Streptopain is a cysteine protease expressed by Streptococcus pyogenes. To study the maturation mechanism of streptopain, wild-type and Q186N, C192S, H340R, N356D and W357A mutant proteins were expressed in Escherichia coli and purified to homogeneity. Proteolytic analyses showed that the maturation of prostreptococcal pyrogenic exotoxin B zymogen (pro-SPE B) involves eight intermediates with a combination of cis- and trans-processing. Based on the sequences of these intermediates, the substrate specificity of streptopain favors a hydrophobic residue at the P2 site. The relative autocatalytic rates of these mutants exhibited the order Q186N > W357A > N356D, C192S, H340R. Interestingly, the N356D mutant containing protease activity could not be converted into the 28-kDa form by autoprocessing. This observation suggested that AsnN356 might involve the cis-processing of the propeptide. In addition, the maturation rates of pro-SPE B with trypsin and plasmin were 10- and 60-fold slower than that with active mature streptopain. These findings indicate that active mature streptopain likely plays the most important role in the maturation of pro-SPE B under physiological conditions.
expressed the wild-type protein and mutants Q186N, C192S, H340R, N356D, and W357A in *Escherichia coli*. Glu186 is the residue outside the substrate-binding pocket and contacts with the loop containing Cys192, Cys340 and His340 are the catalytic residues; and Asn356 and Trp357 are the residues near the active site of streptopain. Studies of the chemical modifications of cysteine, histidine, and tryptophan also indicate that these residues are essential for the protease activity of streptopain (5). Based on the x-ray structure of the streptopain C192S mutant (Fig. 1) (27), the catalytic site of streptopain has a catalytic Cys-His dyad, which differs from most other cysteine proteases containing a Cys-His-Asn catalytic triad. Previous studies have shown that a mutation at Cys192 or His340 of streptopain leads to a complete loss of protease activity (4, 23, 25). Asn175 of papain is not essential for its protease activity, under varying induction conditions, 10 ml of cells were collected by centrifugation and suspended in 1 ml of lysis buffer (20 mM Tris-HCl, 200 mM NaCl, and 1 mg/ml lysozyme, pH 8.0). Each lysate was centrifuged at 10,000 × g for 10 min. The proteins in the supernatant (soluble) were collected, and the proteins in the pellet (insoluble) were dissolved in 1 ml of sample buffer. SDS-PAGE was performed to analyze the relative proportions of overexpressed proteins in the soluble and insoluble fractions.

Recombinant wild-type streptopain was converted into a 28-kDa active enzyme during the course of purification. To prevent the conversion, mercuric chloride was added to the wild-type extract to a final concentration of 1 mM and was kept present throughout the purification. In addition, most of the expressed W357A mutant was present as an insoluble inclusion body, and a standard procedure using a denaturing condition was performed to refold the protein (41). The inclusion body of the W357A mutant was solubilized in denaturing solution (4.5 mM urea, 20 mM Tris-HCl, and 200 mM NaCl, pH 8.0), and the solution was diluted to A_{280} < 0.1. The protein was renatured by dialysis against 20 mM Tris-HCl and 200 mM NaCl, pH 8.0. The recombinant protein was purified by Ni2+ chelating chromatography (Amersham Biosciences) with a gradient of 20–200 mM imidazole. The proteins were concentrated by Amicon ultrafiltration using a 10-kDa cutoff membrane and then exchanged with PBS. The final solutions were stored at -20 °C.

**Purification of Streptopain from *Streptococcus* Strain A20—Native streptopain** was purified from strain A20 as previously described (8). One volume of 1 ml of bacterial culture was first grown overnight at 35 °C in 20 ml of TSBY medium (3% tryptic soy broth and 0.5% yeast extract). The culture was then added to 100 ml of TSBY medium. Streptopain was produced by growing cells at 37 °C for 22–24 h. The supernatant was collected by centrifugation and filtered through a 0.45-μm membrane filter. The filtrate was diluted with 400 ml of cold distilled water, and the pH was adjusted with 1 N NaOH to 8.0. Then, 25 g of pre-equilibrated DEAE-Sepharose resin (Amersham Biosciences) with 20 mM Tris-HCl, pH 8.0, were added to the filtrate. The solution was left for 30 min with occasional mixing, and the unbound protein was collected by filtration. The filtrate was concentrated to 100 ml by Amicon ultrafiltration using a 3-kDa cutoff membrane. The buffer was exchanged by ultrafiltration with 1 liter of 20% ethanol and 20 mM Tris-HCl, pH 7.0. The final solution was loaded onto a Red A column (Dynamex gel, Millipore Corp.). Streptopain was eluted using a linear gradient of 400 ml of 0–2 M NaCl with a flow rate of 20 ml/h, and 5-ml fractions were collected. SDS-PAGE showed that streptopain with a molecular mass of 25 kDa was homogeneous.

**Azocasein Assay**—The azocasein assay was used to test for proteolytic activity of streptopain and mutant proteins. The assay was modified as previously described (28). Activity was determined by measuring the hydrolysis of azocasein based on the absorbance increase at 666 nm against time as described below. The reaction was initiated by adding 20 μl of streptopain or mutant protein to 180 μl of reaction mixture containing 2.7 mg/ml azocasein, 5 mM dithiothreitol (DTT), and 5 mM EDTA (Sigma) in PBS. After incubating the solution at 37 °C for the designated time intervals ranging from 0 to 24 h, the reaction was stopped by addition of 40 μl of 15% ice-cold trichloroacetic acid. Absorbance was measured using a Beckman Model DU640 spectrophotometer. One unit of the enzyme is defined as the amount of protease which releases 1 μg of soluble azopeptide/min. The specific absorption coefficient (A_{666}) = 40) of the azocasein solution was calculated by measuring its absorption after total digestion (28).

**Processing of the Pro-SPE B C192S Mutant by Wild-type and Mutant**

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**EXPERIMENTAL PROCEDURES**

**Streptopain Expression, Mutant Construction, and Purification**—The genomic DNA of GAS was extracted from strain A20. The structural gene of pro-SPE B was amplified by PCR using the sense primer 5'-GATCCGGATCCATCATCATCATCATATGCAAATTTCCT-GTAAAGGAA-A with a His_{6} tag and BamHI recognition and by the antisense primer 5'-GGATCCGGATCCCTAAGGTTTGATGCCTACAA-CAG-3' with BamHI recognition. The PCR product was purified and then cloned into the BamHI site of the pET-21a vector. The recombinant plasmid was transformed into the *E. coli* BL21(DE3) pLys strain, and the system was under the control of a strong T7 promoter. The wild-type construct was used to produce Q186N, C192S, H340R, N356D, and W357A mutations using overlap extension PCR (27). Cells were grown at 37 °C for 6–8 h in LB medium (1 liter of 10 g of Bacto-Tryptone, 5 g of Bacto-yeast extract, and 10 g of NaCl) that was adjusted to pH 7.2 with 3 N NaOH. The cells were cultured to A_{600} = 0.5–1.0. To the culture was added isopropyl-1-thio-β-D-galactopyranoside (1 mM), and the culture was further incubated at 15–37 °C for 2–24 h to induce protein production. Cells were harvested by centrifugation and lysed by liquid shear with a French press to obtain the extract.

To obtain soluble proteins, the conditions of protein expression were optimized by lowering the temperature and by varying induction periods. Under varying induction conditions, 10 ml of cells were collected by centrifugation and suspended in 1 ml of lysis buffer (20 mM Tris-HCl, 200 mM NaCl, and 1 mg/ml lysozyme, pH 8.0). The structure figure was prepared using the program MOLMOL (39).

**Fig. 1. Ribbon drawing of the three-dimensional structure of pro-SPE B.** The α-helices, β-strands, and loop regions of streptopain are shown in red, blue, and gray, respectively. The residues of the P1 (Asn186, Tyr187, Val188, Ala118, and Lys129) and P2 (Val67, Met78, Ile117, and Ile128) cleavage sites of the proregion observed in the maturation processing of pro-SPE B are shown in dark blue. The mutated Gin186, Cys192, His340, Asn356, and Trp357 residues of streptopain are shown in green. The structure figure was prepared using the program MOLMOL.
Expression and purification of wild-type streptopain and its Q186N, C192S, H340R, N356D, and W357A mutants

| Clones | Protein expression conditions | Yielda | Specific activityb |
|--------|-------------------------------|--------|-------------------|
|        |                               | mg/liter | units/mg |
| Wild-type | 37 °C, 24 h | 45 | 652 ± 52 |
| Q186N | 37 °C, 15–17 h | 40 | 366 ± 30 |
| C192S | 28 °C, 12–14 h | 380 | ND |
| H340R | 28 °C, 12–14 h | 210 | ND |
| N356D | 25 °C, 12–14 h | 15 | 1.02 ± 0.2 |
| W357A | 15 °C, 12–14 h | 8 | 0.033 ± 0.005 |

a Yield indicates total soluble protein after purification by Ni2+-chelating chromatography.
b One enzyme unit is defined as the amount of protease required to release 1 μg of soluble azoprotein/min. ND, not detectable.

Proteolytic Activities of Wild-type and Mutant Streptopain—We used azocasein as the substrate to examine the proteolytic activities of the wild-type and mutant proteins of streptopain. As shown in Table I, mutants C192S and H340R of SPE B completely lost their proteolytic activities, consistent with recent reports (4, 17, 29, 35). We found that the presence of extra amino acids at the N terminus did not affect the proteolytic activity of streptopain. Compared with the wild-
type enzyme, the activities of mutants Q186N, N356D, and W357A of SPE B decreased by 1.8-, 359-, and 50,000-fold, respectively (Table I). The inactive C192S mutant was used as a substrate to examine the proteolytic activities of the native and recombinant streptopain proteins (17). Fig. 4 shows the results of SDS-PAGE analyses of the processing of the pro-SPE B C192S mutant using the native, wild-type, Q186N, N356D, and W357A proteins (Fig. 4). Our results show that recombinant streptopain was as active as the native form (Fig. 4, A and B). Interestingly, native SPE B was less efficient at processing the 42-kDa C192S mutant than recombinant SPE B. Based on the N-terminal sequence analyses, recombinant and native SPE B contain a mixture of products 8 and 9. Different 28-kDa
products may have effects on their trans-processing activities.

The times required for the conversion from the 42-kDa C192S mutant to the 28-kDa form with recombinant streptopain and the Q186N, N356D, W357A mutants were 1, 2.5, 14, and 24 h, respectively. Compared with the wild-type enzyme, the proteolytic activities of the Q186N, N356D, and W357A mutants decreased by 3-, 10-, and 30-fold, respectively. Therefore, the digestion rates of the 42-kDa C192S mutant were 1, 2.5, 14, and 24 h, respectively. Compared with the wild-type enzyme, the proteolytic activities of the Q186N, N356D, and W357A mutants decreased by 3-, 10-, and 30-fold, respectively. The times required for the conversion from the 42-kDa C192S mutant to the 28-kDa form with recombinant streptopain were 5 residues apart. The accuracy of the signal peptide of streptopain was position 1. The boxed sequence represents the proregion. The thin, double-tailed, thick, and gray arrows designate the sites of cleavage by streptopain; by trypsin, and plasmin; by streptopain and trypsin; and by trypsin and plasmin, respectively.

Processing of the Pro-SPE B C192S Mutant by 28-kDa Active Streptopain—The maturation of pro-SPE B goes through multiple-step processing involving six intermediates and one final product (17). Similar intermediates are involved in the trans-processing of the pro-SPE B C192S mutant by the 28-kDa active form of streptopain (17). Fig. 4A shows that the processing of the C192S mutant by streptopain involved at least nine cleavages. The cleavage sites of these products were determined by an N-terminal sequencer and are summarized in Table II. Product 9 was identified as an N-terminal sequencer because the azocasein assay, giving the relative proteolytic activities of the mutant proteins on the order of Q186N > N356D > W357A > C192S, H340R. As shown in Fig. 4, the trans-processing of the pro-SPE B C192S mutant by the wild-type and Q186N and N356D mutant proteases generated at least eight intermediates. In contrast, the trans-processing of the pro-SPE B C192S mutant by the W357A mutant gave rise to only two visible intermediates. This finding indicates that Trp357 plays an important role in substrate recognition. This result is consistent with the reported x-ray structure of pro-SPE B, which shows that Trp357, Phe 342, and Phe 367 form a hydrophobic substrate-binding site (26).

Processing of the Pro-SPE B C192S Mutant by 28-kDa Active Streptopain—The maturation of pro-SPE B goes through multiple-step processing involving six intermediates and one final product (17). Similar intermediates are involved in the trans-processing of the pro-SPE B C192S mutant by the 28-kDa active form of streptopain (17). Fig. 4A shows that the processing of the C192S mutant by streptopain involved at least nine cleavages. The cleavage sites of these products were determined by an N-terminal sequencer and are summarized in Table II. Product 9 was identified as the 28-kDa active form of streptopain, consistent with previous reports (8, 17). However, although previous studies reported the presence of six intermediates, four of which have been characterized, we identified a total of eight intermediates. We also found that, based on the sequences of all intermediates and the final product, the substrate specificity of streptopain was similar to the substrate preference of the papain-like family, with a preference for a hydrophobic residue (isoleucine (5/9), tyrosine (2/9), methionine (1/9), or valine (1/9)) at the P2 site. Analysis of the cleavage sites also revealed trends, with an asparagine residue (3/9) at the P3 site, a lysine residue (3/9) at the P1 site, a glycine residue (3/9) at the P2’ site, a glycine or alanine residue (2/9) at the

Table II

| Cleavage site | P4 | P3 | P2 | P1 | P1’ | P2’ | P3’ | P4’ |
|--------------|----|----|----|----|-----|-----|-----|-----|
| By streptopain | | | | | | | | |
| 1’ | A | A | I | K | A | G | A | R |
| 2 | D | K | V | N | E | G | E |
| 3 | S | N | M | Y | V | E | Y | N |
| 4 | N | M | Y | V | 6 | Y | N | I |
| 5 | Y | N | I | S | T | G | E | F |
| 6 | E | N | I | A | 1 | S | F | M |
| 7 | E | Q | I | K | 2 | G | E | K |
| 8 | T | T | Y | A | 3 | G | E | T |
| 9 | A | E | I | K | 4 | G | P | V |
| C | A | E | N | I | K | X | G | A |
| F | 2 | 9 | 3 | 9 | 5 | 9 | 3 | 9 |

By plasmin

| 1 | A | G | A | R | 5 | S | A | E |
| 2 | K | E | N | K | 3 | K | L | D |
| 3 | P | V | V | K | 5 | S | L | D |

These cleavage sites were reported by Doran et al. (17).
These intermediates were identified in this study.
C represents the preferred amino acids at different sites.
X represents any amino acid.
F represents the fractions of the preferred amino acids at different sites.

![Fig. 5.](http://www.jbc.org/)

**Fig. 5.** Primary sequence of recombinant streptopain and the sites of cleavage of pro-SPE B by mature streptopain, plasmin, and trypsin (A) and schematic representation of pro-SPE B (B).

The sequence of recombinant streptopain is shown and contains 11 extra vector residues, 6 histidine residues, and sequences without signal peptide. For purposes of comparison with previous mutagenesis studies, in this study, we numbered the first methionine of the signal peptide of streptopain as position 1. The boxed sequence represents the proregion. The thin, double-tailed, thick, and gray arrows designate the sites of cleavage by streptopain; by trypsin, and plasmin; by streptopain and trypsin; and by trypsin and plasmin, respectively.

P3’ site, and a glutamate residue (3/9) at the P4’ site (Table II). This pattern of intermediate accumulations and disappearances indicates that products 8 and 9 of streptopain were the major final products from the processing of the pro-SPE B C192S mutant by streptopain. The primary sequence of pro-SPE B and the resulting cleavage sites are shown in Fig. 5.

**Processing of the Pro-SPE B C192S Mutant by Plasmin and Trypsin—**The inactive C192S mutant of pro-SPE B was also used to examine its digestion process with plasmin and trypsin. To compare the processing of this mutant with that of the 28-kDa active form of streptopain, we used the same reaction substrate/enzyme ratio of 20:1. Fig. 6 shows that trypsin converted the 42-kDa C192S mutant into the 28-kDa form, which contained two products, and that plasmin converted the 42-kDa C192S mutant into the 28-kDa form, which contained only one product. Both processes involved the same two intermediates; however, the final product differed from the 28-kDa active mature streptopain, and they were 5 residues apart. The accumulation of fewer intermediates was in contrast with the results observed for the processing by streptopain.

The trypsin and plasmin cleavage sites in the primary sequence of pro-SPE B are shown in Fig. 5, and the N-terminal sequences of the reaction products with plasmin are summarized in Table II. Trypsin and plasmin are known to prefer lysine or arginine at the P1 site. The sequences of the intermediates and the final product are consistent with their specificity. In contrast, intermediates 1 and 7 (with lysine at the P1 site) observed in streptopain were not found. The times required for the complete digestion of the 42-kDa C192S mutant by streptopain, trypsin, and plasmin were 1, 12, and 72 h, respectively. Therefore, the digestion rates of the 42-kDa C192S mutant by the 28-kDa active form of streptopain, trypsin, and plasmin were 2, 0.2, and 0.033 nmol/h, respectively.
These findings indicate that the 28-kDa active form of streptopain likely plays the most important role in activating pro-SPE B under physiological conditions.

**Autocatalysis of Pro-SPE B Mutants**—To study the effect of mutation on the autocatalysis of pro-SPE B, we analyzed the conversion of the 42-kDa mutant protein to the 28-kDa active form. As shown in Fig. 7, the C192S and H340R mutants, which had no proteolytic activities, also had no autoprocessing activities. The Q186N mutant was converted into the 28-kDa form within 1 h, and the W357A mutant was converted within 14 h. Note that the conversion rate of the W357A mutant was $10^{-15}$-fold slower than that of the Q186N mutant. In contrast, the 42-kDa N356D mutant showed no autoprocessing even after 14 h, and the protease activity of the N356D mutant was higher than that of the W357A mutant (Table I). These results indicate that Asn356 of pro-SPE B is involved in the cis-processing of the propeptide. Based on the reported x-ray structure of the C192S mutant, Asn356 does not have direct contact with the propeptide region because the Asn356 side chain does not face the propeptide region (26). Interestingly, the corresponding residue of papain is not essential for activity and likely plays a role in positioning the catalytic residues (18). This study shows a new functional role for this conserved asparagine residue of the cysteine protease family.

**cis- and trans-Processing of Pro-SPE B**—As described above, the autocatalysis of pro-SPE B is either a cis- or trans-processing mechanism or, more likely, involves a combination of the two. The cis-processing portion of the mechanism is likely characterized by a zero-order reaction, where the precursor-processing rate is independent of the precursor concentration. In contrast, the trans-processing portion of the mechanism is likely characterized by a higher order reaction, where the precursor-processing rate is dependent on the precursor concentration. In this study, we used pro-SPE B to measure the initial rate of precursor processing at concentrations of 0.2, 0.35, 0.5, 0.75, 1, 2, 3, and 4 µM. The initial rate of precursor processing was determined by measuring the depletion of the precursor over time, and changes in the precursor bands were quantified using an imaging system. We found that the rate of transformation from the 42-kDazymogen to the 28-kDa enzyme was faster at higher concentrations, indicating trans-processing. Fig. 8 shows the plot of the relative rate of activation versus the concentration of precursor as fitted by linear regression. This
plot demonstrates that the reaction mechanism involves cis-processing because the extrapolated rate was not null at the zero pro-SPE B concentration. This plot also demonstrates that the reaction mechanism additionally involves trans-processing because the rate of activation was pro-SPE B concentration-dependent. From these data, we can conclude that the autoactivation of pro-SPE B results from a combination of cis- and trans-processing.

Fig. 8 shows that the extrapolated rate is 0.7583 when the concentration of pro-SPE B is zero and the slope is 22.833. Therefore, it can be calculated that the relative rates of cis- and trans-processing (0.7583) are equal at a pro-SPE B concentration of 33.2 nM. This concentration is 3.3-fold lower than with propapain, which is 110.8 nM (20). These results indicate that the maturation of streptopain is similar to that of propapain and involves both cis- and trans-processing.

**DISCUSSION**

To understand the role of streptopain in the pathogenesis of GAS, one must first understand the maturation processing of pro-SPE B to the 28-kDa active form of streptopain. The steps involved in the maturation ofzymogens for the papain-like family of cysteine proteases have been well documented (18–20, 31–33). On the basis of the consensus sequence and the sizes of the prodomain, streptopain belongs to the C1 family of cysteine protease clan CA, and papain belongs to the C1 family (20, 21, 34, 35). To better understand the maturation of the streptopain protein, we expressed the wild-type and mutant proteins of streptopain in E. coli and purified them to homogeneity. Our CD studies showed that the wild-type and mutant proteins of streptopain expressed in E. coli retained their structural integrity. Recombinant streptopain was expressed as a 42-kDa zymogen and converted into a 28-kDa enzyme during the course of purification. 1 mM HgCl₂ can be used as inhibitor during purification to prevent the conversion. Our protease assays showed that recombinant 28-kDa streptopain was as active as native streptopain. The presence of extra amino acids at the N terminus did not appear to affect the protease activity of recombinant streptopain or the maturation processing of pro-SPE B mutants. CD analyses showed that the secondary structures of the mutant proteins are similar to the reported three-dimensional structure of the pro-SPE B C192S mutant. To our knowledge, these findings provide the first direct evidence that highly labile streptopain can be expressed in E. coli and purified to homogeneity while maintaining its structural integrity and full activity.

Many studies have shown that the maturation of cysteine protease family members, including propapain and procathepsins B, K, and L, involves both cis- and trans-processing (20, 31–33). The effect of the pro-SPE B concentration on the rate of processing and the proteolytic processing of pro-SPE B mutants as shown in this study demonstrates that the autoactivation mechanism of pro-SPE B is stepwise and involves at least eight intermediates with a combination of cis- and trans-processing. Based on the sequences of these intermediates, the specificity pocket of streptopain is similar to that of the papain-like family, with preferences for a hydrophobic residue at the P2 site. In the maturation of pro-SPE B mutants, only Q186N and W357A were transformed fromzymogens into the 28-kDa active forms. In contrast, N356D was not autocatalytically converted into the 28-kDa active form. However, N356D exhibited protease activity, which converted the pro-SPE B C192S mutant into the 28-kDa form. Based on the reported x-ray structure of the C192S mutant, Asn³⁵⁶ does not have direct contact with the propeptide region because the Asn³⁵⁶ side chain does not face the propeptide region (26). These results suggest that Asn³⁵⁶ of pro-SPE B is involved in the cis-processing of the propeptide.

Although the maturation processing of pro-SPE B by exogenous proteases has been shown (12, 14, 17), no studies have been performed to compare the maturation processing of streptopain by both autocatalysis and exogenous proteases. In this study, we found that both trypsin and plasmin could convert pro-SPE B into the 28-kDa active form with three and two intermediates, respectively. Although the maturation processing of the pro-SPE B C192S mutant by exogenous proteases produced fewer intermediates, the rates of digestion of the 42-kDa C192S mutant by trypsin and plasmin were 10- and 60-fold slower than that of the 28-kDa active form. Analyses of the numbers and sequences of all intermediates and the final products created by streptopain, plasmin, and trypsin revealed that streptopain is a relatively promiscuous protease. We speculate that 28-kDa active form of streptopain plays the most important role in converting pro-SPE B into 28-kDa streptopain under physiological conditions. However, sequence analysis of the SPE B gene from 200 GAS isolates showed that 20% of these variants contain an Arg-Gly-Asp motif that preferentially binds integrins α₅β₁ and α₅β₁ (28). Angiostatin, a plasmin fragment containing three to four N-terminal kringle domains, is a ligand for integrin α₅β₁ (40). Therefore, integrin binding may concentrate both streptopain and plasmin at the human cell surface, thereby accelerating the maturation of streptopain. These interactions may be important because they may provide a means for GAS invasion.

Compared with the maturation of procathepsins, the maturation processing of pro-SPE B involves many more identifiable intermediates. In contrast, the accumulation of multiple intermediates is not found in propapain or procathepsins (20, 31–33). The biological significance of having so many intermediates involved in the autocatalytic processing of pro-SPE B is still unclear. Because the propeptide region of pro-SPE B is very close to the substrate-binding site, it is likely that these intermediates with different prosegment sizes likely have different substrate specificities. This is consistent with the results from our proteolytic studies that streptopain has diverse substrate specificity. The relative rates of the cis- and trans-processing of procathepsin L, propapain, and pro-SPE B are equal at concentrations of 0.24, 110.8, and 33.2 nM, respectively (20, 32). These results indicate that the maturation of pro-SPE B, propapain, and procathepsin L involves both cis- and trans-processing. Regulation of the cysteine protease activity of the papain-like family has been implicated in a wide variety of human diseases (35, 36). Specifically, rapidly accumulating evidence suggests that the growth and proliferation of pathogenic bacteria depend on proteolytic enzymes of the invading organism (2, 33, 37, 38). Because the 28-kDa active form of streptopain plays an important role in GAS pathogenesis (12, 14, 17), continuing studies regarding the maturation of pro-SPE B are increasingly important.

In conclusion, we expressed wild-type and Q186N, C192S, H340R, N356D, and W357A mutant streptopain in E. coli and purified them to homogeneity. We found that streptopain produced in E. coli possesses the same function and structure as the native protein. This is the first report to show that labile streptopain can be expressed in E. coli with the correct fold. We also found that the maturation of pro-SPE B is stepwise and involves at least eight intermediates that involve a combination of cis- and trans-processing. Like other papain-like family members of cysteine proteases, including propapain and procathepsin L, the maturation processing of pro-SPE B involves a combination of cis- and trans-processing. Compared with other exogenous proteases, the 28-kDa active form of streptopain is the most effective protease for processing pro-SPE B. We speculate that a similar mechanism occurs in vivo. Because strep-
topain is a possible drug target in the control of streptococcal infection, this study serves as the basis for gaining insight into streptococcal infections by exploring the structure-function relationships of streptopain. This study also extends our understanding of the molecular basis of the maturation mechanism of streptopain under physiological conditions.

REFERENCES

1. Hoge, C. W., Schwartz, B., Talkingon, D. F., Breiman, R. F., MacNeill, E. M., and Englender, S. J. (1983) *J. Am. Med. Assoc.* 250, 384–389
2. Molinari, G., and Cursharan, S. C. (1999) *Curr. Opin. Microbiol.* 2, 56–61
3. Bisno, A. L. (1991) *N. Engl. J. Med.* 325, 783–793
4. Gubba, S., Low, D. E., and Musser, J. M. (1999) *Infect. Immun.* 67, 765–770
5. Tsai, P. J., Kortt, A. A., Lin, T. Y., and Elliott, S. D. (1976) *J. Biol. Chem.* 251, 1955–1959
6. Burns, E. H., Lukoms, S., Jr., Rurangirwa, J., Podbielski, A., and Musser, J. M. (1999) *Microb. Pathog.* 24, 333–339
7. Kazmi, S. U., Kansal, R., Houshalan, M., Norby-Teiglund, A., Low, D. E., Halim, A. B., and Koth, M. (2001) *Infect. Immun.* 69, 4988–4995
8. Kuo, C.-F., Wu, J.-J., Lin, K. Y., Tsai, P. J., Lee, S. C., Jin, Y. T., Lei, H. Y., and Lin, Y.-S. (1998) *Infect. Immun.* 66, 3931–3935
9. Kuo, C.-F., Wu, J.-J., Tsai, P. J., Kao, F. J., Lei, H. Y., Lin, M. T., and Lin, Y.-S. (1999) *Infect. Immun.* 67, 126–130
10. Lukoms, S., Montgomery, C. A., Rurangirwa, J., Geske, R. S., Barrish, J. P., Adams, G. J., and Musser, J. M. (1999) *Infect. Immun.* 67, 1779–1788
11. Ohara-Nemoto, Y., Sasaki, M., Kaneko, M., Nemoto, T., and Ota, M. (1994) *Can. J. Microbiol.* 40, 930–936
12. Hauser, A. R., and Schlievert, P. M. (1990) *Infect. Immun.* 58, 501–507
13. Matsuzaka, Y. V., Pilias, S., Gubba, S., Musser, J. M., and Olmsted, S. B. (1999) *Infect. Immun.* 67, 426–433
14. Musser, J. M., and Wu, J.-J. (1998) *Proc. Natl. Acad. Sci. U. S. A.* 95, 2235–2240
15. Tsai, P. J., Kuo, C.-F., Lin, K. Y., Tsai, P. J., Lee, S. C., Jin, Y. T., Lei, H. Y., and Lin, Y.-S. (1998) *Infect. Immun.* 66, 3931–3935
16. Nomizu, M., Pietrzynski, G., Kato, T., Lachance, P., Menard, R., and Ziomek, E. (2001) *J. Biol. Chem.* 276, 44551–44556
17. Doran, J. D., Nomizu, M., Takebe, S., Menard, R., Griffith, D., and Ziomek, E. (1999) *Eur. J. Biochem.* 256, 145–151
18. Vernet, T., Berti, P. J., de Montigny, C., Musil, R., Tessier, D. C., Menard, R., Magno, M. C., Storer, A. C., and Thomas, D. Y. (1995) *J. Biol. Chem.* 270, 10838–10846
19. Vernet, T., Chatellier, J., Tessier, D. C., and Thomas, D. Y. (1993) *Protein Eng.* 6, 213–219
20. Vernet, T., Khouri, H. E., Lafframme, P., Tessier, D. C., Musil, R., Gour-Salin, B. J., Storer, A. C., and Thomas, D. Y. (1991) *J. Biol. Chem.* 266, 21451–21457
21. Groves, M. R., Coulombe, R., Jenkins, J., and Cyliger, M. (1998) *Proteins* 32, 504–514
22. Poow-King, R., Bannan, J., Viteri, A., Cu, G., and Zabriskie, J. B. (1993) *J. Exp. Med.* 178, 759–763
23. Gubba, S., and Musser, J. M. (2000) *Infect. Immun.* 68, 3716–3719
24. Matsuzaka, Y. V., Pilias, S., Gubba, S., Musser, J. M., and Olmsted, S. B. (1999) *Infect. Immun.* 67, 426–433
25. Musser, J. M., Stockbauer, K., Kapur, V., and Rudgers, G. W. (1996) *Infect. Immun.* 64, 1913–1917
26. Kagawa, T. F., Cooney, J. C., Baker, H. M., McSweeney, S., Liu, M., Gubba, S., Musser, J. M., and Baker, E. N. (2000) *Proc. Natl. Acad. Sci. U. S. A.* 97, 2235–2240
27. Aiyar, A., Xiang, Y., and Leis, J. (1996) *Methods Mol. Biol.* 57, 177–191
28. Stockbauer, K. E., Magoun, L., Liu, M., Burns, E. H., Jr., Gubba, S., Renish, S., Pan, X., Bodary, S. C., Baker, E., Coburn, J., Leong, J. M., and Musser, J. M. (1999) *Proc. Natl. Acad. Sci. U. S. A.* 96, 242–247
29. Perczel, A., Hollosi, M., Tesnady, G., and Fasman, G. (1991) *Protein Eng.* 4, 669–679
30. Perczel, A., Park, K., and Fasman, G. D. (1992) *Anal. Biochem.* 203, 83–93
31. McQueney, M. S., Amegadzie, B. Y., D’Alessio, R., Hanning, C. R., McLaughlin, M. M., McNulty, D., Carr, S. A., Ijames, C., Kurdyla, J., and Jones, C. S. (1997) *J. Biol. Chem.* 272, 13955–13960
32. Menard, R., Carmona, E., Takebe, S., Dafoir, E., Floufle, C., Mason, P., and Mort, J. S. (1998) *J. Biol. Chem.* 273, 4478–4484
33. Rozman, J., Stojan, J., Kuhelj, R., Turk, V., and Turk, B. (1999) *FEBS Lett.* 459, 358–362
34. Karrer, K. M., Peiffer, S. L., and DiTommaso, M. E. (1993) *Proc. Natl. Acad. Sci. U. S. A.* 90, 3063–3067
35. Berquin, I. M., and Sloane, B. F. (1996) *Adv. Exp. Med. Biol.* 389, 281–284
36. Turk, R., Turk, V., and Turk, D. (1997) *Biol. Chem. Hoppe-Seyler* 378, 141–150
37. Gordon, V. M., and Leplaa, S. H. (1994) *Infect. Immun.* 62, 333–340
38. Travis, J., Potempa, J., and Maeda, H. (1995) *Trends Microbiol.* 3, 405–407
39. Koradi, R., Billeter, M., and Wuthrich, K. (1996) *J. Mol. Graph.* 14, 51–55
40. Tarui, T., Majumdar, M., Miles, L. A., Ruf, W., and Takada, Y. (2002) *J. Biol. Chem.* 277, 33564–33570
41. QIAGEN Inc. (1992) *QIAGEN Manual*, 2nd Ed., QIAGEN Inc., Chatsworth, CA
