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Purification and Characterization of Additional Low-Molecular-Weight Basic Proteins Degraded During Germination of *Bacillus megaterium* Spores

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Dormant spores of *Bacillus megaterium* contained a group of low-molecular-weight (5,000 to 11,000) basic (pI > 9.4) proteins (termed D, E, F, and G proteins) which could be extracted from disrupted spores with strong acids. These proteins were distinct from the previously described A, B, and C proteins which are degraded during spore germination. However, the D, E, F, and G proteins were also rapidly degraded during spore germination, accounting for 10 to 15% of the protein degraded. Proteins similar to the D, E, F, and G species were also present in spores of other bacterial species. In *B. megaterium*, the D, E, F, and G proteins were low or absent (<15% of the spore level) in vegetative and young sporulating cells and appeared only late in sporulation. The D, E, F, and G proteins were purified to homogeneity, and all contained a high percentage of hydrophilic amino acids; one protein (G) contained 31% basic amino acids and also contained tryptophan. All four proteins were rapidly degraded in vitro by dormant spore extracts. Two proteins (D and F) were degraded in vitro by the previously described spore protease which initiates degradation of the A, B, and C proteins in vivo; the spore enzyme(s) degrading proteins E and G have not been identified.

Approximately 20% of the protein of dormant spores of *Bacillus* and *Clostridium* species is degraded in the first minutes of spore germination (12, 18). During germination of spores of *Bacillus megaterium*, the degradation products of this proteolysis are free amino acids which are utilized in part for protein synthesis by germinating and outgrowing spores (16). Generation of amino acids by proteolysis is necessary for protein synthesis during this period, since most free amino acids, as well as many amino acid biosynthetic enzymes, are absent from dormant spores (4, 16). Three proteins, termed A, B, and C proteins, account for the majority (~85%) of the protein degraded during spore germination (12). The A, B, and C proteins are found only in dormant and developing spores (12, 19). All three species have been purified, and all are low-molecular-weight (7 to 9,000) basic proteins which are located in the spore core (12, 13). The degradation of these proteins during spore germination is initiated by an endoproteolytic activity found only in dormant spores and developing forespores (5, 15, 19). This activity, which is due to at least two enzymes, is absolutely specific for the A, B, and C proteins (5, 15).

One as yet unexplained facet of this system is the source of the tryptophan needed for protein synthesis during spore germination and outgrowth. Dormant spores contain no free tryptophan and also lack tryptophan synthetase (4, 16); this enzyme is not synthesized until 40 min after the initiation of spore germination (16). Before this time tryptophan for protein synthesis must be generated by proteolysis, yet the A, B, and C proteins lack tryptophan (13, 16). However, it was reported previously that extraction of disrupted dormant spores with strong acid reveals an additional group of spore proteins which are degraded during germination and that at least one of these species contains tryptophan (16). This paper describes the identification, purification, and characterization of these additional proteins degraded during germination of spores of *B. megaterium*.

MATERIALS AND METHODS

Spores, cells, and enzymes. The majority of the work reported in this communication utilized *B. megaterium* QM B1551 (originally obtained from Hillel S. Levinson, U.S. Army Natick Laboratories, Natick, Mass.). Spores of this organism were prepared by growth at 30°C in supplemented nutrient broth, harvested, washed, and stored as previously described (15). Cells of this organism were also grown at 30°C in supplemented nutrient broth, harvested by centrifugation (10 min; 10,000 × *g*), frozen, and lyophilized. Spores of *Bacillus cereus* T (originally obtained from Harlyn O. Halvorson, Brandeis University, Waltham,

Mass.) were also prepared in supplemented nutrient broth as previously described (12). Spores of *B. subtilis* 168 and *Clostridium bifermentans* were the generous gifts of Donald J. Tipper (University of Massachusetts Medical School, Worcester, Mass) and William M. Waites (Agricultural Research Office, Norwich, England).

The proteolytic activity (a mixture of at least two proteases [5]) from spores of *B. megaterium*, which is specific for degradation of the A, B, and C proteins, was purified and assayed as previously described (14). Aminopeptidase from spores of *B. megaterium* was also assayed and partially purified as previously described (14). Crude extracts of dormant or germinated (10 min) spores were prepared by sonification of 3 ml of a spore suspension (~75 mg/ml) in 50 mM tris(hydroxymethyl)aminomethane (Tris)-hydrochloride (pH 7.4) and 5 mM $CaCl_2$ with glass beads as previously described (14). The disrupted spores were centrifuged (30 min; 40,000 × *g*), and the supernatant fluid was dialyzed at 4°C against 10 mM Tris-hydrochloride (pH 7.4) and 5 mM $CaCl_2$ containing 20% glycerol. The final solution was frozen and stored at −20°C.

Extraction of proteins A through G from spores or cells. All dialyses were carried out with Spectrapor tubing (Spectrum Industries, molecular-weight cutoff, 3,500). Extraction of proteins A through G from cells or spores routinely followed prior disruption of the cells or spores. Cells or spores (30 to 100 mg, dry weight) were dry ruptured in a dental amalgamator (Wig-L-Bug) with glass beads (100 to 200 mg) as the abrasive (9, 13). The dry powder was suspended in 4 ml of cold 3% acetic acid; after 30 min at 4°C this mixture was centrifuged (10 min; 10,000 × *g*), and the pellet was reextracted with 3 ml of 3% acetic acid. The supernatant fluids were pooled, lyophilized, and dissolved in water. The pellet was extracted with 3 ml and then with 2 ml of 0.1 N H_2SO_4 for 30 min at 4°C. The two supernatant fluids were pooled, dialyzed exhaustively against 3% acetic acid, lyophilized, and dissolved in water. In later experiments, extraction with 4 ml and then with 3 ml of 2 N HCl for 30 min at 4°C was substituted for both the acetic acid and sulfuric acid extractions. The supernatant fluids from the HCl extractions were dialyzed against 1 liter of water (one change) and lyophilized. The proteins in the HCl extract were the sum of the proteins found in both the acetic and sulfuric acid extracts.

Because of the rather small amounts of proteins D, E, F, and G in spores, we needed to prepare large amounts of spores to obtain sufficient quantities of these protein species in pure form. However, dry rupture of large quantities of spores was an extremely tedious procedure. Consequently, for purification of proteins D, E, F, and G from dormant spores of *B. megaterium*, we resorted to the "acid popping" procedure described by Robinow (7). Spores (100 mg/ml) in cold water were rapidly diluted 1/1 with cold 4 N HCl and stirred for 30 min at 4°C. The suspension was centrifuged (10 min; 12,000 × *g*), and the pellet was reextracted with 2 N HCl (12 ml/g of dry spores). Both supernatant fluids were pooled and dialyzed extensively (five changes of 2 liters each) at 4°C against 10 mM Na acetate (pH 4.6). This extraction procedure removes all small molecules from dormant spores of *B. megaterium* (P. Setlow, unpublished data, 1978) and extracts as much of the A through G proteins from spores as does the extraction of disrupted spores described above (see Results). However, this procedure was rather inefficient with germinated spores (data not shown).

Spore coat protein was removed from dormant spores by extraction at high pH in the presence of urea and dithiothreitol as previously described (extraction procedure 3 [12]).

Purification of proteins D, E, F, and G. All steps in the purification of proteins D, E, F, and G were carried out at 4°C, and all dialyses were carried out in Spectrapor tubing. Individual protein species were identified by disc gel electrophoresis at low pH (6, 12). 5 g of intact dormant spores were extracted directly with 2 N HCl as described above. The supernatant fluid was dialyzed (24 h with four changes of 2 liters each) against 10 mM sodium acetate (pH 4.6) and then applied to a column (1.4 by 15 cm) of phosphocellulose equilibrated in 10 mM sodium acetate (pH 4.6). Protein was eluted with a linear gradient of 0.2 to 1.0 M NaCl in 10 mM sodium acetate (pH 4.6) by using 250 ml of each solution and collecting 6-ml fractions. Fractions from the phosphocellulose column which contained protein B were pooled, dialyzed, (~14 h, three changes) against 5 mM Tris-maleate (pH 6.0) and further purified by chromatography on carboxymethyl-cellulose (CM-cellulose) as previously described (13). Fractions containing protein G were pooled, dialyzed against 5 mM Tris-maleate (pH 6.0), and applied to a column (1.2 by 10 cm) of CM-cellulose in 5 mM Tris-maleate (pH 6.0). Protein G was eluted with a linear gradient of 0.05 to 0.35 M NaCl in 5 mM Tris-maleate (pH 6.0) by using 125 ml of each solution and collecting 5-ml fractions. Fractions containing homogeneous protein G were pooled, dialyzed briefly against water to remove salts, lyophilized, dissolved in a small volume of 88% formic acid, and stored frozen.

Fractions containing proteins E and F, which were poorly separated by the phosphocellulose column, were pooled together and dialyzed against 5 mM Tris-maleate (pH 6.0); and separated by chromatography on CM-cellulose as described above for protein G, but with a gradient of 0 to 0.30 M NaCl. Fractions which contained homogeneous protein E or F as determined by analyses on gels were pooled and treated as described above for protein G.

Proteins A, C, and D were not eluted from the phosphocellulose column by the salt gradient, but were eluted together by washing the column with ~100 ml of 4 M urea in 1 M NaCl, 10 mM 2-mercaptoethanol (to destroy cyanate in the urea), and 10 mM sodium acetate (pH 4.6). This elute was dialyzed, and D protein was purified by CM-cellulose chromatography as described above for proteins E and F.

Spore germination. Dormant spores of *B. megaterium* (20 mg/ml) in water were heated for 15 min at 60°C and then cooled. Spores were germinated at 2.5 mg/ml and at 30°C in 50 mM KPO_4 (pH 7.4) and 100 mM glucose. Greater than 95% of the spores had initiated germination after 15 min, as judged by observation in the phase-contrast microscope. Germination of spores of *B. cereus* was carried out as previously

described (17).

Analytical procedures. Proteins A through G were quantitated after their electrophoretic separation on acrylamide gels at low pH as previously described (12). In all experiments two different concentrations of extract were run on gels to ensure that the assay response was roughly linear with protein content. Assays for degradation of proteins A through G in vitro followed the disappearance of the protein by acrylamide gel electrophoresis as described above (10), and/or the conversion of the protein to ninhydrin-positive material in the presence of the spore aminopeptidase as described previously (14).

Protein molecular weights were determined by electrophoresis on sodium dodecyl sulfate-acrylamide (12.5%) gels with markers of ovalbumin, chymotrypsinogen, myoglobin, cytochrome C, and the B-chain of insulin (20). Protein pI's were estimated from the direction of migration of a protein during electrophoresis on cellulose-acetate strips in 25 mM arginine (pH 9.4). Amino acid analyses were carried out on acid hydrolyzed samples as previously described (13). Cysteine and cystine were determined similarly after performic acid oxidation (2, 13). The ratio of tyrosine to tryptophan in a protein was determined by the method of Beaven and Holliday (1), and these results were confirmed by fluorescence spectra recorded on a Perkin Elmer spectrofluorometer. Protein was determined by the procedure of Lowry et al. (3), and dipicolinic acid (DPA) was determined by the method of Rotman and Fields (8).

RESULTS

Identification of proteins D, E, F, and G. Disc gel electrophoretic analysis of acetic acid extracts of disrupted dormant spores of *B. megaterium* gave two major protein bands as found previously (Fig. 1a) (12). These two bands are due to proteins A and C, which migrate concurrently, and protein B (12). In addition to these two major bands, faint bands which were farther towards the cathode were sometimes observed on these gels (Fig. 1a). However, extraction of acetic acid treated disrupted spores with dilute sulfuric acid produced much larger amounts of

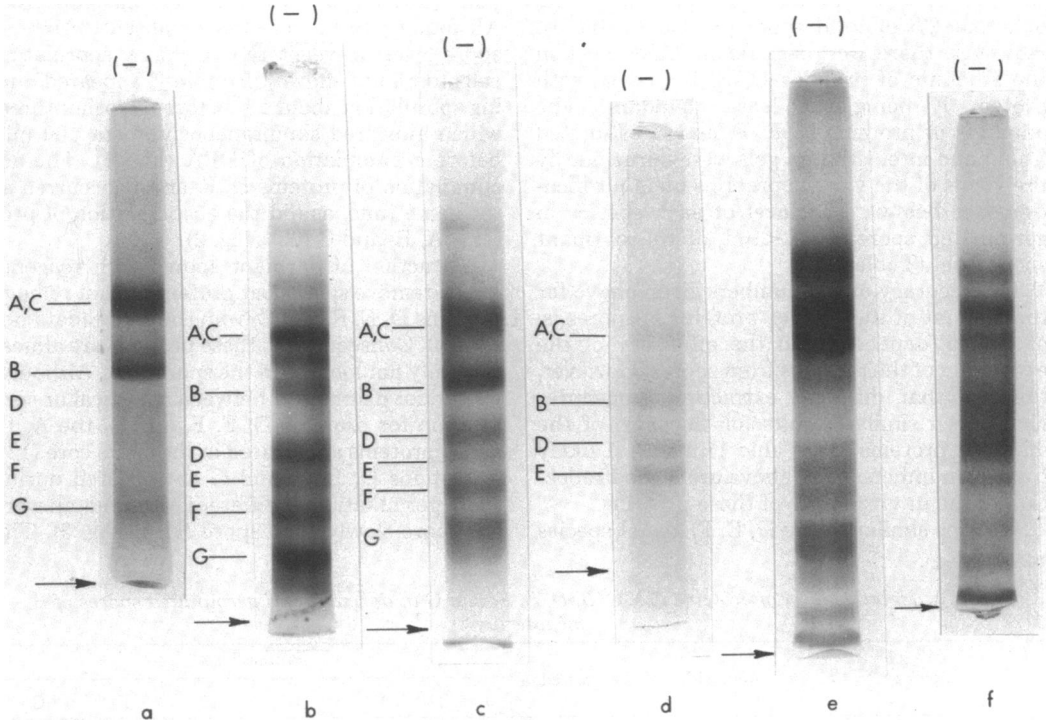

FIG. 1. *Disc gels of various spore extracts. Extracts were prepared and acrylamide gels were run at low pH as described in the text. The arrows at the bottom of the gels give the position of the dye front. (a) Acetic acid extract from 0.5 mg of dry-ruptured dormant spores of B. megaterium; (b) sulfuric acid extract from 1.25 mg of dry-ruptured, acetic acid-extracted dormant spores of B. megaterium; (c) 2 N HCl extract from 0.35 mg of dry-ruptured spores of B. megaterium; (d) acetic acid extract from 5.0 mg of dry-ruptured germinated (30 min) spores of B. megaterium combined with the sulfuric acid extract from 5.0 mg of dry-ruptured acetic acid-extracted germinated (30 min) spores of B. megaterium; (e) acetic acid extract from 0.8 mg of dry-ruptured dormant spores of B. cereus; (f) sulfuric acid extract from 1.9 mg of dry-ruptured, acetic acid-extracted spores of B. cereus.*

these additional proteins which migrated faster than the A, B, and C proteins, as well as some of the A, B, and C proteins themselves (Fig. 1b). These additional species, termed proteins D, E, F, and G, could also be extracted from disrupted spores with 2 N HCl, but in this case almost all of the A, B, and C proteins were extracted as well (Fig. 1c). 2 N HCl also extracted all seven proteins from intact dormant spores (Table 1; see below).

Proteins D, E, F, and G were not found at significant levels in acetic acid-sulfuric acid extracts (Table 1, Fig. 1d) or 2 N HCl extracts (data not shown) of germinated spores. The proteins were not excreted during germination (see below). Presumably these proteins were degraded during spore germination, as is known to be the fate of the A, B, and C proteins (12).

Level of proteins, D, E, F, and G in spores of *B. megaterium* and other species. The combined amount of proteins D, E, F, and G in dormant spores of *B. megaterium* was ~17% of the level of the A, B, and C proteins combined, or about 2% of total spore protein (Table 1). However, there were significant differences in the amounts of proteins D, E, F, and G, with protein E being the least abundant. The amounts of proteins D, E, F, and G estimated from band intensities on gels were borne out by the yields of the various proteins upon purification (see below). The level of each species in germinated spores was <16% of the dormant spore level (Table 1).

The accuracy of the numbers given above for the amount of the various proteins in spores is, of course, dependent on the efficiency of the extraction of the proteins from spores. However, the fact that different extraction procedures have given similar results for the level of the different proteins (12; Table 1) makes it likely that the numbers given above are a good reflection of the in vivo levels of these proteins.

Proteins similar to the D, E, F, and G species were also identified in spores of *B. cereus* (Fig. 1e and f), although in this organism most of these faster migrating minor species were extractable with acetic acid. The three major bands in acetic acid extracts of *B. cereus* spores correspond to the A, B, and C proteins of *B. megaterium* spores (Setlow, unpublished data, 1978). In *B. cereus* spores the minor species totaled ~15% of the level of the three major proteins, and all disappeared upon spore germination (data not shown). Proteins similar to the D, E, F, and G species were also identified in dormant spores of *B. subtilis* and *C. bifermentans*, where they represented 11 and 7% of the major protein species, respectively (data not shown).

Appearance of proteins D, E, F, and G during sporulation, their loss in germination, and their absence from spore coats. The presence of significant levels of proteins D, E, F, and G in dormant spores of *B. megaterium* made it of obvious interest to determine at what point during sporulation the proteins appeared. All four proteins were low or absent (<15% of spore level) in vegetative or young sporulating cells (data not shown). Protein E appeared during sporulation about 2 h before the other three, which appeared simultaneously about 100 min before accumulation of DPA (Fig. 2). The accumulation of proteins D, F, and G occurred at the same time, as did the accumulation of proteins A, B, and C (12) (Fig. 2).

Extraction of dormant spores with reagents which remove spore coat protein did not remove proteins D, E, F, and G from the spore (data not shown). Consequently, these proteins are almost certainly not located in the spore coat. Although we cannot distinguish between a cortical or core location for proteins D, E, F, and G, the A, B, and C proteins are located in the spore core (12).

Proteins D, E, F, and G disappeared during spore germination at different rates, but all were lost more slowly than spore DPA (Fig. 3). The

TABLE 1. *Relative level of proteins A + C, B, D, E, F, and G in dormant and germinated spores of B. megaterium*[a]

Spores	Acid with which extracted	Protein level					
		A + C	B	D	E	F	G
Disrupted dormant	Acetic	100	46	<0.5	<0.5	<0.5	1
Disrupted dormant	Sulfuric after acetic	10	3	6	3	10	8
Disrupted dormant	Hydrochloric	125	51	7	3	11	8
Intact dormant	Hydrochloric	92	39	5	3	8	6
Disrupted germinated	Acetic	3	<0.5	<0.5	<0.5	<0.5	<0.5
Disrupted germinated	Sulfuric after acetic	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5

[a] Samples were prepared and analyzed for various proteins as described in the text. All values are averages of two determinations on two separate extracts, and all have been normalized by setting A + C in acetic acid extracts of disrupted dormant spores as 100. This latter value represents ~22 mg of protein/g of dry spores.

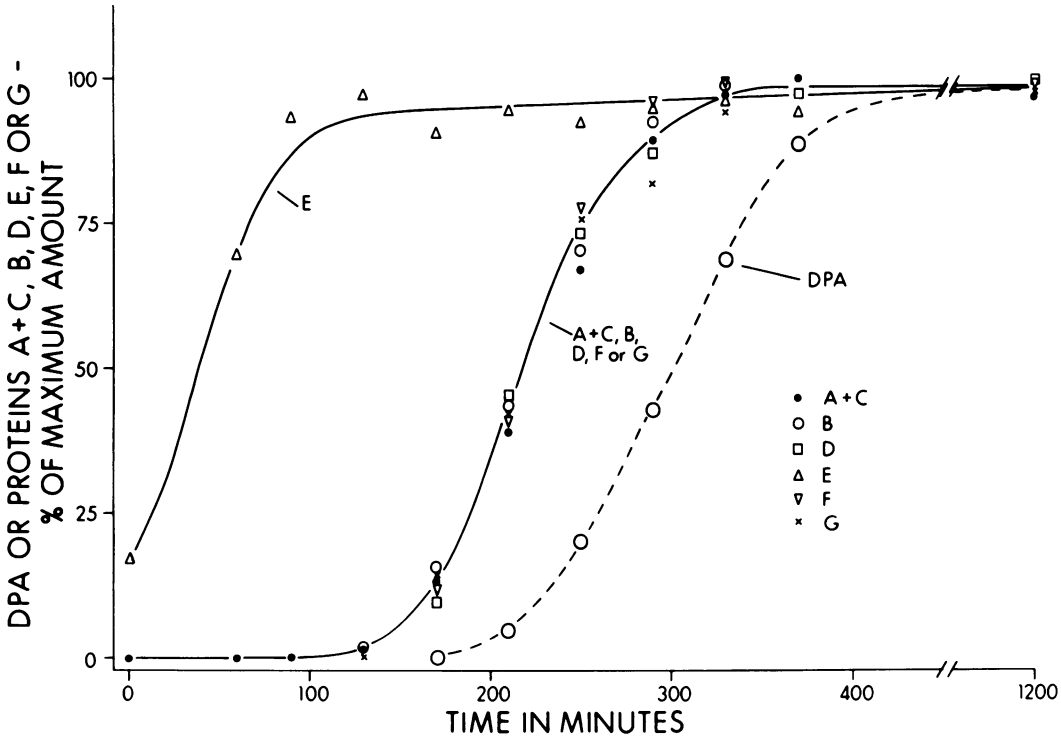

FIG. 2. *Appearance of DPA and proteins A through G during sporulation. Cells were grown at 30°C in supplemented nutrient broth, and at various times two samples (5 and 20 ml) were centrifuged (10 min; 10,000 × g) and frozen. The 5-ml sample was extracted with 2 ml of water for 15 min at 100°C and centrifuged (10 min; 10,000 × g), and the supernatant fluid was analyzed for DPA. The 20-ml sample was lyophilized, dry ruptured, extracted with 2 N HCl, dialyzed, lyophilized, and dissolved in 0.5-ml of water, and portions were analyzed for proteins A through G as described in the text.*

loss in protein B was faster than proteins A plus C, as previously noted (14). The loss of proteins D, E, F, and G during germination was not affected by blockage of all ATP production, as was previously found for loss of the A, B, and C proteins (11; data not shown). None of the proteins was excreted into the medium during germination, and none was extracted from intact spores by acetic acid (data not shown).

Purification of proteins D, E, F, and G. With the identification of additional protein species which appeared to be degraded during germination of *B. megaterium* spores, it was of obvious interest to purify these proteins and compare them with the previously characterized A, B, and C proteins. A key observation in facilitating purification of proteins D, E, F, and G was that they, as well as proteins A, B, and C, could be readily extracted from intact spores with HCl (7). Purification of proteins D, E, F, and G (as well as proteins A, B, and C) from this extract was then relatively straightforward.

Proteins were initially fractionated by phosphocellulose chromatography using a steep gradient, followed by elution of proteins A, C, and D from the column with urea plus high salt (Fig. 4). Purification of proteins D, E, F, and G to homogeneity was then accomplished by chromatography on CM-cellulose of appropriate fractions (Fig. 5a to d). Proteins B and A + C were also obtained from this procedure, and the yields of these latter proteins were similar to those reported previously (13) (Table 2). The overall procedure gave relative yields of each of the proteins as predicted from the amount of each species estimated from band intensities on acrylamide gels (Tables 1 and 2). Proteins D, E, F, and G obtained from this procedure were all homogeneous (>90%) on disc gel electrophoresis at low pH, on sodium dodecyl sulfate-gel electrophoresis and on electrophoresis on cellulose-acetate strips at pH 9.4 (data not shown).

Throughout the purification procedure, analysis of column fractions by gel electrophoresis revealed that the D, E, F, and G species which were purified were the major (>75%) species

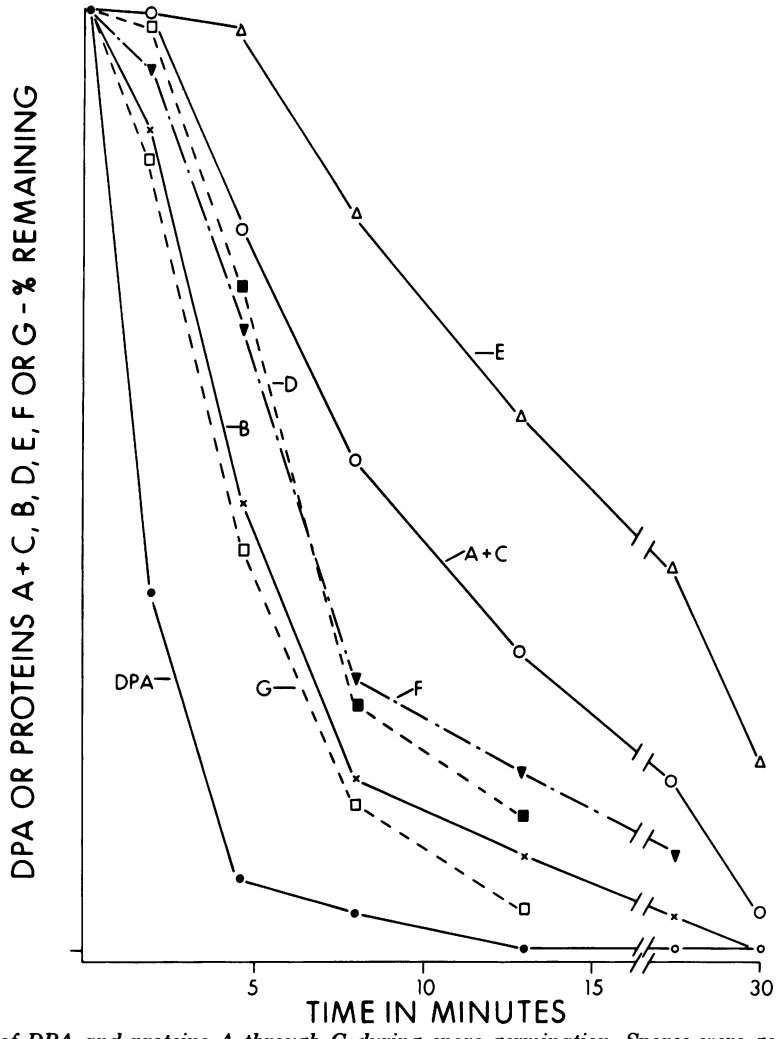

FIG. 3. *Loss of DPA and proteins A through G during spore germination. Spores were germinated as described in the text, and at various times samples (20 ml) were made 3% in acetic acid to halt further germination. This mixture was centrifuged (10 min; 10,000 × g), and the supernatant fluid was saved for analysis of DPA. The pellet fraction was lyophilized and dry ruptured, and proteins A through G were extracted with 2 N HCl and analyzed as described in the text.*

which migrated in the position of bands D, E, F, or G. However, several minor species were noted which purified differently than the major species but which migrated concurrently with proteins D, F, or G. However, the total of these minor proteins was less than 25% of the major protein with which they concurrently migrated (data not shown).

Physical and chemical properties of proteins D, E, F, and G. Like proteins A, B, and C, proteins D, E, F, and G also had relatively low-molecular weights and high pI's (Table 3). Proteins D, E, F, and G also contained a high percentage of hydrophilic amino acids and a high percentage of basic amino acids, with the latter values ranging as high as 31% in protein G (Table 4). All four proteins lacked cysteine and cystine, and one protein (G) contained tryptophan. There were significant differences among proteins D, E, F, and G in amino acid composition, and these proteins also differed significantly from proteins A, B, and C, particularly in their basic amino acid content (Table 4).

Degradation of proteins D through G in vitro. One characteristic feature of proteins A, B, and C is that all three are rapidly degraded by extracts of dormant and germinated spores (10, 13) (Table 5). Proteins D, E, F, and G were

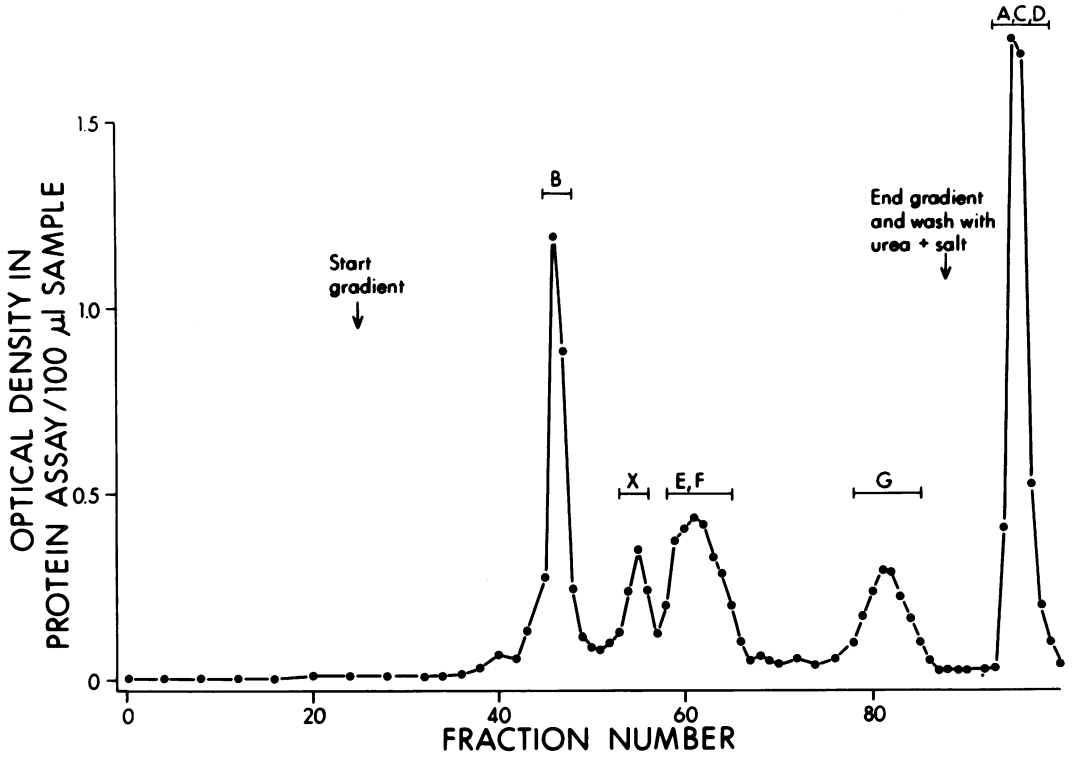

FIG. 4. *Phosphocellulose chromatography of crude HCl extract of dormant spores. Five grams of intact dormant spores were extracted with HCl, dialyzed, and run on phosphocellulose chromatography as described in the text. The letters over the peaks denote the proteins present. The peak designated X had no protein which could be visualized on gel electrophoresis and was not studied further.*

also degraded by spore extracts at a rate fast enough to account for their degradation in vivo, as previously shown for the A, B, and C proteins (10) (Table 5). In addition, two of the proteins were degraded in vitro by the purified spore protease shown to initiate degradation of the A, B, and C proteins in vivo and in vitro (5, 14) (Table 5).

DISCUSSION

The combined level of proteins D, E, F, and G in spores was about 15% that of proteins A, B, and C, as indicated both by analysis of band intensities on disc gels of crude extracts and the yields of these proteins after purification. Coupled with earlier data indicating that proteins A, B, and C comprise ~15% of the protein of spores of *B. megaterium* (12, 13), this indicates that proteins D, E, F, and G comprise ~2 to 2.5% of total spore protein. Since proteins A, B, and C are known to comprise ~85% of the protein degraded to free amino acids during spore germination, proteins D, E, F, and G together comprise most of the remaining protein degraded. That the D, E, F, and G species are indeed degraded during spore germination is indicated by (i) their absence from germinated spores even when extraction is carried out under several conditions; (ii) their degradation by spore extracts in vitro at a rate fast enough to account for their degradation in vivo; and (iii) the fact that two of these proteins are degraded in vitro by a spore protease shown to be involved in initiating degradation of the A, B, and C proteins during germination (5, 14). This spore protease has been previously found to act in vitro only on the A, B, and C proteins (14).

An obvious question to ask concerning proteins D, E, F, and G is: are these proteins derived in some fashion in vitro from the more abundant A, B, and C proteins? This possibility seems unlikely for the following reasons. (i) The spore protease which acts on the A, B, and C proteins acts only at a specific primary sequence in these species (Setlow, unpublished data, 1978); presumably, this sequence is absent in proteins E and G. (ii) All of these proteins lack cysteine and

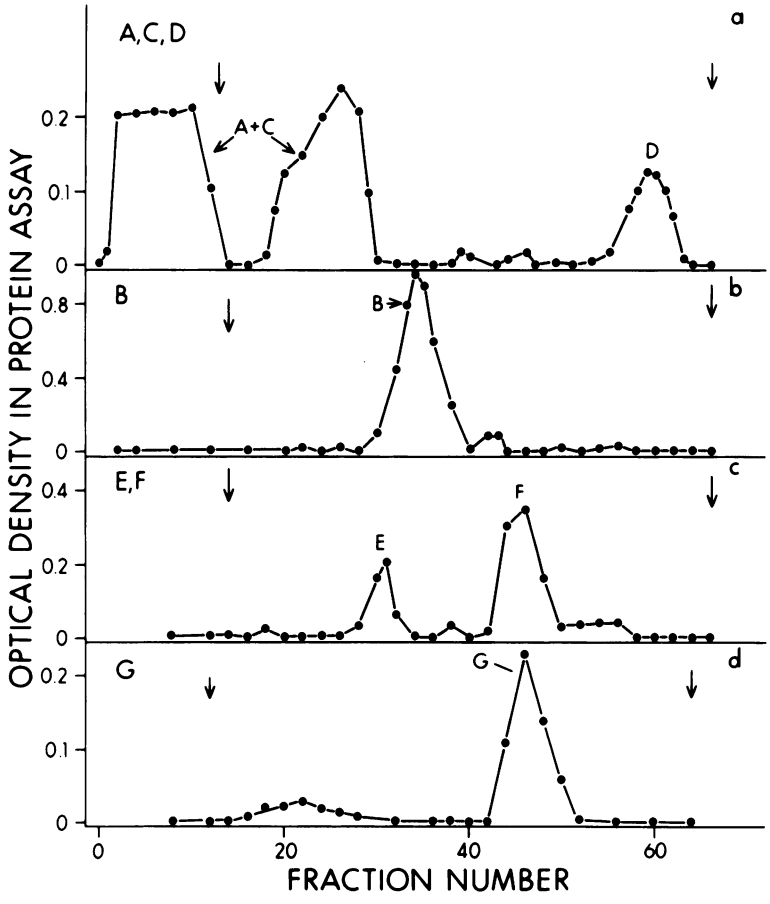

FIG. 5. *(a to d) CM-cellulose chromatography of proteins A to G. Peaks from the phosphocellulose column were run on CM-cellulose as described in the text. Letters denote individual protein species. In (a), the first 16 fractions were 15 ml.*

TABLE 2. *Purification of proteins A + C, B, D, E, F, and G from dormant spores of B. megaterium*[a]

Step	Proteins present	Protein (mg)[b]
HCl extract after dialysis	A → G	49
Phosphocellulose chromatography[c]	B	9.3
	E, F	2.2
	G	1.3
	A, C, D	18
CM-cellulose chromatography[d]	A + C[e]	11
	B	6
	D	0.5
	E	0.2
	F	0.8
	G	0.6

[a] Protein was purified from 5 g of intact dormant *B. megaterium* spores as described in the text.
[b] Values are for 1 g of spores as starting material.
[c] Peaks are those in Fig. 4.
[d] See Fig. 5a to d.
[e] Fractions containing A + C proteins were pooled.

cystine, whose presence could lead to artifactual multiple forms of proteins. (iii) The same additional proteins are seen when spores are extracted with H_2SO_4 or HCl with or without prior spore disruption. (iv) There are significant differences in amino acid content between these proteins. Protein G contains tryptophan, a residue not found in proteins A through F. Furthermore, 31% of the amino acids in protein G are basic residues, a percentage much higher than in proteins A through F (13). This high percentage of basic residues is of course reminiscent of eucaryotic histones. (v) Proteins A, B, and C, which are extracted from spores by the same procedures as are proteins D, E, F, and G, are distinct species, as shown by determination of their primary sequence (P. Setlow, C. Gerard, and J. Ozols, unpublished data, 1978). We cannot, of course, rule out the possibility that two or more of proteins A through G are derived in

vivo from a common precursor by some type of proteolytic processing.

The presence of tryptophan in protein G clarifies one question concerning proteolysis during spore germination—that of the source of tryptophan. Since spores lack tryptophan synthetase, as well as free tryptophan, this amino acid must be supplied by protein degradation in order for outgrowing spores to carry out protein synthesis. The absence of tryptophan from proteins A, B, and C, the major species degraded during germination, made the source of the tryptophan for protein synthesis during germination and outgrowth unclear. However, with the identification of protein G as a species degraded during germination, one source of tryptophan for protein synthesis early in spore outgrowth is now identified. Using data in Tables 1, 3, and 4, one can calculate that degradation of protein G will provide ~650 nmol of tryptophan per g of dry spores.

TABLE 3. *Molecular weights and pI's of proteins A through G*[a]

Protein	Mol wt	pI
A[b]	7,100	>9.8
B[b]	8.450	>9.8
C[b]	9,650	>9.8
D	10,800	>9.4
E	9,000	>9.4
F	10,300	>9.4
G	5,500	>9.4

[a] Molecular weights and pI's were determined as described in the text.

[b] Data taken from reference 13.

Certainly a major issue concerning proteins D, E, F, and G, as well as A, B, and C, is their function in the dormant and germinated spore. Previous work has indicated that one function of the A, B, and C proteins is to be degraded during spore germination in order to generate amino acids needed for protein synthesis. It

TABLE 5. *Degradation of proteins A through G by dormant spore extracts or the spore protease*

Protein	Dormant spore extract[a]	Spore protease[b]
A + C	41.5	100[c]
B	16	32
D	12	50
E	6	<3
F	34	80
G	19	<2

[a] Values expressed as micrograms of substrate degraded per hour per milligram of protein in extract. Proteolysis was carried out at 37°C in 0.4 ml of 50 mM Tris-hydrochloride (pH 7.4) and 5 mM $CaCl_2$ containing 100 μg of the appropriate protein. Proteins D, E, F, and G were first dried to remove formic acid and then dissolved in a small volume (~100 μl) of 5 mM NaOH before addition of buffer, water, and extract. The reaction was started by addition of dormant spore extract (~1 mg of protein), and portions (50 μl) of the mixture were taken at various times, made 3% in acetic acid, and lyophilized. Protein degradation was measured by following loss of the proteins by acrylamide gel electrophoresis.

[b] Proteolysis was carried out as described in footnote *a*, but with 0.1 to 0.5 μg of spore protease. All values for rates of degradation have been normalized to the value for proteins A + C.

[c] Value set at 100.

TABLE 4. *Amino acid composition of proteins A, B, C, D, E, F, and G*[a]

Residue	A[b]	B[b]	C[b]	D	E	F	G
Lysine	5.7	10.3	6.6	13.8	8.9	14.2	23.0
Histidine	<0.3	1.3	1.1	2.3	4.4	2.6	5.3
Arginine	2.9	1.3	3.3	3.1	9.4	4.6	3.0
Aspartic acid	8.6	14.1	12.1	15.2	10.4	13.4	9.0
Threonine	5.7	9.0	6.6	2.5	2.6	4.7	2.7
Serine	4.3	7.7	4.4	7.2	8.6	9.0	15.3
Glutamic acid	15.7	25.6	16.5	17.4	22.2	17.0	15.2
Proline	2.9	<0.25	2.2	1.8	4.5	3.3	<0.5
Glycine	12.9	6.4	11.0	6.3	7.9	8.3	7.7
Alanine	17.1	15.4	14.3	11.5	6.3	9.0	4.0
Valine	7.1	3.8	5.5	3.8	3.3	3.5	3.4
Methionine	2.9	<0.15	2.2	2.0	<0.3	1.2	<1
Isoleucine	4.3	<0.15	3.3	<0.4	<0.3	1.8	2.7
Leucine	7.1	<0.15	6.6	1.5	8.5	3.1	2.4
Tyrosine	1.4	1.3	2.2	3.0	1.6	1.6	4.2
Phenylalanine	1.4	3.8	2.2	2.9	<0.2	1.8	<0.5
Cysteine/cystine	<0.15	<0.15	<0.1	<0.3	<0.3	<0.2	<0.5
Tryptophan	<0.15	<0.15	<0.1	<0.3	<0.3	<0.2	2.1

[a] Amino acid compositions were determined as described in the text. Values are expressed as percentages of total residues.

[b] Calculated from data in reference 13.

seems likely that this is one function of proteins D, E, F, and G as well, almost certainly so for protein G as noted above. However, it seems unlikely that if this were the sole function of these proteins that there would be so many of them. Consequently, it is tempting to speculate that these multiple protein species serve some key function or functions in dormant spores, functions which can be done away with upon spore germination (13). The high concentration of these species in the spore core (~30 mg/ml total [13]) certainly lends support to such speculation. However, at present, the identity of such other functions for proteins A through G (if indeed such functions exist) is unknown.

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