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The Bacillus subtilis Signaling Protein SpoIVB Defines a New Family of Serine Peptidases

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The protein SpoIVB plays a key role in signaling in the σk checkpoint of Bacillus subtilis. This regulatory mechanism coordinates late gene expression during development in this organism and we have recently shown SpoIVB to be a serine peptidase. SpoIVB signals by transiting a membrane, undergoing self-cleavage, and then by an unknown mechanism activating a zinc metalloprotease, SpoIVFB, which cleaves pro-σk to its active form, σk, in the outer mother cell chamber of the developing cell. In this work we have characterized the serine peptidase domain of SpoIVB. Alignment of SpoIVB with homologues from other spore formers has allowed site-specific mutagenesis of all potential active site residues within the peptidase domain. We have defined the putative catalytic domain of the SpoIVB serine peptidase as a 160-amino-acid residue segment at the carboxyl terminus of the protein. His236 and Ser378 are the most important residues for proteolysis, with Asp363 being the most probable third member of the catalytic triad. In addition, we have shown that mutations at residues Asn290 and His394 lead to delayed signaling in the σk checkpoint. The active site residues suggest that SpoIVB and its homologues from other spore formers are members of a new family of serine peptidases of the trypsin superfamily.

The protein SpoIVB has recently been identified as a serine peptidase that plays a central role in a regulatory checkpoint (the σk checkpoint) which coordinates gene expression during the later stages of spore formation in Bacillus subtilis (36). Proteolytic activity has been demonstrated in vitro as well as in vivo and this activity is essential to SpoIVB’s role as the signaling molecule that activates σk.

SpoIVB is synthesized in the forespore chamber of the sporingulating cell and is secreted across the inner forespore membrane. At this point SpoIVB becomes proteolytically active and self-cleaves into at least three distinct species, of 46, 45, and 44 kDa. These are thought to be the active forms which signal proteolytic processing of the transcription factor σk. Signaling leads to activation of a processing complex embedded in the outer forespore membrane which cleaves the N-terminal leader sequence (the pro sequence) from pro-σk. The sigma factor σk is then competent to direct the final stages of gene expression in the mother cell. Reminiscent of the blood clotting cascades, SpoIVB is also subject to secondary proteolysis, which presumably inactivates SpoIVB by cleaving the active species into 42- and 40-kDa forms (36). How SpoIVB activates processing of pro-σk is not yet clear, but genetic evidence has shown that SpoIVB most likely interacts with one or more members of the pro-α domain complex which is embedded in the outer forespore membrane (7, 8, 10). These proteins are SpoIVF, a zinc metalloprotease which cleaves pro-α (10, 28), and the BofA and SpoIVF proteins, both of which are required to inhibit activity of SpoIVB (25, 26). Interestingly, both SpoIVFA and BofA inhibit SpoIVFB using their C termini, which are exposed to the space between the inner and outer forespore membranes, and removal of either the BofA or SpoIVFA C terminus renders SpoIVFB constitutively active (12, 27, 34).

SpoIVB also carries a PDZ domain in the N-terminal half of the polypeptide (21). PDZ domains are used by signaling molecules for protein targeting and protein-protein interactions (18, 21, 22). In other work we have shown that this SpoIVB PDZ domain could be involved in multiple interactions including oligomerization, interaction with an inhibitor protein, BofC, and activating processing of pro-σk (14). The bacterial Prc (also called Tsp) and HtrA (also called DegP) serine peptidase families carry both a PDZ and peptidase domain (19, 21) and it has been shown that substrate recognition is mediated by the PDZ domain (3). An attractive model for how SpoIVB signals is that SpoIVB uses its PDZ domain to target one or both of the SpoIVFB inhibitors (SpoIVFA and BofA) followed by cleavage of the C termini of these inhibitors. An alternative role for the serine peptidase activity is simply to enable secretion of SpoIVB across the inner forespore membrane since SpoIVB does not carry a normal N-terminal signal sequence.

Homologues of SpoIVB proteins have been identified in a number of spore-forming organisms, revealing a string of strictly conserved residues. In this work we have examined the serine peptidase domain of SpoIVB with the aim of defining amino acid residues involved in catalysis. It appears that SpoIVB is a distinctive variant of trypsin-like proteases and that PDZ domains commonly have a specialized role in bacteria for the activation and substrate recognition of proteases that have to cross a membrane.

MATERIALS AND METHODS

Bacterial strains. Strains used in this work are listed in Table 1 and were all congenic with the prototrophic spo+ strain PY79. To construct lysogens of
Established strains
PY79   spo + 38
SC433  spoIBΔ::spc 9
SC1836 spoIBΔ::spc 17
NH578  spoIBΔ::spc amyE::spoIB + pNH470 into SC1836
NH577  spoIBΔ::spc amyE::pDG364 pDG364 into SC1836

Nonconservative mutants
NH1377 spoIBΔ::spc amyE::spoIBDL213 pNH1375 into SC1836
NH1320 spoIBΔ::spc amyE::spoIBHN236 pNH1308 into SC1836
NH1345 spoIBΔ::spc amyE::spoIBDL240 pNH1342 into SC1836
NH1346 spoIBΔ::spc amyE::spoIBDL242 pNH1334 into SC1836
NH1347 spoIBΔ::spc amyE::spoIBN290 pNH1344 into SC1836
NH1380 spoIBΔ::spc amyE::spoIVBK321 pNH1376 into SC1836
NH1326 spoIBΔ::spc amyE::spoIBDL363 pNH3134 into SC1836
NH1328 spoIBΔ::spc amyE::spoIVBK378 pNH3136 into SC1836
NH1455 spoIBΔ::spc amyE::spoIVBK387 pNH1445 into SC1836
NH1330 spoIBΔ::spc amyE::spoIVBH394 pNH3138 into SC1836

Conservative mutants
NH1451 spoIBΔ::spc amyE::spoIBHF326 pNH1441 into SC1836
NH1452 spoIBΔ::spc amyE::spoIBDN242 pNH1442 into SC1836
NH1453 spoIBΔ::spc amyE::spoIBDN363 pNH1443 into SC1836
NH1454 spoIBΔ::spc amyE::spoIVSA378 pNH1444 into SC1836

* Mutations were created in pBluescript clones carrying the spoIB gene, sequenced, and subcloned into pDG364 to give the plasmid clones indicated. These subclones were then linearized and DNA was introduced into the amyE locus of SC1836 cells as described in Materials and Methods.

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**RESULTS**

Site-directed mutagenesis of putative peptidase catalytic residues. Figure 1 shows a sequence alignment of the C-terminal domains (residues 188 to 425 in *B. subtilis* SpoIVB) of SpoIVB homologues from four *Bacillus* species (*B. subtilis, B. anthracis, B. stearothermophilus, and B. halodurans*), two *Clostridium* species (*C. acetobutylicum* and *C. difficile*), and *Carboxydothermus* hydrogenoformans. This region lies downstream of the PDZ domain (residues 102 to 186 in *B. subtilis* SpoIVB). The availability of sequences from such divergent sources aids the definition of strictly conserved residues. A single serine (Ser378) is conserved among these proteins. Proteolysis in vivo and in vitro is inhibited by the serine peptidase inhibitor diisopropylfluorophosphate and a mutation at this serine (SA378) inhibits autoproteolysis of SpoIVB (36). In addition, residues (GMSG) flanking this serine are conserved and are reminiscent of the SA clan of serine peptidases, which comprise the trypsin superfAMILY (1, 23) which has recently been reclassified as clan PA(S) (24). As has been discussed previously, this is the most likely candidate for the active site serine (36). The catalytic domain of most (but not all) serine peptidases consists of a triad of three residues (histidine, aspartic acid, and serine), with their order and spacing defining the clan and family of the enzymes.
peptidase (23). Clans indicate a common evolutionary origin, usually assigned following determination of three-dimensional structure, while families are primarily defined by sequence homology. The alignment reveals four conserved Asp residues (positions 213, 240, 242, and 363) that could form the acidic part of the catalytic triad. Only two His residues (positions 326 and 394) are conserved which could represent the base that enhances the nucleophilicity of the active site serine. Conserved lysine residues (positions 321 and 387) were also targeted for mutagenesis as they could potentially perform the role of a base. Examples include β-lactamase and Tsp, which have a catalytic Ser-Lys dyad (1). Similarly, the conserved asparagine at position 290 was mutated, since Asn residues are known to be involved in oxyanion stabilization in some proteases (e.g., subtilisin) or are important in orienting catalytic His residues in cysteine proteases. A number of conservative replacements were introduced into the sites predicted to be important in catalytic activity (Table 2).

### In vivo effects of SpoIVB mutations

All mutations were created in a pBluescript spoIVB clone, which carried the entire spoIVB gene. The mutated spoIVB clone was sequenced to verify the presence of a single base change and then subcloned into the plasmid pDG364. When linearized, this plasmid enables cloned DNA to be introduced at the amyE locus by a double-crossover recombination event (11). The mutant spoIVB alleles were incorporated at amyE in cells carrying a null mutation at the spoIVB locus, spoIVB::spc. Using isogenic control strains with (NH578) or without (NH577) a copy of the wild-type spoIVB gene at amyE we examined sporulation and signaling.

As shown in Table 2 there were three distinct classes of mutant phenotype. The first group (group I) consisted of spoIVBHN236, spoIVBHF236, spoIVBDL242, spoIVBDL363, spoIVBSA378, and spoIVBSK378 mutants which caused a severe effect on sporulation, with the failure to produce heat- or lysozyme-resistant spores. Spore formation was arrested at stage III-IV with the production of limited numbers of phase-grey spores. This phenotype was essentially asporogenous and indistinguishable from the null spoIVB::spc mutant. The pDG364 plasmids carrying these mutant alleles were introduced into spo mutants which caused a severe effect on spore formation, with the failure to produce heat- or lysozyme-resistant spores and were essentially indistinguishable from the isogenic Spo + strain, NH578. Spores produced in these mutants were able to germinate normally.

The second phenotypic class (group II) was made up of strains carrying the spoIVBDL213, spoIVBDL240, spoIVBDN242, spoIVBKA321, spoIVBDN363, and spoIVBKA387 alleles. Cells carrying these mutations produced normal levels of heat- and lysozyme-resistant spores and were essentially indistinguishable from the null spoIVB::spc mutant. The final and most interesting mutant class (group III) comprised the spoIVBNI290 and spoIVBHD394 mutations. They formed phase-bright spores, and so were Spo +, but there was a marked and reproducible reduction in the level of spores produced (approximately 30% fewer spores than in the wild-type strain). For these mutants we examined the capacity of spores to germinate and found that they were germination defective. Further analysis revealed that for spoIVBNI290 this germination defect was temperature sensitive, since the mutant was unable to germinate properly at 37°C. In other work
we have shown that bofA and bofB mutations (in BofA and SpoIVFA, respectively) allow constitutive activation of SpoIVB and therefore pro-σK processing (8). This, in turn, leads to premature σK-controlled gene expression, a reduction in spore-forming efficiency, and for those spores that are formed (approximately 10% of the total CFU), a defective germination response. This phenotype is most probably caused by a defect in spore coat assembly, which is controlled by the σK regulon. We reasoned that the reduced spore efficiency and defective germination response of the NI290 and HD394 mutants could be caused by a defect in signaling.

**σK-directed gene expression.** To examine signaling in the σK checkpoint we used a σK-transcribed reporter gene, gerE-lacZ (9). Lysogenized cells carrying mutant alleles with the SpoIVB null mutant (SC1836 spoIVB::spc) we detected low levels (less than 15%) of σK-directed gene expression, in

<table>
<thead>
<tr>
<th>Straina</th>
<th>Relevant allele</th>
<th>Mutationb</th>
<th>Heat resistancec</th>
<th>Lysozyme resistancec</th>
<th>Germination phenotyped</th>
<th>Signalingc</th>
</tr>
</thead>
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<tr>
<td>NH577</td>
<td>spoIVBΔ:spc</td>
<td>Insertion-deletion</td>
<td>4.9 × 10⁻⁵</td>
<td>1.56 × 10⁻²</td>
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<td>–</td>
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<tr>
<td>NH578</td>
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<td>98</td>
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<td>NH1320</td>
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<td>His236 to Asn</td>
<td>1.18 × 10⁻⁴</td>
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<td>–</td>
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<td>6.9 × 10⁻³</td>
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<td>–</td>
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<tr>
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<td>Asp242 to Leu</td>
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<td>2.19 × 10⁻²</td>
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<td>–</td>
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<td>spoIVBN290</td>
<td>Asn290 to Ile</td>
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<td>29</td>
<td>+ (30°C), – (37°C)</td>
<td>Delayed</td>
</tr>
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<td>86</td>
<td>88</td>
<td>+</td>
<td>+</td>
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<tr>
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<td>Asp363 to Leu</td>
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<td>3.7 × 10⁻²</td>
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<td>–</td>
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<tr>
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<td>1.77 × 10⁻²</td>
<td>ND</td>
<td>–</td>
</tr>
<tr>
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<td>–</td>
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<td>57</td>
<td>+</td>
<td>+</td>
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<tr>
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<td>spoIVBHD394</td>
<td>His394 to Asp</td>
<td>26.7</td>
<td>34.7</td>
<td>– (30°C), – (37°C)</td>
<td>Delayed</td>
</tr>
</tbody>
</table>

*a See Table 1.

*b See Fig. 1.

*c Heat or lysozyme resistance of cultures 24 h after the initiation of sporulation in DS medium (16). Values are expressed as percentages of CFU per milliliter in the untreated culture.

*d Five-day-old colonies grown on sporulation agar were tested for their capacity to germinate normally on tetrazolium agar plates as described previously (16). All strains that were phenotypically Spc⁺ were not tested for a germination phenotype (ND).

*e Signaling was defined according to the following two criteria: firstly, formation of pigmented colonies (Pig⁺); secondly, expression of the σK-controlled reporter gene, gerE-lacZ, in cells containing an SP[gerE-lacZ] lysogen (see Fig. 2) as described previously (9).

![FIG. 2. gerE-lacZ expression in spoIVB mutants. β-Galactosidase synthesis was measured at the indicated times following the initiation of sporulation in cells carrying the SP[gerE-lacZ] reporter phage.](image-url)
agreement with previous work, while for spo+ cells, gerE-lacZ expression started at about 3.5 to 4 h after the initiation of spore formation. Our results define three clear classes of mutant gene expression, with (i) a block in σK-directed gene expression, (ii) no effect on σK-directed gene expression, and (iii) delayed and reduced levels of expression. These mutant phenotypes correspond exactly to the groups classified above according to their ability to sporulate.

The group I mutant strains with a null sporulation phenotype have essentially abolished or severely reduced gerE-lacZ expression (Fig. 2A and C). The second group comprised the mutants with no detectable sporulation defect. These strains expressed gerE-lacZ at the same time and at the same levels as in spo+ cells (Fig. 2B and C). The third group caused a clear delay (1 h) and reduction in the level of gerE-lacZ expression (Fig. 2B). As mentioned above, defective signaling can lead to defects in spore coat assembly and spore germination. Here, gene expression was delayed, in contrast to mutations in bofA and bofB that allow constitutive processing of pro-σK, leading to premature σK-directed gene expression. Although other alleles within spoIVB have been shown to lead to delayed signaling (14, 17), the clear delay seen in the spoIVBHIN394 and spoIVBHIN290 mutations reinforces the notion that the role of this checkpoint is as a timing mechanism. That signaling does occur accounts for the levels of phase-bright spores that are produced.

**Proteolysis of SpoIVB.** To determine the effect of our collection of mutations on proteolysis we examined SpoIVB during spore formation using a polyclonal antiserum (Fig. 3). Previous work has shown that SpoIVB is synthesized as a 50-kDa polypeptide at about the second hour of sporulation (36). Starting at hour 3, SpoIVB is cleaved into a number of products (approximately 46, 45, and 44 kDa), one or more of which is the active form that signals SpoIVFB-mediated processing of pro-σK. Almost simultaneously, these forms are subject to secondary proteolysis, which presumably inactivates the active forms by cleavage to 42- and 40-kDa species which are then cleaved further. During sporulation, then, in wild-type cells, we see the 50-kDa species briefly but at hour 3 autoproteolysis has begun producing the active species as well as the inactive forms.

The effects on SpoIVB proteolysis are shown in Fig. 3. It must be stressed that these Western blots are particularly difficult to interpret due to the appearance of intermediate species in the blots and our accuracy in sampling. Regardless, we have repeated these blots at least twice and are confident of the following classifications by residue.

**FIG. 3.** Proteolysis of SpoIVB in B. subtilis. Sporulation was induced in wild-type (spo+), spoIVBΔ:spc (Δspo), and spoIVB mutant strains. Panel A shows an experiment examining strains carrying nonconservative mutations, and panel B shows strains carrying conservative changes as well as the alteration KA387. Samples of sporulating cells were taken at the times indicated, cells were lysed, and equivalent amounts of cellular protein were fractionated by SDS-PAGE (12%) and examined with a polyclonal antiserum to SpoIVB (2-min enhanced chemiluminescence exposure time). The full-length, 50-kDa, unprocessed form of SpoIVB is marked, as are 46-, 45-, and 44-kDa intermediate SpoIVB cleavage products and the 42- and 40-kDa cleavage products produced by secondary cleavage.
SpoIIB is a key component of the SpoIIB protease, which plays a crucial role in the sporulation process of Bacillus subtilis. The spoIIB gene is essential for sporulation and is involved in the processing of the SpoIIB protein. The SpoIIB protease is composed of a serine peptidase domain and a β-thiogalactopyranoside (IPTG)-inducible serine protease domain. The serine peptidase domain contains a catalytic residue, His394, which is essential for the proteolytic activity of the enzyme. The β-thiogalactopyranoside (IPTG)-inducible serine protease domain is inactive and undergoes self-cleavage under certain conditions.

The SpoIIB protease is active in the presence of IPTG, which induces the expression of the spoIIB gene. The enzyme cleaves the SpoIIB protein at specific sites, releasing the SpoIIB-D1 domain and the SpoIIB-D2 domain. The SpoIIB-D1 domain is further processed by the SpoIIB protease to release the SpoIIB-D1a and SpoIIB-D1b domains, which are required for the activation of the Spore-specific sigma factor, σK.

The SpoIIB protease is activated by the SpoIIB-D1 domain, which is released by the D1 domain of SpoIIB. The SpoIIB-D1 domain contains a serine protease domain, which is active in the presence of IPTG. The SpoIIB-D1 domain is further processed by the SpoIIB protease to release the SpoIIB-D1a and SpoIIB-D1b domains, which are required for the activation of the Spore-specific sigma factor, σK.

The SpoIIB protease is inhibited by the SpoIIB-D2 domain, which is released by the D2 domain of SpoIIB. The SpoIIB-D2 domain contains a serine protease domain, which is inactive in the presence of IPTG. The SpoIIB-D2 domain is further processed by the SpoIIB protease to release the SpoIIB-D2a and SpoIIB-D2b domains, which are required for the activation of the Spore-specific sigma factor, σK.

The SpoIIB protease is activated by the SpoIIB-D1 domain, which is released by the D1 domain of SpoIIB. The SpoIIB-D1 domain contains a serine protease domain, which is active in the presence of IPTG. The SpoIIB-D1 domain is further processed by the SpoIIB protease to release the SpoIIB-D1a and SpoIIB-D1b domains, which are required for the activation of the Spore-specific sigma factor, σK.

The SpoIIB protease is inhibited by the SpoIIB-D2 domain, which is released by the D2 domain of SpoIIB. The SpoIIB-D2 domain contains a serine protease domain, which is inactive in the presence of IPTG. The SpoIIB-D2 domain is further processed by the SpoIIB protease to release the SpoIIB-D2a and SpoIIB-D2b domains, which are required for the activation of the Spore-specific sigma factor, σK.

The SpoIIB protease is activated by the SpoIIB-D1 domain, which is released by the D1 domain of SpoIIB. The SpoIIB-D1 domain contains a serine protease domain, which is active in the presence of IPTG. The SpoIIB-D1 domain is further processed by the SpoIIB protease to release the SpoIIB-D1a and SpoIIB-D1b domains, which are required for the activation of the Spore-specific sigma factor, σK.

The SpoIIB protease is inhibited by the SpoIIB-D2 domain, which is released by the D2 domain of SpoIIB. The SpoIIB-D2 domain contains a serine protease domain, which is inactive in the presence of IPTG. The SpoIIB-D2 domain is further processed by the SpoIIB protease to release the SpoIIB-D2a and SpoIIB-D2b domains, which are required for the activation of the Spore-specific sigma factor, σK.

The SpoIIB protease is activated by the SpoIIB-D1 domain, which is released by the D1 domain of SpoIIB. The SpoIIB-D1 domain contains a serine protease domain, which is active in the presence of IPTG. The SpoIIB-D1 domain is further processed by the SpoIIB protease to release the SpoIIB-D1a and SpoIIB-D1b domains, which are required for the activation of the Spore-specific sigma factor, σK.
other trypsin family members, suggesting that SpoIVB must be a member of a new family of the PA(S) clan.

We have identified one Asp residue, Asp363, for which mutation to Leu leads to a lack of SpoIVB self-cleavage and signaling properties. Another Asp residue, Asp242, when mutated to Leu also blocked signaling yet failed to accumulate any protein at all, suggesting that the mutation rendered the protein unstable. We have noticed a DxxLL motif which is present in a number of serine peptidase families, especially those of the PA(S) clan (23, 24), and is found at position 363, and we take this as evidence that the SpoIVB serine peptidase has a catalytic triad with Asp363 as its third member. Intriguingly, a conservative change at this position does not disrupt proteolysis of the SpoIVB protein. The acidic residue is the least important component of the active site triad in serine peptidases, and Asp is replaced by Glu in carboxyl peptidase (37) and aspartyl dipeptidase (13). In the case of cytomegalovirus protease, histidine acts as the acid in a unique Ser-His-His triad. Mutation of cytomegalovirus protease His157 reduces activity only 5- to 10-fold (33). In contrast, catalysis by trypsin-like enzymes is severely impaired when the acidic residue is replaced by mutation, suggesting that the negative charge of the Asp side chain balances the positive charge that develops on the histidine during catalysis (6). In some systems, however, the Asp simply serves to maintain the correct position of the active site histidine. Indeed, in cysteine proteases, a conserved Asn residue (e.g., Asn175 in papain) serves this very purpose (35) and Asn156 appears in the hydrolase catalytic triad of E. coli outer membrane phospholipase A (15). We assume that the role of Asp363 is to stabilize the appropriate His263 rotamer or tautomer in SpoIVB catalysis, a function that could potentially be performed by Asn. The unusually close spacing of Asp363 to Ser378, its ability to be replaced by Asn, and the lack of a consensus motif around His236 all argue that SpoIVB represents a new family of serine peptidases.

One observation we have made here is that mutations that block signaling of pro-αK processing underwent proteolysis in B. subtilis to yield intermediate forms. We have not noticed this before with a different spoIVBSA378 mutant which was placed at the spoIVB locus (36). We believe that one possible explanation is that even though an active site residue is mutated, the protease can still retain residual proteolytic activity (20). Despite mutation of all three members of the catalytic His-Asp-Ser triad of subtilisin, enzymatic activity is still approximately 10^3 to 10^4 times the noncatalyzed rate (5). Our in vivo work shows that changes at His236, Asp363, and Ser378 do appear to allow limited self-cleavage of SpoIVB to forms of approximately 46, 45, and 44 kDa. We had previously predicted that

![Graphical representation of bacterial proteases with PDZ domains.](image-url)
these might represent active forms but we must now question this model. It is possible, though, that the 44- to 46-kDa species observed in these mutants are actually inactive forms brought about by incorrect cleavage or by secondary proteolysis. Alternatively, residual enzyme activity is sufficient to permit self-cleavage but at levels too low to permit signaling. Our work does show clearly, though, that all nonsignaling mutants show delayed cleavage of the full-length form of SpoIVB, and we wonder whether signaling can occur only during a precise window of time concurrent with release of SpoIVB from the inner forespore membrane by self-cleavage. This latter event would occur following the first cut, and for signaling this is presumably then the most important event, enabling release of SpoIVB. In the nonsignaling mutants, though, we reason that self-processing is substantially reduced, leading to a delayed appearance of intermediate species, at which time the SpoIVFB processing complex is no longer responsive (or competent) to cleave pro-\(\alpha^K\).

The results of mutation at residues Asn290 and His394 are more difficult to interpret. Our work suggests that proteolysis of these mutants was less potent, leading to delayed signaling. Possible explanations include roles for these residues in ground state substrate binding or stabilization of the reaction transition state by forming an oxyanion hole reminiscent of Asn155 in subtilisin. Mutation of this sidechain leads to a 200- to 300-fold reduction in turnover number of this serine peptidase (4). A similar role for Asn290 might explain a delay in signaling.

We are presently attempting to determine the crystal structure of SpoIVB, which will enable us to test our prediction that Aasp363 is suitably placed to comprise the third member of a catalytic triad and to assess the importance of residues Asn290 and His394 in SpoIVB function. We will also explore the role of the PDZ domain in relation to the peptidase activity. A survey of bacterial proteins with PDZ domains suggests that of 187 non-SpoIVB bacterial PDZ-containing proteins, remarkably only 9 are predicted not to contain transit peptides or transmembrane segments. More surprising is that only 28 are not associated with a protease activity, i.e., 85% of all known PDZ-containing bacterial proteins are also proteases (Fig. 5), indicating that they have evolved a specialized role in association with peptidase activity due to their ability to traverse a membrane for (i) localization and (ii) substrate specificity, and thus are ideal for the signaling function of SpoIVB.

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