Protease Deficiency and Its Association with Defects in Spore Coat Structure, Germination and Resistance Properties in a Mutant of *Bacillus subtilis*

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Spores formed by *Bacillus subtilis* carrying the mutation *ger-36* are lysozyme-sensitive and germination-defective. The coats of these spores lack a number of polypeptides normally found in the spore coat, and four additional polypeptides are present that are not normally found in the coats of wild-type spores. The *ger-36* mutation also prevents the synthesis (at about stage V) and the incorporation into the spore outer layers, of an intracellular protease (protease B, approx. mol wt. of monomer 25000). A partial revertant of a strain carrying *ger-36* was isolated and this had an additional mutation in a locus distinct from *gerE*. The suppressing mutation restored the protease activity, but not the germination defect. It partly restored resistance to lysozyme and the spores formed had apparently normal coat protein composition except for the absence of one polypeptide (mol wt. 36000).

**INTRODUCTION**

During the late stages of sporulation (stages V and VI) in *Bacillus subtilis*, the coat proteins are deposited on the forespore (Jenkinson et al., 1981) and the spore germination and resistance properties develop (Dion & Mandelstam, 1980; Jenkinson et al., 1980). The proper assembly of the twelve or so polypeptides in the spore coat requires proteolytic processing of at least some of the components (Jenkinson et al., 1981), and a possible precursor to one of these proteins (the alkali-extractable protein, molecular mass about 12000 Dal) has recently been identified (Goldman & Tipper, 1981).

Evidence suggests that the formation of the coat and the development of the spore resistance and germination properties are interrelated, since spores formed with altered coat structure by sporulation mutants also have altered properties in these respects (Jenkinson, 1981, 1983). A germination mutant carrying the mutation *ger-36*, isolated by Moir et al. (1979), also produces spores which have defective coats (as seen by electron microscopy), and which are highly sensitive to lysozyme (Moir, 1981). These phenotypic properties fit in with the suggestion that the assembly of the coat proteins may determine them.

The results in this paper show that the *ger-36* mutation leads to the formation of spores that lack several of the polypeptides normally found in the coat of wild-type spores. In addition, this mutation prevents the formation of an intracellular protease which is normally associated with stage V of sporulation, and which is usually found in the outer layers of mature spores. This protease appears to play no role in germination, but is probably required for processing of the coat polypeptides and their assembly into the spore coat.

**METHODS**

*Bacteria.* The bacterial strains used are listed in Table 1. Strain CU267 which sporulates normally is referred to as the wild-type strain.

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Sporulation. Cells were induced to sporulate by the resuspension method of Sterlini & Mandelstam (1969). Exponentially growing bacteria at 37 °C in hydrolysed-casein medium were harvested by centrifugation when the cell density was about 0.25 mg dry wt ml⁻¹ and suspended in an equal volume of sporulation salts medium (Sterlini & Mandelstam, 1969) containing glutamate, and amino acid supplements (200 µg ml⁻¹ except for tryptophan, 20 µg ml⁻¹). After incubation with shaking for 8 h at 37 °C, 80 to 90% of the cells contained phase-bright spores, as seen by phase-contrast microscopy. Times after resuspension (h) are denoted t₁, t₂, etc.

Measurement of sporulation marker events. Alkaline phosphatase activity was assayed by the method of Glenn & Mandelstam (1971) with Tris/HCl buffer (1 M, pH 8.0). Glucose dehydrogenase was measured as described by Sadoff (1966), and 2,6-dipicolinic acid by the method of Janssen et al. (1958).

Preparation of spores. Spores were prepared from cultures at t₂₀, by first allowing the cultures to stand for 24 h at 4 °C (this ensured complete lysis of mother cells), and then harvesting the spores by centrifugation (8000 g, 10 min). They were washed and purified by centrifugation through 50% (w/v) Urografin as described by Jenkinson (1981). Spores from cultures at t₁₀ were freed from sporangia by passage through a French pressure cell at 83 MPa, and washed as previously described (Jenkinson et al., 1981).

Extraction of spore coat proteins. Except where otherwise stated all procedures were carried out at 4 °C. Spores (approx. 5 mg dry wt ml⁻¹) were suspended in Tris/HCl buffer (50 mM, pH 7.2) containing 5 mM-EDTA and 2 mM-phenylmethylsulphonyl fluoride (10 ml) and broken with glass beads at 4 °C in a Braun model MSK cell homogenizer (4 min, full speed). The insoluble fraction obtained after centrifugation (12000 g, 20 min) of the broken spore suspension was extensively washed and the coat proteins were extracted with DS buffer [0.3 ml of 20% (w/v) acrylamide slabs containing 0.1% (w/v) SDS as described by Jenkinson et al. (1981)]. Before electrophoresis (see below) the extracts were clarified by centrifugation (5000 g, 5 min) and mixed with 0.05% (w/v) bromophenol blue tracking dye in 66% (v/v) glycerol (0.085 ml extract with 0.015 ml dye/glycerol).

Measurement of spore resistance properties. The numbers of spores surviving treatment with toluene or heat (80 °C, 15 min) were determined as described by Jenkinson et al. (1980). Lysozyme resistance was determined by suspending mature (t₁₅₀) spores in sporulation salts medium (see above) at a density of 5 × 10⁷ spores ml⁻¹, adding lysozyme (EC 3.2.1.17; 13 µg ml⁻¹ final concen) and measuring at various times during incubation at 37 °C (1) the loss of survivors by plating suitable dilutions on nutrient agar (Oxoid) and (2) the loss in absorbance (A₅₅₀) of the suspension.

SDS-PAGE. Proteins were fractionated by electrophoresis in 15% (v/v) acrylamide slab gels containing 0.1% (w/v) SDS as described by Jenkinson et al. (1981) using the system of Laemmli & Favre (1973). Gels were stained with Coomassie blue, destained (Jenkinson et al., 1981), and scanned with a microdensitometer (Joyce, Loeb & Co., Gateshead). Molecular weights of proteins were estimated by reference to the migration distances of six marker proteins (Pharmacia) in the range 94000-14400 Dal.

Extraction of protease activity from sporulating cells or spores. A sample (10 ml) of sporulating culture was centrifuged (5000 g, 5 min, 4 °C), and the cells were washed twice with NaCl (1 M, 4 °C). The pellet was suspended in Tris/HCl buffer (62.5 mM, pH 6.8) containing 0.1% (w/v) SDS and passed through a French pressure cell at 83 MPa. A solution of 20% (w/v) SDS containing 1% (v/v) 2-mercaptoethanol (5 µl) was added to 0.5 ml of the broken cell suspension, and the mixture was incubated at 37 °C for 30 min. Purified t₂₀ spores (about 2 mg dry wt)
were extracted in a similar way, by suspending them in 0.02 ml Tris/HCl buffer (see above), adding a solution of SDS containing 2-mercaptoethanol (2 µl; same composition as above) and incubating the suspension for 30 min at 37 °C, unless otherwise stated. At the end of the incubation the mixtures were centrifuged at room temperature (5000 g, 5 min) and a portion of the supernatant was mixed with a solution of bromphenol blue/glycerol (see above) and subjected to SDS-PAGE on a 15% (w/v) acrylamide slab gel containing fibrinogen (Sigma, 0.2 mg ml⁻¹). After electrophoresis, the gel was soaked at 37 °C for 60 min with shaking in Tris/HCl buffer (0.04 M, pH 7.6) containing MgCl₂ (2 mM) and CaCl₂ (2 mM) (500 ml). Buffer solution was discarded, replaced by fresh buffer (1 litre), and the incubation was continued for a further 16 h at 30 °C. This treatment washes the SDS from the gel and allows proteolytic enzymes to renature and hydrolyse the fibrinogen incorporated into the gel (Lacks & Springhorn, 1980). The gel was then stained with Coomassie blue and destained, and bands of protease activity were seen as unstained regions on a blue (stained) background.

Surface protein iodination of spores. Spores (about 5 mg dry wt) prepared from cultures at tₐ₀ were surface-iodinated with ¹²⁵Iodine (New England Nuclear) in the presence of lactoperoxidase (EC 1.11.1.7; Sigma) (Jenkinson et al., 1981). The spores were broken with glass beads, and the coat proteins were extracted and fractionated by SDS-PAGE. The gel was stained and scanned, and the individual lanes were excised. Slices (1 mm thick) of each lane were counted for radioactivity in an LKB Wallac 1270 Rackgamma counter.

Germination properties. Spores from cultures at tₐ₀ were purified (see above) and suspended at 37 °C to an initial absorbance (A₅₅₀ nm) of 0.35 in Tris/HCl buffer (10 mM, pH 8.0) containing L-alanine (10 mM). Germination was followed by measuring absorbance loss at intervals for up to 3 h.

Transformation. DNA for transformation was prepared by the method of Ward & Zahler (1973). Competent cells were obtained as described by Jenkinson (1983). Transformants were selected on lactate/glutamate minimal agar (Piggot, 1973) with appropriate supplements.

Scoring of recombinants. Transformants were picked on to lactate/glutamate minimal agar, incubated for 24 h at 37 °C, and then picked on to potato-extract/glucose/yeast extract (PGYE) agar and scored for germination defect by the tetrazolium overlay method of Lafferty & Moir (1977).

Mutagenesis. An exponentially growing culture of cells of strain 522 in 20 ml antibiotic medium (no. 3, Difco) containing about 4 × 10⁸ c.f.u. ml⁻¹, was centrifuged (5000 g, 10 min, 20 °C) and the cells were suspended in sporulation salts medium (10 ml) containing glutamate, MgSO₄, CaCl₂, and tryptophan (Sterlini & Mandelstam, 1969), and N-methyl-N'-nitro-N-nitrosoguanidine (250 µg ml⁻¹). After 30 min at 37 °C, the cells were collected by centrifugation (5000 g, 10 min at room temperature), and washed twice with warm (37 °C) Schaeffer's sporulation medium (Schaeffer et al., 1965). The treatment reduced the viable count to about 5% of the initial number of cells. The bacteria were suspended in 50 ml Schaeffer's medium at 37 °C, portions (5 ml) were distributed between 10 tubes, and these were shaken for 16 h at 37 °C to allow sporulation to occur.

To select spores that were lysozyme-resistant, 0.5 ml lysozyme solution (2 mg ml⁻¹) was added to each of the five cultures, and the tubes were incubated at 37 °C for 4 h. A control (unmutagenized) culture of strain 522 was taken through the whole procedure. The suspensions were centrifuged (5000 g, 15 min), and resuspended in 5 ml fresh Schaeffer's medium, and 0.2 ml portions were plated on to nutrient agar, and incubated at 37 °C for 16 h. Between 20 and 30 colonies appeared on each plate from the mutagenized cultures, while only between two and ten colonies appeared on plates of the control (unmutagenized strain 522) cultures. Approximately 400 colonies were picked from the plates on to lactate/glutamate minimal agar containing tryptophan, and these were then scored for defective germination by the tetrazolium overlay (see above). One Ger⁺ clone was subsequently purified. It formed spores which germinated normally, and was designated strain 558.

To select spores that were lysozyme-resistant but still germination-defective, the spores from the remaining five mutagenized cultures were harvested by centrifugation (5000 g, 10 min). Each pellet was suspended in sporulation salts medium (3 ml) containing L-alanine (20 mM) and the spores were allowed to germinate for 2 h at 37 °C. Lysozyme was added (final concn 200 µg ml⁻¹) and the incubation was continued for a further 2 h at 37 °C. The tubes were then heated at 80 °C for 15 min to kill germinated spores, the suspensions were centrifuged (5000 g, 15 min) and each pellet was suspended in 2 ml sporulation salts medium containing L-alanine (10 mM). After a further 2 h at 37 °C, the spores were heated at 80 °C for 15 min, and portions of the suspensions were plated on to lactate/glutamate minimal agar containing tryptophan. After 2 d at 37 °C, each plate contained up to 25 colonies; no colonies appeared on plates on which the mutagenized culture of strain 522 had been taken through the procedure. A number of colonies were picked and inoculated into 5 ml portions of Schaeffer's medium. These were grown for 16 h at 37 °C with shaking to allow sporulation to occur, heated (80 °C, 15 min), and then treated with lysozyme (final concn 200 µg ml⁻¹) at 37 °C for 30 min. Any cultures which did not visibly clear on this treatment were retained. Of 60 tested in this way, one was found to produce spores which more resistant to lysozyme treatment than those of the parent strain 522. The strain was designated number 551, and was found to be negative (Ger⁻) in the tetrazolium reaction (see above).

Chemicals. Acrylamide and SDS were obtained from BDH. Other chemicals were from Sigma unless otherwise stated.
RESULTS

Phenotypic properties and coat protein composition of spores formed by strain 522 (ger-36)

The ger-36 mutation originally described by Moir et al. (1979) is approximately 15% linked by transformation to leuB16 (Jenkinson, 1983). We transferred the ger-36 mutation from strain 4673 (see Table 1) into strain CU267 (leuB16 ilvB2 trpC2) by transformation and selection for Leu+ recombinants. One Ger− colony identified by the tetrazolium overlay method of Lafferty & Moir (1977) was subsequently purified. It was found to be Ilv+ (ilvB2 and leuB16 are approximately 60% linked by transformation (Ward & Zahler, 1973; Jenkinson, 1983) and was designated strain 522.

Cells of this strain sporulated normally at 37 °C, and produced the same amounts of alkaline phosphatase, glucose dehydrogenase and 2,6-dipicolinic acid as the isogenic Spo+ parent strain CU267. The spores formed by strain 522 were resistant to toluene and to heating (80 °C, 15 min); however, they were lysed extremely rapidly when suspended in a solution of lysozyme (see Introduction) and they were slow to germinate in L-alanine.

The polypeptides present in the spore coat of spores formed by strain 522 (ger-36), were identified by SDS-PAGE (see Methods). The coat protein profile obtained was compared with that obtained with the wild-type (Spo+) strain CU267. About 14 polypeptides have been shown to constitute the normal spore coat in B. subtilis (Jenkinson et al., 1981). In Fig. 1 (lane 1) 11 of these polypeptides are identified by their positions in the gel. Comparison of the coat polypeptide components of spores produced by strain 522 (Fig. 1, lane 2) with those from the wild-type strain shows that the patterns are grossly different. A number of polypeptides (36K, 24K, 20K, 19K, 11K, 9K and 8K polypeptides; K denotes kDal) normally found in the spore coat were either missing in the coat of spores from strain 522, or were present in obviously very reduced amounts. In addition, four new protein bands were visible, and these had approximate molecular masses of 22K, 18K, 14K and 13.K (Fig. 1, lane 2).

One possible explanation for the missing polypeptides in the spore coat of the mutant is that they might have been less strongly bound to the structure than in the wild-type and that they had been lost during preparation of the spore coats for extraction. (This in itself would have indicated at least some difference in coat formation.) However, when intact spores of strain 522 were extracted with DS buffer and the proteins separated by SDS-PAGE, the pattern of protein bands was identical (results not shown) to that obtained when the purified insoluble coat material was extracted (Fig. 1, lane 2). Thus the altered coat protein profile in spores of strain 522 was not an artefact of the method of extracting proteins.

Intracellular protease activities during sporulation

A possible explanation for the large difference observed in the coat protein composition of strain 522 spores was that the ger-36 mutation caused some defect in coat protein processing. To investigate this further, we looked at the production of intracellular proteases during sporulation of both the wild-type and strain 522. Samples (10 ml) of a sporulating culture were taken at various times (t0, t2, t4, etc.) during sporulation and the cells were broken by passage through a French pressure cell. The proteins were solubilized (see Methods) and separated by SDS-PAGE, and proteolytic activity within the gel was detected as described in Methods. Two major bands of protease activity were found which reproducibly appeared in sporulating cell extracts at t7. One band (possibly made up of two components) had a molecular mass of approximately 22K, and the other had a molecular mass of about 25K (Fig. 2, lane 1). The former activity began to appear at about t2 (stage II) in sporulating cells of the wild-type strain, while the second band began to appear about t5 (stage V) (results not shown). Three additional bands of protease activity corresponding to molecular masses 34K, 37K and 49K were also occasionally seen, but they were not investigated further because they were not always detected.

The activity with the lower molecular mass (22K; referred to hereafter as protease A) was not found at t7 in sporulating cells of a mutant blocked at stage II in sporulation (Fig. 2, lane 2), but it was formed by three strains tested which were blocked at stages III, IV and V in sporulation
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**Fig. 1.** SDS–PAGE of spore coat proteins (stained with Coomassie blue) extracted from t10 spores of wild-type strain CU267 (lane 1) and strain 522 (ger-36) (lane 2). The coat polypeptides are identified by their approximate molecular masses (K, kDal).

*Fig. 2, lanes 3, 4 and 5.* The second protease activity (protease B) was not produced by any of the sporulation mutants tested, except for a strain (513) carrying a mutation in the spoVIA locus (Jenkinson, 1981) (Fig. 2, lanes 3, 4, 5 and 6). When strain 522 (ger-36) was tested for protease production, no protease B activity was detected (Fig. 2, lane 7) and only a small amount (about 20% of the wild-type activity) of protease A was present (Fig. 2, lane 7).

*Extraction of protease from spores*

Both protease A and protease B activities were detected in extracts obtained by incubating washed mature (t20) wild-type spores in SDS/2-mercaptoethanol (see Methods) at 37 °C (Fig. 3, lane 1). This suggested that the protease activities were located in the spore outer layers. When spores were extracted at 50 °C, only protease A activity was detected (Fig. 3, lane 2), protease B being apparently more heat-sensitive. When mature spores were heated in deionized water at 70 °C for 15 min, and then extracted at 37 °C, neither of the proteolytic activities was found (Fig. 3, lane 3).

As expected from the previous results with extracts of sporulating cells, spores of strain 522 contained no protease B activity and only about 20% of the wild-type protease A activity (Fig. 3, lane 4).
Fig. 2. Intracellular protease activity in sporangia at $t_i$. Sporulating cells were broken by passage through a French pressure cell, the proteins were solubilized in buffer containing SDS, and then the extracts were subjected to SDS-PAGE. Protease activity was detected as described in Methods. Each lane had approximately the same amount (40 μg) of protein applied as determined by the Lowry method. Lane 1, wild-type strain CU267; lane 2, strain 1.3 (spoIHA1); lane 3, strain 2.1 (spoIIH2); lane 4, strain 133.5 (spoIVC133); lane 5, strain 91 (spoVB91); lane 6, strain 513 (spoVIA513); lane 7, strain 522 (gerE36). The bands of protease activity are identified by their approximate molecular masses (K, kDal). Faint bands of apparent activity are not noted as the significance of these is uncertain (see text).

Isolation of strains carrying additional mutations that partially or wholly suppress the phenotypic effects of the ger-36 mutation

Since these results suggested a correlation between protease activity, coat protein assembly and spore resistance and germination properties, we attempted to isolate partial revertants of strain 522 which, while still carrying the original ger-36 mutation, might display some or even all of the normal wild-type spore properties. The procedures used for isolating such strains are described in Methods.

The first such strain isolated (strain 551) formed spores that were more resistant to lysozyme than spores of strain 522 but less resistant than spores of the wild-type strain (Fig. 4). After 10 min incubation with lysozyme the viable count of spores from strain 522 was less than 5% of the initial count; the viable count of spores of strain 551 was, after the same time, 50% of the initial count (results not shown). The spores of strain 551 germinated in L-alanine (10 mM) at the same rate as those of strain 522 (ger-36) (Fig. 5). When the coat proteins of strain 551 were extracted and separated by SDS–PAGE, all the polypeptides normally found in the coat of wild-type spores were present, except for the 36K polypeptide (Fig. 6) the amount of which was much reduced being about 10% of the wild-type amount. Both protease A and protease B were produced by sporulating cells of strain 551 and these were extractable from mature spores with SDS/2-mercaptoethanol.

Strain 558, the second revertant of strain 522 produced spores that were fully resistant to lysozyme (Fig. 4) and which germinated in L-alanine (10 mM) at the same rate as wild-type spores (Fig. 5). The coat protein profile as analysed by SDS–PAGE (Fig. 6) was identical to that
Fig. 3. Protease activities extracted from mature \(t_{20}\) spores. Spores were extracted, the proteins subjected to SDS-PAGE and the proteolytic activities detected as described in Methods. Lane 1, spores from the wild-type strain CU267 extracted at 37 °C; lane 2, wild-type spores extracted at 50 °C; lane 3, wild-type spores heated at 70 °C in distilled water, and then extracted at 37 °C; lane 4, spores from strain 522 \(\text{(ger-36)}\) extracted at 37 °C.

Fig. 4. Effect of lysozyme treatment on the optical density of suspensions of \(t_{20}\) spores of wild-type strain CU267 (○), strain 522 \(\text{(ger-36)}\) (●), strain 551 (□), and strain 558 (■).

Fig. 5. Germination of spores of wild-type strain CU267 (○), strain 522 (●), strain 551 (□) and strain 558 (■) in L-alanine (10 mM). Spores were suspended at 37 °C in Tris/HCl buffer (10 mM, pH 8.0 containing L-alanine, 10 mM) and the progress of germination was followed by measuring absorbance \(A_{650}\) as described in Methods.
Fig. 6. SDS–PAGE patterns of spore coat proteins extracted from $t_{10}$ spores of strain 551 (lane 1) and strain 558 (lane 2).

Fig. 7. Protease activities extracted at 37 °C from mature ($t_{20}$) spores of strain 551 (lane 1) and strain 558 (lane 2). See legend to Fig. 2 for details.

of the wild-type pattern (compare Fig. 6 lane 2 and Fig. 1 lane 1), and both proteases A and B were produced (Fig. 7).

To check whether these strains (551 and 558) still carried the ger-36 mutation, DNA was prepared from each of them and used at a concentration of 1 μg ml$^{-1}$ to transform strain 4823 (leu-2 citF2) to Cit$. The ger-36 mutation in strain 522 itself was found to be about 65% linked to citF2 by transformation. When DNA from strain 551 was used as donor, about 68% (142/208) of the Cit$^+$ transformants were Ger$. Several of these Ger$^+$ transformants were purified, and when spores were prepared from them they were shown to be lysed rapidly by lysozyme, suggesting that they carried the ger-36 mutation. Thus the phenotype is the result of a suppressor mutation in strain 551 in a locus which is unlinked to the ger-36 mutation.

When DNA from the second strain, 558, was used to transform strain 4832, only one out of the 520 Cit$^+$ transformants tested was Ger$^-$ and formed lysozyme-sensitive spores. It thus appeared that the suppressing mutation in this strain was closely linked to the ger-36 mutation.

Surface protein iodination of spores

The arrangement of the coat proteins on mature spores can be determined by surface-labelling with $^{125}$Iodine in the presence of lactoperoxidase. Four of the major coat proteins (molecular
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Fig. 8. Surface iodination of coat proteins of t10 spores from strain CU267 (wild-type) (top) and strain 522 (ger-36) (bottom). Spores were isolated from sporangia, washed and their surface labelled with $^{125}$I as described in Methods. The coat proteins were extracted, separated by SDS-PAGE, and the stained gel was scanned with a densitometer (——). The lanes were then excised, sliced into 1 mm sections, and these were counted for radioactivity in a gamma counter (---).

mass 36K, 12K, 9K and 8K; see Fig. 1) have been shown to be the predominant surface components of wild-type spores (Jenkinson et al., 1981). When spores of strain 522 were iodinated, the 12K polypeptide was highly labelled as it is in the wild-type (Fig. 8). Additionally, three of the abnormal polypeptides (the 18K, 14K and 13.5K bands) were iodinated, and the other proteins in the coat were all partly labelled (Fig. 8).

Iodination of spores produced by strain 551 gave a labelling pattern of coat polypeptides indistinguishable from that previously obtained for mutant spores which lack the 36K polypeptides (Jenkinson, 1981). Thus, those proteins normally covered by the 36K protein – the 24K, 19K and 15K bands – are accessible to the labelling agents and are iodinated (results not shown).

DISCUSSION

The ger-36 mutation behaves as if it were a single mutation, as evidenced by the mapping carried out by Moir (1981) and by the fact that it can be transferred to other backgrounds by transformation (Jenkinson, 1983). The consequences of this mutation are that it affects simultaneously the germination and resistance properties of the spores and, therefore, for reasons outlined in the Introduction, it was expected that the spore coat of mutant spores would be altered either in polypeptide composition or in arrangement of the proteins or both. In fact, seven of the normal polypeptides are missing from the coat of mutant spores, and four abnormal proteins are present (Fig. 1), and the arrangement of the proteins on the spores is unusual in that...
they are all – except for the 26K protein – surface-exposed (Fig. 8). These results would be
difficult to explain on the basis of the presence of a single mutation were it not for the
observation that the mutant fails to synthesize one of two intracellular proteases (Fig. 2). The
isolation of the partial revertant strain 551 which now produces this protease, and in which the
coat polypeptide composition is apparently restored to normal (except for the 36K protein),
strongly implies a role for this enzyme in the processing of coat proteins.

The unusual pattern of coat polypeptides (Fig. 1) extracted from spores of strain 522 (ger-36)
may thus be explained by suggesting that some of the ‘abnormal’ proteins found in the mutant
are precursors of some of the ‘normal’ proteins that are missing. An alternative explanation is
that when a processing enzyme is absent, some of the normal proteins are not deposited because
they have not been processed properly, and their places are taken by other proteins that are not
precursors at all.

The two protease activities identified in this paper have been denoted protease A and
protease B. Srivastava & Aronson (1981) also identified two proteases in sporulating cells of B.
subtilis. Protease A referred to here, like the protease I activity purified by Kerjan et al. (1979)
and by Srivastava & Aronson (1981), is relatively resistant to heating at 50 °C and it appears
during sporation, but the molecular weight (22K) is somewhat lower than that of the
protease (30K) identified by the other authors. Protease B referred to here, like protease II of
Srivastava & Aronson (1981) is heat-sensitive and appears late during sporation. The
molecular weight (25K) of protease B would fit with that (47K) determined by Srivastava &
Aronson (1981) for protease II if the protein normally exists as a dimer, since the molecular
weight determinations by these authors were done using gel filtration under non-denaturing
conditions. Both protease A and protease B are found in the outer layers of mature spores of the
wild-type strain. However, neither activity can be positively correlated with any of the structural
components of the spore coat identified previously (Jenkinson et al., 1981; see Fig. 1, lane 1).

Neither protease A nor protease B appears to play a direct role in the germination process
since heating mature wild-type spores at 70 °C for 15 min (a treatment which normally increases
the rate of germination, Powell & Hunter, 1955) destroys their activities (Fig. 3). It follows that it
is the defective spore coat in strain 522 (ger-36) and not the mere absence of protease B from the
coat that causes the germination defect. When the protease activity is restored (as in the partial-
revertant strain 551), the spores remain germination-defective – presumably because their coats
are still not complete [they lack the 36K polypeptide which has been shown to be required for
normal germination; Jenkinson (1981)].

From the results presented it is not possible to tell whether the gene in which the ger-36
mutation resides is the structural gene for protease B or whether the gerE locus regulates protease
production. The latter possibility seems more likely in view of the fact that the partial revertant
strain 551, whose protease B activity has been restored, still fails to deposit the 36K polypeptide
in normal amounts. It is possible, therefore, that the gerE locus encodes some regulatory
molecule required for expression of late sporulation genes, e.g. those for protease B and the 36K
polypeptide.

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