence of ethidium bromide and by agarose gel electrophoresis. Large molecular size DNAs

in agarose gels were electroeluted into hydroxylapatite (25), and small molecular size DNA fragments in polyacrylamide gels were eluted by immersing the crushed or ground gels.

DNA nucleotide sequence analysis

5' ends of DNA fragments were labeled with polynucleotide kinase and [γ-^{32}P]ATP. Sequencing reactions of the labeled DNA fragments were performed according to the method of Maxam and Gilbert (26). The cleaved products were separated on 8 % or 20 % polyacrylamide gels containing 8.3 M urea. The gels were autoradiographed at -70^{o}C using an intensifying secreen (DuPont).

Transformation of B. subtilis

Transformation of B. subtilis 207-25 by the recombinant plasmids was performed according to the protoplast transformation method of Chang and Cohen (27).

Assay of β-lactamase activity

β-lactamase activity in the culture media and the washed cells was determined spectrophotometrically using nitrocefin (BBR Biological Systems, Maryland, USA) by the method of O'Callaghan et al (28). One unit of β-lactamase hydrolyzed one μmol of nitrocefin in one min at 37^{o}C. β-lactamase activity in plates was detected by the presence of red halos around the colonies after the nitrocefin solution (0.5 mg/ml of 0.1 M phosphate buffer, pH 7.5) was sprayed onto the plates.

Enzymes

Restriction enzymes, bacterial alkaline phosphatase, polynucleotide kinase, DNA polymerase I (Klenow fragment) and T4 DNA ligase were purchased from Takara Shuzo Co., Ltd., (Kyoto, Japan) or from the Bethesda Research Lab., (Maryland, USA). Each enzyme was used according to the manufacturer's specification.

Chemicals

HindIII linker DNA, d(CAAGCTTG), was purchased from Takara Shuzo Co., Ltd. Fully protected dideoxyribonucleotides were purchased from Yoshitomi Pharmaceutical Co., Ltd (Osaka, Japan), and protected deoxyribonucleotide resins from Yamasa Shoyu Co., Ltd. (Choshi-shi, Japan). Kanamycin was purchased from Meiji Seika Co., Ltd. (Tokyo, Japan). All other chemicals were of the reagent grade.

RESULTS

Construction and analyses of plasmids pTUB231, pTUB249 and pTUB256

In order to prepare different lengths of signal sequences of B. subtilis

α-amylase, plasmids pTUB231, pTUB249 and pTUB256 were constructed according to the scheme shown in Fig.1. The α-amylase promoter-signal sequece and the β-lactamase structural gene in pTUB231 were in the opposite orientation with respect to the vector as compared with those of pTUB249 and pTUB256. The physical maps of plasmids pTUB231, pTUB249 and pTUB256 were compared with those of plasmids pTUB4 and pTUB228 (Fig. 2). The molecular size of the promoter and signal sequence coding regions of plasmids pTUB228, pTUB231, pTUB249 and pTUB256 was 428, 395, 398 and 404 bp respectively (Fig. 3). In order to ascertain the location of the α-amylase signal sequence coding regions and the E. coli β-lactamase structural gene, DNA nucleotide sequences at and near the junctions with these structures were analysed. The results and the deduced amino acid sequences of the four plasmids are shown in Fig. 4. pTUB228 contained the complete length of the signal sequence of B. subtilis α-amylase. The HindIII linker DNA, whose sequence is encased, was found at the 3' side of the sequence for the position 41 Glu residue. The signal sequence coding region of pTUB231 was lacking 39 bp from the original nucleotide sequence of pTUB228. The signal sequence of pTUB231 contained 28 amino acid residues from the initiator Met to position 28 (Pro) of the 41 amino acids. The signal sequences of pTUB249 and pTUB256 contained 31 and 33 amino acid residues of the α-amylase signal sequence, respectively. In pTUB249 and pTUB256, the tetrapeptide, Gln-Ala-Cys-Pro, which was encoded in the linker region, was located downstream of the Ala-X-Ala sequence while the tetrapeptide in pTUB228 and pTUB231 was not. The β-lactamase proper in the four plasmids starts at position 2, the Pro residue.

The secondary structure of the four signal sequences was calculated from the amino acid sequences using the method of Chou and Fasman (29)(Fig. 4). The core parts of the signal sequences (position 1 through 24 in the four signal sequences) have high potential for either the α-helix conformation (H), $\langle P\alpha \rangle = 1.11$, or the β-sheet conformation, $\langle P\beta \rangle = 1.14$, although the core parts contain the random coil conformation (C) from position 12 - 16. These conformations were blocked by Gly at position 27 and Pro at position 28 in the four signal sequences, and the β-turn conformation (T) was predicted from positions 25 to 27. The predicted secondary structure from position 1 through 27 was the same in the four signal sequences. Then the α-helix conformation was predictable from position 28 to 33 ($\langle P\alpha \rangle = 1.17$) in the signal sequence of pTUB228, from positions 28 to 33 ($\langle P\alpha \rangle = 1.17$) in the signal sequence of pTUB256, and also from positions 28 to 31 ($\langle P\alpha \rangle = 1.21$) in the signal sequence of pTUB249.

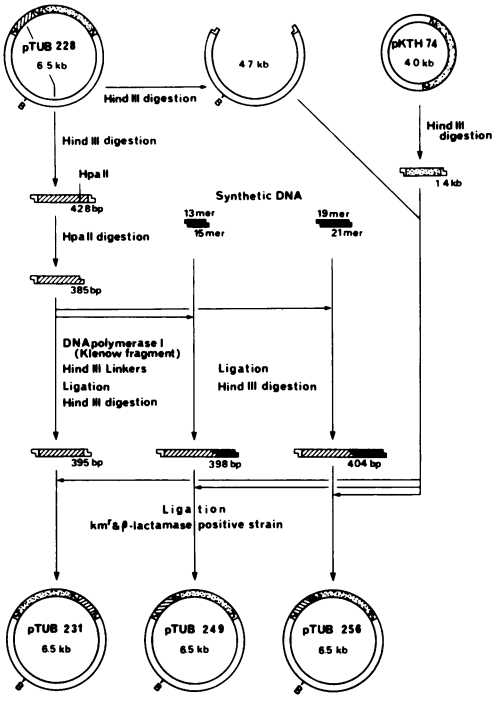

Fig. 1. Scheme for the construction of plasmids, pTUB231, pTUB249 and pTUB 256, which have different lengths of the signal sequences of B. subtilis α-amylase. The plasmid pTUB228 produces three DNA fragments (4.7, 1.4 and 0.43 kb) by HindIII-digestion. The 0.43 kb fragment, which contained the DNA sequence for the promoter and signal sequence of the α-amylase, was digested by HpaII and a 385 base pair (bp) fragment was obtained. After the 385 bp fragment was treated with polymerase I (Klenow fragment) and dXTP, it was ligated to HindIII linkers, redigested by HindIII and purified. Then the DNA fragment was ligated with 4.7 kb fragment, which was treated with bacterial alkaline phosphatase, and with the E. coli β-lactamase gene which was isolated from a HindIII-digest of pKTH74. The constructed plasmid was transferred into B. subtilis 207-25. A kanamycin-resistant (Km^r) and β-lactamase-positive transformant was selected. The plasmid harbored in the transformant was designated as pTUB231. The chemically synthesized oligonucleotide, 13mer and 15mer, and 19mer and 21mer, were annealed, and ligated to the 385 bp fragment. After the DNAs were digested by HindIII, a 398 bp fragment for 13mer and 15mer, and 404 bp fragment for 19mer and 21mer were purified. The DNA fragments were religated to the 4.7 kb fragment and the E. coli β-lactamase gene. The constructed plasmids were transferred into B. subtilis 207-25. Km^r and β-lactamase-positive transformants were isolated. One of the transformants carrying the 398 bp fragment was selected, whose plasmid was designated as pTUB249. pTUB256 was extracted from one of the other transformants carrying the 404 bp fragment. ▨ , promoter and signal sequence coding regions of the B. subtilis α-amylase gene. ▩ , E. coli β-lactamase structural gene from pKTH74. ▭ , vector DNA. ⊏⊐ , HindIII linkers. ▬ , synthetic DNA. B, BglII sites in plasmid pUB110.

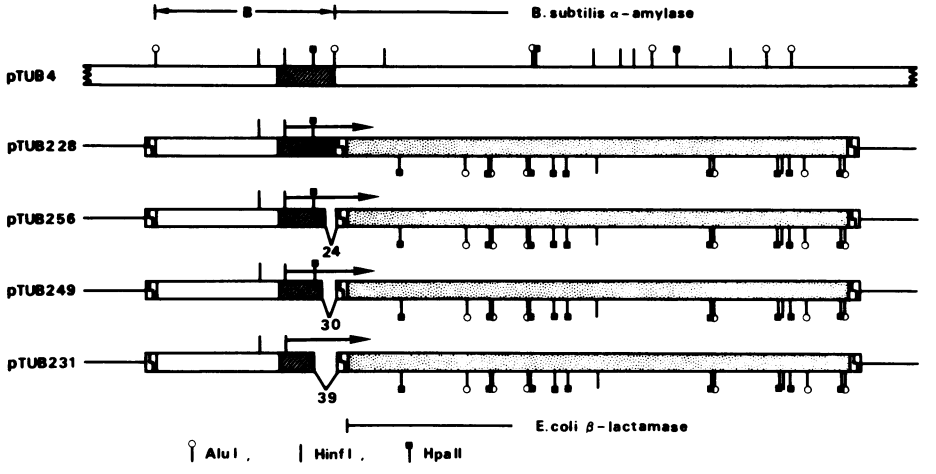

Fig. 2. Restriction maps of the promoter and signal sequence coding regions constructed and the structural genes of B. subtilis α-amylase and E. coli β-lactamase in pTUB228, pTUB231, pTUB249 and pTUB256. ⟵ B ⟶ , the 424 bp AluI fragment of pTUB4, which contains the promoter and signal sequence coding regions of the B. subtilis α-amylase. ▭, B. subtilis α-amylase DNA. ▨, signal sequence coding regions of the plasmids. ▩, E. coli β-lactamase DNA. ⊔, HindIII linkers. ———, vector DNA. The arrows (⟵⟶) indicate the direction and extent of the DNA nucleotide sequences determined.

However, the α-helix conformation could not be predicted for the signal sequence of pTUB231 downstream of position 28.

Production and location of E. coli β-lactamase expressed by B. subtilis

β-lactamase activity curves for the B. subtilis strains containing pTUB249 and 256 were quite similar (Fig. 5-B and -C). The maximum activity

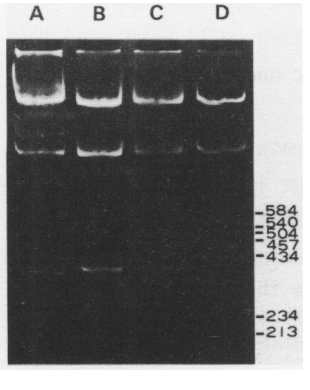

Fig. 3. Polyacrylamide gel electrophoresis of the DNA fragments containing the promoter and signal sequence coding regions of B. subtilis α-amylase in pTUB231 (A), pTUB249 (B), pTUB256 (C) and pTUB228 (D). A HaeIII-digest of pBR322 was used as the molecular weight marker shown in bp. Samples were digested by HindIII, electrophoresed in 5 % polyacrylamide gel and stained with 0.5 μg/ml of ethidium bromide.

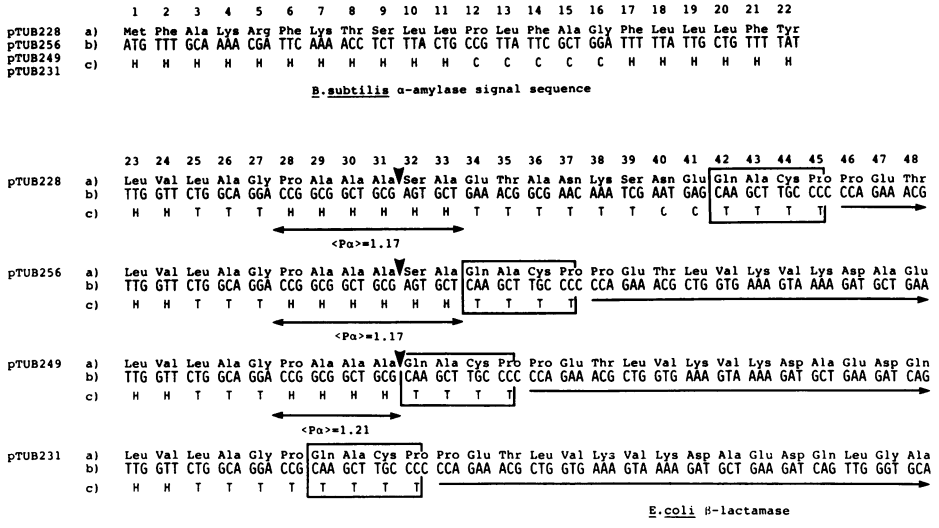

Fig. 4. DNA nucleotide sequences (line b) and amino acid sequences (line a) deduced from the nucleotide sequences at the junction regions of the promoter and the signal sequence coding regions of B. subtilis α-amylase gene and E. coli β-lactamase gene. The nucleotides of the linker are encased (CAAGCTTGC is the HindIII linker and CC is a part of EcoRI linker (18)). The most probable cleavage sites in the signal sequences are indicated by the arrows (▼). Peptide secondary structure predictions were made as described by Chou and Fasman (26)(line c). The letters H, C, and T stand for the α-helix, random coil and β-turn conformations, respectively. <Pα> is the average of the α-helix conformation parameters in the regions indicated by the arrows ⟷.

in the supernatants, about 700 units/ml, was observed 10 - 11 h after inoculation, and is approximately twice as much as pTUB228 carrying cells. The activity curves of the cell-bound β-lactamase in the three strains were the same up to about 20 units/ml. Thus the three strains secreted approximately 95 % of the enzyme into the culture medium. Washed cells were used to measure the cell-bound β-lactamase activity because it decreased during sonication.

On the other hand, more than 90 % of the β-lactamase activity in pTUB231-carrying strain was found to be cell bound. The maximum activity in the washed cells, 33 units/ml, was observed in the early stationary growth phase, 7 h after inoculation.

Growth curves of the four strains were similar to those of B. subtilis 207-25 and 207-25 harboring pTUB218.

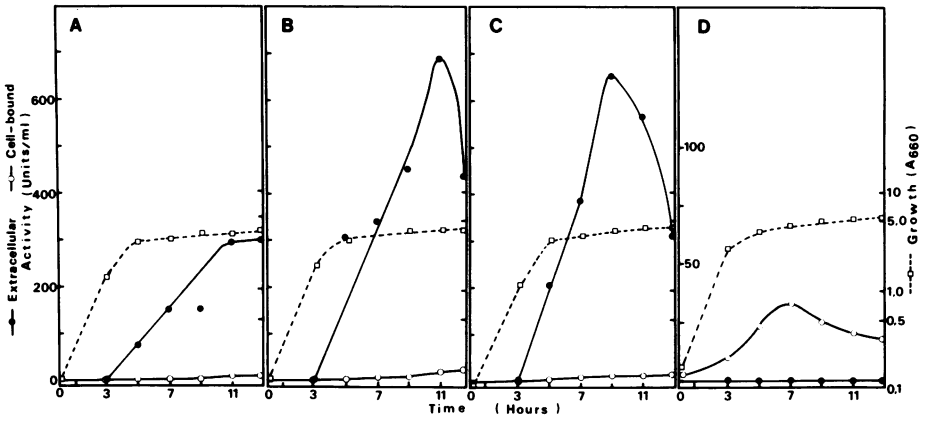

Fig. 5. Production and localization of E. coli β-lactamase by B. subtilis strains harboring pTUB228 (A), pTUB256 (B), pTUB249 (C) and pTUB231 (D). The cells were grown in LG-medium containing 10 μg/ml of kanamycin at 37°C. —●— , β-lactamase activity in the culture supernatants. —○— , β-lactamase activity in the washed cells.---□-----, growth measured in terms of absorbance at 660 nm.

Characterization of the E. coli β-lactamase produced by B. subtilis

The β-lactamase activity in the culture supernatnats of strains containing pTUB228, pTUB249 and pTUB256, and in the washed cells of the pTUB231-strain were inhibited by a rabbit antiserum raised against E. coli β-lactamase in the same way as the activity from the E. coli plasmide pBR322 was inhibited by the serum. The molecular weights of the extracellular β-lactamases from pTUB228 (β-lactamase-pTUB228), from pTUB249 (β-lactamase-pTUB249) and from pTUB256 (β-lactamase-pTUB256) were estimated to be approximately 28,200, 27,200 and 27,400, respectively (Fig. 6). The difference in the molecular weights of the β-lactamases was detected repeatedly. The size of the cell-bound β-lactamase from pTUB231 (β-lactamase-pTUB231) was estimated to be approximately 31,000. The major 43 k bands in lanes E and F (Fig. 6) were not correspond to the β-lactamase because the band was also detected in the cell preparations of 207-25 and 207-25 carrying pTUB218. No band corresponding to β-lactamase activity was detected in the culture supernatant of the strain with pTUB231. The Western blotting patterns of the supernatants of strains 207-25 and 207-25 carrying pTUB218 were the same to that of pTUB231-strain. These results indicate that the signal sequence of

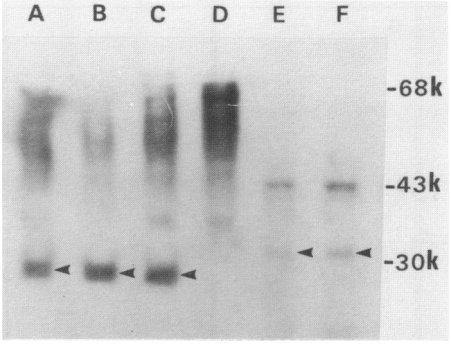

Fig. 6. Molecular weight determination of β-lactamases synthesized by B. subtilis strains containing pTUB228, pTUB231, pTUB249 and pTUB256. The molecular weights were estimated by SDS polyacrylamide gel electrophoresis in combination with the Western blotting method. (A), culture supernatant of pTUB228. (B), culture supernatant of pTUB256. (C), culture supernatant of pTUB249. (D), culture supernatant of pTUB231. (E), cell-bound β-lactamase of pTUB231. (F), cell-bound β-lactamase of pTUB231, two volumes of E were used for the electrophoresis. Bovine serum albumin (mol.wt., 68,000), ovalbumin (mol.wt., 43,000) and carbonic anhydrase (mol.wt., 30,000) were used as the molecular weight markers.

β-lactamase-PTUB231 was not cleaved and that the enzyme was not secreted into the culture medium. The molecular weight of the β-lactamase, which was isolated from the periplasmic space of an E. coli containing pBR322, was approximately 27,000.

DISCUSSION

The amino acid sequence of the precursor protein of B. subtilis α-amylase, deduced from the DNA nucleotide sequence, has an NH_2-terminal extension of 41 amino acid residues (11, 15). We speculate that the precursor of the α-amylase will at first be cleaved between the positions 33 (Ala) and 34 (Glu) from the initiator Met, and subsequently between the positions 41 (Glu) and 42 (Leu) to form extracellular α-amylase (11). The core peptide of the signal sequence from the initiator Met to position 24 (Val) has a high potential for either the α-helix or the β-sheet conformation. It is possible, however, that the core peptide has strong potential for the α-helix conformation in the intramembranous environment as the predicted secondary structure of the synthetic signal peptide of the preprothyroid hormone (9) and that of cytochrome P450 (30). The α-helix conformation of the signal sequence of the α-amylase was predicted to be inhibited by Gly and Pro at the positions 27 and 28 because the formation of the β-turn conformation from positions 25 to 27.

Then the α-helix conformation could be predicted from positions 28 (Pro) to 33 (Ala), whose <Pα>=1.17, as in the signal sequence of pTUB228. In this region, there are two Ala-X-Ala sequences, Ala-Ala-Ala (positions 29 - 31) and Ala-Ser-Ala (positions 31 - 33) and it has been shown that Ala-X-Ala sequence is the most frequent sequence preceding signal-peptidase cleavage (31). Thus we prepared three DNA fragments which were encoded from the initiator Met (position 1) to 28 (Pro), from positions 1 to 31 (Ala) and from positions 1 to 33 (Ala). The E. coli β-lactamase structural gene was ligated downstream of the three DNA fragments using HindIII linker. pTUB231 which contains 28 amino acid residues of the signal sequence of the B. subtilis α-amylase, pTUB249 (31 amino acid residues) and pTUB256 (33 amino acid residues) were constructed. The expression and secretion of E. coli β-lactamase by these plasmids in B. subtilis cells were compared to those by pTUB228 which contained the original length (41 amino acid residues) of the signal sequence of B. subtilis α-amylase.

The E. coli β-lactamase structural gene in the plasmids pTUB249 and 256 was expressed by the aid of the α-amylase promoter in B. subtilis cells and active β-lactamase was secreted into the culture medium from the cells by the action of the short signal sequences in the same way as the β-lactamase gene in pTUB228 was expressed. In the secondary structure of the signal sequences from these three plasmids, the α-helix conformation could be predicted downstream of the position 28 (Pro). On the other hand, the β-lactamase gene in pTUB231 was expressed but almost all of the β-lactamase activity was found in the B. subtilis cells. The α-helix conformation downstream of the position 28 was not able to predicted in the secondary structure of the signal sequence of pTUB231.

These results indicate that the α-helix conformation downstream of the position 28 in the signal sequence of B. subtilis α-amylase is closely related to the secretion of E. coli β-lactamase protein. It is conceivable that the α-helix conformation of the signal sequences plays an important role in the cleavage of the signal peptide of the precursor proteins.

Many signal peptides were readily cleaved behind the Ala-X-Ala sequence (31) and between the α-helix conformation and the β-turn conformation (9, 31). The differences in the molecular weights of the β-lactamases may indicate that the precursors of β-lactamase-pTUB228, -pTUB249 and pTUB256 were cleaved equally between positions 31 (Ala) and 32 (Ser) to produce extracellular enzymes. This site is located behind the Ala-X-Ala sequence in the three signal sequences and between the α-helix conformation and the

β-turn conformation in the signal sequence of pTUB249. Although positions 32 (Ser) and 33 (Ala) in the signal sequences of pTUB228 and pTUB256 are located in the α-helix conformation (Fig. 4), it is likely that the α-helix conformation is weak at the position 32 (Ser), because the Pα (the α-helix conformation parameter) of the Ser residue is very low (0.71 i). However the NH_2-terminal amino acid analysis of the secreted β-lactamases should be completed before drawing conclusion because it is also possible that the precursors of extracellular β-lactamase are cleaved at other sites in their signal sequences such as positions 33 and 34.

In contrast, the different secondary structure of the signal sequence of the β-lactamase-pTUB231 shows it was not cleaved because of the deletion of the Ala-X-Ala sequence. It is possible that the β-lactamase-pTUB231 crossed the cell membrane, but was not secreted into the culture medium because its signal sequence was not cleaved.

These results suggest that the signal peptide of B. subtilis α-amylase consists of 31 amino acid residues from the initiator Met. Such a signal peptide might be favorable for the production of extracellular E. coli β-lactamase in B. subtilis cells which was twice as much in strains carrying pTUB249 and pTUB256 as in the strain with pTUB228.

It is possible that the B. subtilis α-amylase precursor is processed in a similar manner as the formation of exo-small penicillinase of Bacillus licheniformis (32).

ACKNOWLEDGMENTS

We thank Prof. H.Saito, Institute of Applied Microbiology, The University of Tokyo, for his helpful discussions. We are grateful to Dr. I.Palva for providing the plasmid pKTH74 and to Dr. M.Inoue for supplying the serum. This work was suported by a Grant-in-Aid for Special Distinguished Research from the Ministry of Education, Science and Culture of Japan.

*To whom correspondence should be addressed

REFERENCES

1. Blobel, G., and Dobberstein, B. (1975) J.Cell Biol. 67, 835-851.
2. Davis, B.D., and Tai, P.-C. (1980) Nature 283, 433-438.
3. Kreil, G. (1981) Annu.Rev.Biochem. 50, 317-348.
4. Devillers-Thiery, A., Kindt, T., Scheele, G., and Blobel, G. (1975) Proc. Natl.Acad.Sci.USA 72, 5016-5020.
5. Neugebauer, K., Sprengel, R., and Schaller, H. (1981) Nucleic Acids Res. 9, 2577-2588.

6. Austen, B.M. (1979) FEBS Lett. 103, 308-313.
7. Cohn, D.V., Smardo, F.L.Jr., and Morrissey, J.J. (1979) Proc.Natl.Acad. Sci.USA 76, 1469-1471.
8. Rosenblatt, M., Beaudette, N.V., and Fasman, G.D. (1980) Proc.Natl.Acad. Sci.USA 77, 3983-3987.
9. Emr, S.D., and Silhavy, T.J. (1983) Proc.Natl.Acad.Sci.USA 80, 4599-4603.
10. Takeichi, Y., Ohmura, K., Nakayama, K., Otozai, K., and Yamane, K., (1983) Agric.Biol.Chem. 47, 159-161.
11. Ohmura, K., Yamazaki, H., Takeichi, Y., Nakayama, K., Otozai, K., Yamane, K., Yamasaki, M., and Tamura, G. (1983) Biochem.Biophys.Res. Commun. 112, 678-683.
12. Yamazaki, H., Ohmura, K., Nakayama, A., Takeichi, Y., Otozai, K., Yamasaki, M., Tamura, G., and Yamane, K. (1983) J.Bacteriol. 156, 327-337.
13. Mäntsälä, P., and Zalkin, H. (1979) J.Biol.Chem. 254, 8540-8547.
14. Yoneda, Y., Yamane, K., and Maruo, B. (1973) Biochem.Biophys.Res.Commun. 50, 765-770.
15. Yang, M., Galizzi, A., and Henner, D. (1983) Nucleic Acids Res. 11, 237-249
16. Ohmura, K., Shiroza, T., Nakamura, K., Nakayama, A., Yamane, K., Yoda, K., Yamasaki, M., and Tamura, G. (1984) J.Biochem. 95, 87-93.
17. Palva, I. (1982) Gene 19, 81-87.
18. Palva, I., Sarvas, M., Lehtovaara, P., Sibakov, M., Kääriäinen, L. (1982) Proc.Natl.Acad.Sci.USA 79, 5582-5586.
19. Palva, I., Lehtovaara, P., Kääriäinen, L., Sibakov, M., Cantell, K., Sehein, C.H., Kashiwagi, K., and Weissman, C. (1983) Gene 22, 229-235.
20. Yamane, K., Takeichi, Y., Masuda, T., Kawamura, F., and Saito, H. (1982) J.Gen.Appl.Microbiol. 28, 417-428.
21. Ohtsuka, E., Ikehara, M., and Soll, D. (1982) Nucleic Acids Res. 10, 6553-6570.
22. Laemmli, U.K. (1970) Nature 227, 680-685.
23. Towbin, H., Staehelin, T., and Gordon, J. (1979) Proc.Natl.Acad.Sci.USA 76, 4350-4354.
24. Birnboim, H.C., and Doly, J. (1979) Nucleic Acids Res. 7. 1513-1523.
25. Tabak, H.F., and Flavell, R.A. (1978) Nucleic Acids Res. 5, 2321-2332.
26. Maxam, A.M., and Gilbert, W. (1980) Methods Enzymol. 65, 499-560.
27. Chang, S., and Cohen, S.N. (1979) Molec.Gen.Genet. 168, 111-115.
28. O'Callaghan, C.H., Morris, A., Kirby, S.M., and Shingler, A.H. (1972) Antimicrob.Ag.Chemother. 1, 283-288.
29. Chou, P.Y., and Fasman, G.D. (1978) Adv. Enzymol. 47, 45-148.
30. Tarr, G.E., Black, S.D., Fujita, V.S., and Coon, M.J. (1983) Proc.Natl. Acad.Sci.USA 80, 6552-6556.
31. Perlman, D., and Halvorson, H.O. (1983) J.Mol.Biol. 167, 391-409.
32. Nielsen, J.B.K., and Lampen, J.O. (1982) J.Biol.Chem. 257, 4490-4495.