JOURNAL OF BACTERIOLOGY, May 1975, p. 642-649

Vol. 122, No. 2
Printed in U.S.A.

Protease and Peptidase Activities in Growing and Sporulating Cells and Dormant Spores of *Bacillus megaterium*

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Received for publication 29 January 1975

Peptidase and protease activities on many different substrates have been determined in several stages of growth of *Bacillus megaterium*. Extracts of log-phase cells, sporulating cells, and dormant spores of *B. megaterium* each hydrolyzed 16 different di- and tripeptides. The specific peptidase activity was highest in dormant spores, and the activity in sporulating cells and log-phase cells was about 1.2-fold and 2- to 3-fold lower, respectively. This peptidase activity was wholly intracellular since extracellular peptidase activity was not detected throughout growth and sporulation. In contrast, intracellular protease activity on a variety of common protein substrates was highest in sporulating cells, and much extracellular activity was also present at this time. The specific activity of intracellular protease in sporulating cells was about 50- and 30-fold higher than that in log-phase cells and dormant spores, respectively. However, the two unique dormant spore proteins known to be the major species degraded during spore germination were degraded most rapidly by extracts of dormant spores, and slightly slower by extracts from log-phase or sporulating cells. The specific activities for degradation of peptides and proteins are compared to values for intracellular protein turnover during various stages of growth.

The rate of protein degradation during log-phase growth of cells of several *Bacillus* species is rather low (~3%/h) (14b, 17), but degradation is much more rapid during sporulation and spore germination. During sporulation in *B. subtilis*, about 18% of the total cellular protein is degraded per hour (17), and the proteins degraded include a large number of enzymes (5). Similarly, during the first 20 min of germination of *B. megaterium* spores about 20% of the protein originally present in the dormant spore is degraded (14b). However, unlike the proteolysis occurring during sporulation, dormant spore enzymes are not degraded during spore germination. Rather, the major substrates are two low-molecular-weight basic proteins, which may be unique to the dormant spore (14a, 14b). These two proteins have previously been termed proteins A and B, and will be referred to by these names in this communication (14a).

As might be expected, the early stages of sporulation in *Bacillus* species are characterized not only by an increased rate of protein turnover, but also by a large increase in intracellular protease activity (9, 10). Indeed, synthesis of a protease is thought to be one of the early steps essential for sporulation (9). Dormant spores have also been reported to have protease activity (3, 7), but the activities reported have been surprisingly low considering the rapid proteolysis which occurs during germination. Furthermore, values for protease activity in the dormant spore have not always been directly compared with values for protease activity in other stages of growth. There is also little information on the levels of peptidase activity throughout the growth cycle of *Bacillus* species. Since protein degradation in log-phase cells, sporulating cells, and germinating spores proceeds to free amino acids (14b, 17), peptidase activity is clearly involved in protein turnover, although it is probably not involved in the rate-limiting step (14b). This report describes measurements of the levels of protease and peptidase activity on a variety of substrates in log-phase cells, sporulating cells, and dormant spores of *B. megaterium*, and compares these values to the rate of intracellular protein turnover during log-phase growth, sporulation, and spore germination.

MATERIALS AND METHODS

Chemicals. Di- and tripeptides were purchased from Sigma Chemical Co., and all were found to be >90% pure on thin-layer chromatography on plastic-backed cellulose sheets with 2-methylpropanol-2-butanone-propanone-methanol-water-ammonia

(40:20:20:1:14:5) as the solvent (6). Potassium [^{14}C]cyanate (11 mCi/mmol) was obtained from New England Nuclear; azocasein, hemoglobin, and azocoll were obtained from Calbiochem; and albumin, azoalbumin, casein, leucine-*p*-nitroanilide, and ninhydrin were purchased from Sigma Chemical Co.

Hemoglobin and albumin were carbamylated with [^{14}C]cyanate by the procedure of Stark (18), and unreacted cyanate was removed by prolonged dialysis. The proteins' amino groups (50 to 75%) were modified by this treatment, giving specific activities for both proteins of 2×10^4 to 6×10^4 counts/min per mg with >98% of the counts precipitated by 5% trichloroacetic acid.

Growth and isolation of spores and cells. All work described in this communication was carried out with *B. megaterium* QM B1551 originally obtained from Hillel Levinson (U.S. Army Natick Laboratories, Natick, Mass.). Dormant spores were prepared at 30 C in supplemented nutrient broth, and the procedures for harvesting, washing, and storing the dormant spores have been previously described (15). All spore preparations used in this study were free of vegetative cells and cell debris and were >95% refractile as observed in the phase-contrast microscope. Log-phase cells and sporulating cells were also grown at 30 C in supplemented nutrient broth, and 80-ml aliquots were harvested by centrifugation (10 min, 15,000 × *g*). The supernatant fluid was saved and the cell pellets were washed once with 20 volumes of cold 0.15 M NaCl and then frozen in dry ice-ethanol. Additional washing of cells before freezing removed <10% of the total protease activity of the cell pellets as determined by assays of sonically treated preparations (Setlow, unpublished data). This indicates that >90% of the protease activity in the final cell pellets was due to intracellular enzymes.

Spore germination was carried out at 30 C at a spore concentration of 1 mg/ml in the minimal medium of Spizizen (16) supplemented with 0.1% Casamino Acids. Spores (20 mg [dry weight] per ml in water) were heat shocked for 10 min at 60 C, cooled, and then germinated. Germination was >95% complete in 15 min as observed in the phase-contrast microscope.

Preparation of cell and spore extracts. Cells (~700 mg [wet weight]) or spores (300 mg [dry weight]) were suspended in 2.5 ml of 50 mM tris(hydroxymethyl)aminomethane (Tris)-hydrochloride (pH 8.0) and 5 mM $CaCl_2$. Glass beads (1.5 g; 120 μm) were then added, and the cells or spores were disrupted by sonic treatment. The tube containing the cells or spores was immersed in ice throughout the period of sonic treatment which was carried out for only 30-s periods with 1- to 2-min pauses. Sonic treatment was continued until ~90% of the cells or spores were broken as observed in the microscope; this required 2 to 3 min for log-phase cells, 4 to 6 min for stationary-phase cells, 5 to 8 min for germinated spores, and 10 to 12 min for dormant spores. Unless otherwise noted, the sonic extracts were then centrifuged (15 min, 15,000 × *g*) and either assayed immediately or frozen in dry ice-ethanol and stored. In a few cases, the supernatant fluid was passed through a small column of Sephadex G-25 equilibrated at 4 C in 0.1 M Tris-hydrochloride and 5 mM $CaCl_2$. Protein was determined in extracts by the Lowry procedure (8).

Peptidase and protease assays. All peptidase assays were carried out in 0.1 ml which was 50 mM in Tris-hydrochloride (pH 8.0) and 1 mM in $MnCl_2$ since these conditions were determined to be optimal for hydrolysis of alanylvaline, glutamylalanine, and leucylglycylglycine. The concentration of peptide in the assays was 5 mM and reaction was for 30 min at 37 C. Samples (10 to 25 μl) of the reaction mix were then incubated at 37 C for 30 min in 1 ml of a 1% ninhydrin solution containing in 100 ml: 100 mg of cadmium acetate, 85 ml of ethanol, and 15 ml of acetic acid. The optical density was then read at 505 nm and corrected for the presence of ninhydrin-positive material in buffers, substrate, or extracts, and the amount of hydrolysis was determined by reference to a calibration curve constructed using the constituent amino acids of the peptide substrate. In all cases several different protein concentrations were tested, and where tested the reactions were linear with both time and protein concentration up to 75% hydrolysis of the substrate. Hydrolysis of leucine-*p*-nitroanilide was measured as described by Roncari and Zuber (13).

Protease assays were carried out in a volume of 0.9 ml at 37 C in 50 mM Tris-hydrochloride (pH 8.0) and 5 mM $CaCl_2$. These conditions of pH and divalent metal ion gave maximal activity in both cell and spore extracts. Azoalbumin or azocasein were present at 2.5 mg/ml and ^{14}C-labeled carbamylated hemoglobin or ^{14}C-labeled carbamylated albumin were present at 0.4 mg/ml. After reaction for 30 min, 0.1 ml of 50% trichloroacetic acid was added, and the mix was chilled in ice and then centrifuged (15 min, 15,000 × *g*). Aliquots of the supernatant fluid were then either counted in Triton-toluene scintillation fluid ([^{14}C]hemoglobin or [^{14}C]albumin assay) or diluted with NaOH (0.5 ml plus 0.5 ml of 1.0 M NaOH), and the optical density was read at 440 nm. These assays were linear with time and protein concentration only up to 10 to 25% solubilization of the substrate. Therefore, several different protein concentrations were always tested to ensure that the assay was in the linear range. Protease activity with Azocoll as the substrate was measured as described by Cabib and Ulane (4), and protease activity with casein as the substrate was measured as described by Njus et al. (11) but with casein at 2.5 mg/ml.

Assay for degradation of unique dormant spore proteins A and B. The fraction containing the two major dormant spore proteins (proteins A and B) degraded during spore germination was isolated by dry rupture of dormant spores followed by extraction with dilute acetic acid, dialysis, and finally lyophilization (14a). The final dry powder was then dissolved in water to give a protein concentration of 3 to 4 mg/ml. The two unique dormant spore proteins, termed A and B, comprise >85% of the protein in this fraction (14b). The degradation of proteins A and B was assayed at 37 C in 0.9-ml reactions which were 50 mM in a solution of Tris-hydrochloride (pH 8.0) and 5 mM in

$CaCl_2$, and 300 μg/ml in proteins A and B combined. Aliquots (200 μl) were removed at 0, 4, 15, and 40 min of reaction, diluted to 1 ml with 0.2 M H_2SO_4, and centrifuged (10 min, 15,000 × *g*). Control experiments showed that this amount of sulfuric acid leaves >95% of both A and B proteins in the supernatant fraction. The supernatant fluids were then dialyzed at 4 C for 24 h against two 1-liter changes of 1% acetic acid and finally lyophilized. The residue was then dissolved in 100 μl of water, 50 μl was run on acrylamide gel (7.5%) electrophoresis at low pH, stained, destained, and the intensities of the A and B protein bands were quantitated as previously described (14a). The rate of degradation of proteins A and B was determined from the decrease in the total intensity of the electrophoretic bands with increasing reaction time. These rates were nearly linear with both time and protein concentration up to ~80% degradation.

Other methods. Spores were dry ruptured in a dental amalgamator (Wig-L-Bug) with glass beads as the abrasive, as described by Sacks and Bailey (14). Spores were ruptured with lysozyme after pretreatment of spores with 8 M urea—10% 2-mercaptoethanol at pH 3.0 for 90 min at 37 C, as previously described (5).

RESULTS

Peptidase activity during growth and sporulation. Enzymes capable of hydrolyzing the dipeptides alanylvaline and glutamylalanine and the tripeptides alanylalanylglycine and leucylglycylglycine were present throughout the growth cycle of *B. megaterium* (Fig. 1 and 2). The specific peptidase activities were relatively constant during log-phase growth, increased 1.5- to 2-fold in late log phase, and then showed a gradual increase throughout sporulation (Fig. 1 and 2). Values in the dormant spore were similar or slightly higher than those found in cells late in sporulation after refractile spores had appeared (Fig. 1 and 2). Throughout the growth cycle, >90% of the peptidase activity was found in the soluble fraction of the cell (20,000 × *g* supernatant fraction), and the amount of extracellular peptidase activity on alanylvaline and leucylglycylglycine was >3% of the intracellular activity (data not shown).

Degradation of different peptides by extracts of spores and log-phase cells. In addition to the four peptides whose degradation was measured throughout the growth cycle, dormant-spore and log-phase cell extracts were also found to degrade an additional 10 dipeptides and 2 tripeptides including those containing acidic, basic, hydrophilic, and hydrophobic amino acids (Tables 1 and 2). The peptide which was degraded most slowly was glycylproline, but prolylglycine was rapidly degraded. As was mentioned above, the peptidase activity of dormant spores was two- to threefold higher than that in log-phase cells (Tables 1 and 2). The aminopeptidase substrate leucine-*p*-nitroanilide was also degraded by cell and spore extracts with ratios of activity in different stages of growth similar to those seen with the di- and tripeptides as substrates (Table 1). The specific activity observed for degradation of leucine-*p*-nitroanilide in *B. megaterium* is similar to that reported in cells of *B. stearothermophilus* (13).

Protease activity during growth and sporulation. In contrast to the small differences observed in peptidase activity throughout the growth cycle of *B. megaterium*, large differences

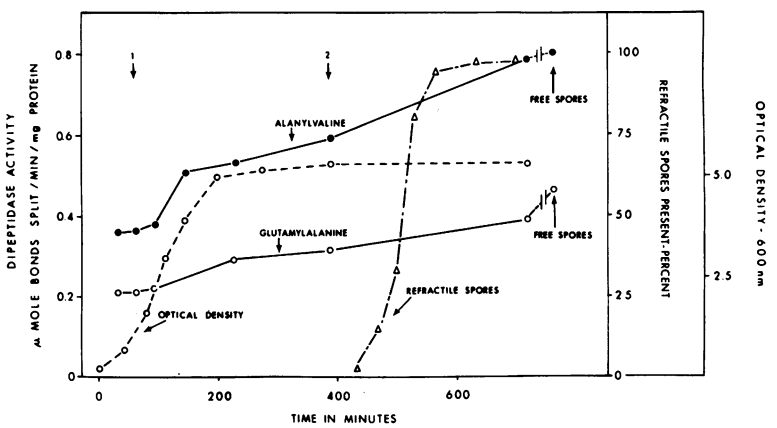

FIG. 1. *Dipeptidase activity during growth and sporulation of B. megaterium. Cells and spores were grown, harvested, extracted, and assayed for hydrolysis of alanylvaline and glutamylalanine. The values for free spores were determined on cleaned dormant spores, and values for time on the abscissa refer to the time of cell harvest.*

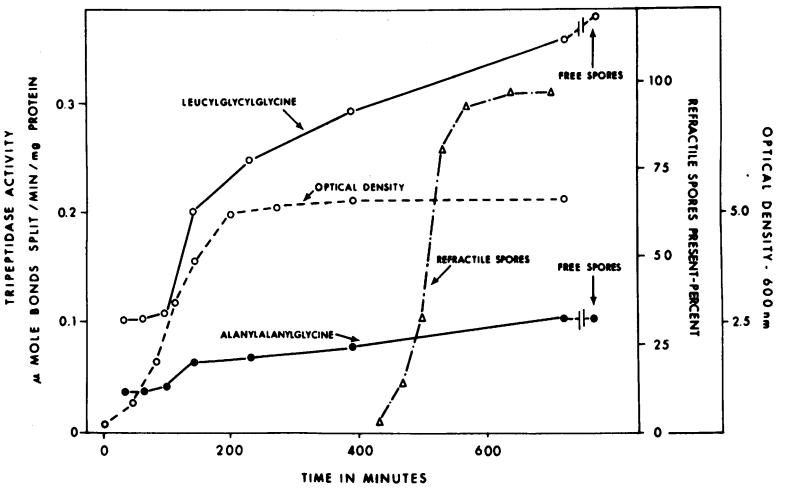

FIG. 2. *Tripeptidase activity during growth and sporulation of B. megaterium. Cells and spores were grown, harvested, extracted, and assayed for hydrolysis of alanylalanylglycine and leucylglycylglycine. The values for free spores were determined on cleaned dormant spores, and values for time on the abscissa refer to the time of cell harvest.*

TABLE 1. *Hydrolysis of dipeptides by extracts of dormant spores and log-phase cells*[a]

Substrate	Hydrolysis of dipeptides[b]	
	Log-phase cells	Dormant spores
Alanyltyrosine	0.28	0.65
Alanylvaline	0.37	0.79
Glutamylalanine	0.22	0.48
Glycylasparagine	0.36	0.57
Glycylglutamate	0.067	0.16
Glycylglycine	0.38	0.64
Glycylmethionine	1.33	2.82
Glycylproline	0.015	0.03
Glycylthreonine	0.39	1.16
Leucylglycine	0.46	0.89
Lysylleucine	0.08	0.19
Prolylglycine	0.34	0.63
Leucine-*p*-nitroanilide	0.036	0.082

[a] Cell and spore extracts were prepared and hydrolysis of dipeptides and leucine-*p*-nitroanilide measured. Log-phase cells were harvested at an optical density of 1.2 (arrow number 1, Fig. 1).

[b] Micromole bonds split per minute per milligram protein.

TABLE 2. *Hydrolysis of tripeptides by extracts of dormant spores and log-phase cells*[a]

Tripeptide	Hydrolysis of tripeptides[b]	
	Log-phase cells	Dormant spores
Alanylalanylglycine	0.039	0.109
Glycylglycylglycine	0.067	0.20
Leucylglycylglycine	0.12	0.38
Glycylglycylphenylalanine	0.08	0.28

[a] Cell and spore extracts were prepared and tripeptide hydrolysis measured. Log-phase cells were harvested at an optical density of 1.2 (arrow number 1, Fig. 1).

[b] Micromoles of bonds split per minute per milligram of protein.

in protease activity were seen. As has been observed previously (10) protease activity was constant during log-phase growth, but then increased rapidly in late log phase, reaching a value ~30-fold higher than that in log-phase cells (Fig. 3). The level of intracellular protease in sporulating cells was also 20- to 30-fold higher than that in free dormant spores (Fig. 3). In addition to this large increase in intracellular protease activity, high levels of extracellular protease also appeared at the end of log-phase growth as has been noted by other workers (1, 10). The extracellular protease activity accumulated slightly after the increase in intracellular protease activity but, late in sporulation, cultures contained ~20-fold more extracellular protease than intracellular protease (Fig. 3, note different scales). Although not shown here, measurements during growth and sporulation of intracellular and extracellular protease activity with azocasein as the substrate gave results very similar to those obtained by using ^{14}C-carbamylated hemoglobin as the substrate (Setlow, unpublished data).

Low protease activity in dormant spores. The high level of intracellular protease activity

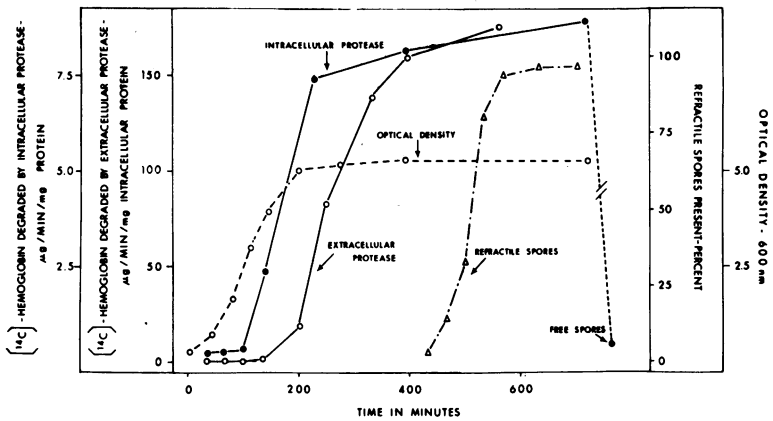

FIG. 3. *Protease activity during growth and sporulation in B. megaterium. Cells were grown and the cells and growth medium were isolated and assayed for hydrolysis of ^{14}C-labeled carbamylated hemoglobin. The values for extracellular protease in the cultures are expressed on the basis of intracellular protein present in the cultures. The values for free spores was determined on cleaned dormant spores, and values for time on the abscissa refer to the time of cell harvest.*

found in sporulating cells was consistent with the rapid protein degradation which is known to occur at this time (17). However, the low protease activity in dormant spores was surprising since protein degradation during spore germination is actually three- to fivefold more rapid then during sporulation, and the enzymes for the protein degradation during germination are known to be present in the dormant spore (14b). Therefore the possibility was examined that the protease activity of dormant spores might indeed be high, but was not extracted or detected using the standard procedure. However, the protease activity on either ^{14}C-labeled carbamylated hemoglobin or azocasein was not increased (i) when several different procedures were used to open dormant spores, (ii) when uncentrifuged extracts or extracts desalted on Sephadex G-25 were assayed, (iii) when spores were first germinated and then opened by sonic treatment, or (iv) when assays were carried out at pH values from 5 to 11, or with and without Ca^{2} or Mn^{2+} (Table 3). Similarly, assays with a number of different protein substrates also gave values for dormant spore protease which were similar to those in log-phase cells, but 20- to 40-fold lower than values obtained in sporulating cells (Table 4). It has also been previously shown that extracts from sporulating cells rapidly inactivate a number of enzymes, whereas dormant spore extracts give much slower inactivation (5).

Degradation of specific proteins A and B by cell and spore extracts. Another possible explanation for the apparent contradiction of rapid proteolysis during spore germination and low protease activity in the dormant and germinated spore is that the protein species degraded during germination are extremely sensitive to proteolysis. Indeed, earlier work has shown that >80% of the protein degraded

TABLE 3. *Effects of several changes in standard procedures on protease activity of dormant spores*[a]

Change in standard procedure	Protease activity[b]	
	[^{14}C]-hemoglobin	Azocasein
None	0.45	1.0
Spores opened with lysozyme	0.31[c]	0.7[c]
Spores opened by dry rupture	0.06	<0.2
Extract not centrifuged	0.40[d]	1.1[d]
Extract passed through Sephadex G-25	0.43	1.1
Assayed at pH 5, 6, 7, 9, 11[e]	<0.34	<0.9
$-Ca^{2+}$, $+Mn^{2+}$ (1 mM)	0.42	1.1
$-Ca^{2+}$	0.17	0.4

[a] Extracts from dormant spores were prepared by sonic treatment or with variations. Assays were carried out with either ^{14}C-carbamylated hemoglobin or with azocasein as the substrate.

[b] Micrograms of substrate degraded per minute per milligram of protein in extract.

[c] Specific activity corrected for the presence of lysozyme.

[d] Specific activity expressed on the basis of soluble protein.

[e] Buffers were used at 100 mM and were: acetate (pH 5.0), Tris-maleate (pH 6.0), Tris-hydrochloride (pH 7.0 and 9.0), and glycine (pH 11.0).

TABLE 4. *Endoprotease activities on several substrates in different stages of growth of B. megaterium*[a]

Substrate	Protease activity[b]		
	Log-phase cell	Sporulating cell	Dormant spore
^{14}C-carbamylated hemoglobin	0.25	9.2	0.45
^{14}C-carbamylated albumin	0.14	6.3	0.24
Azocasein	0.62	22.4	1.03
Azoalbumin	0.18	4.8	0.32
Casein	0.29	33.5	0.50
Azocoll	0.84	34.5	1.21

[a] Cell and spore extracts were prepared and protease activity measured with different substrates. Log-phase cells and sporulating cells were harvested at times indicated by arrows number 1 and 2, respectively, in Fig. 1.

[b] Micrograms of substrate degraded per minute per milligram of protein in extract.

during spore germination can be accounted for by a few (possibly two) low-molecular-weight, basic proteins which may be unique to the dormant spore (14a, 14b). These low-molecular-weight proteins might be expected to be extremely sensitive to proteolysis, and in fact they are rapidly degraded by extracts of both dormant and germinated spores (Table 5). Furthermore, the specific protease activity on these substrates is actually slightly higher in dormant spores than in either log-phase cells or sporulating cells (Table 5).

DISCUSSION

Comparison of rates of proteolysis in vitro and in vivo. Since protein degradation at all stages of growth of *Bacillus* species proceeds to the free amino acids, it is not surprising that di- and tripeptidase activities are demonstrable throughout growth. Indeed, if one assumes low average values for di- or tripeptide hydrolysis in log-phase cells, sporulating cells, and dormant spores, one can calculate that peptidase activity can cleave 0.6 to 2.4 mg of peptide/h per mg of cell or spore protein (Table 6). Although these calculated values are probably underestimates of the potential peptidase activity in vivo, they are 1.5- to 30-fold higher than the actual rate of in vivo protein degradation in either log-phase cells, sporulating cells, or germinating spores (Table 6). Therefore, hydrolysis of small peptides is probably not the rate-limiting step in protein turnover.

Indeed, the rate-limiting step in intracellular protein degradation not only in *Bacillus* species but also in other organisms has been proposed to be the initial cleavage of the substrate by an endoprotease (12, 14b). Clearly, protease assays in vitro may not be an accurate indicator of the true rate of protein turnover, since protease activity may be regulated in vivo and thus be much lower than predicted by in vitro assays (2). However, protease assays in vitro should provide a rough value for the maximal rate of protein degradation in vivo, if similar types of proteins are degraded both in vivo and in the protease assays. It is known that during sporulation in *Bacillus* species a large number of proteins are degraded including many enzymes (5, 17). If one assumes that azocasein, casein, hemoglobin, etc. represent reasonable models of these proteins degraded in sporulation and averages the data obtained with these different substrates (Table 6), one can calculate a value for protease activity in sporulating cells which is greater than the rate of in vivo proteolysis (Table 6). Similarly, although the nature of the proteins degraded during log-phase growth is not known, the rate of degradation of average proteins by log-phase cells in vitro is close (within a factor of two) of the rate of in vivo proteolysis (Table 6). This comparison breaks down, however, in dormant spores, since protease activity on average proteins is ~40-fold lower than the rate of in vivo proteolysis during spore germination (Table 6). It appears likely that the explanation for this discrepancy is that average proteins are not being degraded during spore germination, but rather that a unique group of very labile proteins is the substrate.

Indeed, the in vitro degradation of these unique proteins by dormant spore extracts takes place

TABLE 5. *Hydrolysis of proteins A and B by extracts from various stages of growth*[a]

Source of extract	Protein degraded[b]
Log-phase cells	11.2
Sporulating cells	21.7
Dormant spores	28.2
Germinated spores	25.5
Boiled dormant spores	<2

[a] Cell and spore extracts were prepared and degradation of proteins A and B measured. Log-phase cells and sporulating cells were harvested at times indicated by arrows number 1 and 2, respectively, in Fig. 1, and germinated spores were harvested after 20 min of germination.

[b] Micrograms per minute per milligram of protein in extract.

TABLE 6. *Comparison of peptidase and protease activities in vitro with the rates of protein degradation in vivo during different stages of growth*

Reaction	Stage of growth[a]		
	Log phase	Sporulation	Spore germination
Protein degradation in vivo	37[b]	257[c]	1,575[d]
Dipeptidase activity[e]	1,200	2,400	2,400
Tripeptidase activity[f]	675	2,160	2,160
Protease activity on[g] average proteins	20	1,110	38
Protease activity on unique[h] dormant spore proteins	672	1,202	1,692

[a] Microgram of protein or peptide degraded per hour per milligram soluble protein in extract.

[b] Calculated from data of Setlow (14b), assuming 80% of log-phase cell protein is soluble (Setlow, unpublished data).

[c] Calculated from data of Spudich and Kornberg (17) assuming 70% of sporulating cell protein is solubilized by sonic treatment.

[d] Calculated from data of Setlow (14b) and assuming 33% of dormant-spore protein is solubilized by sonic treatment (Setlow, unpublished data).

[e] Calculated by assuming average values for dipeptide hydrolysis of 0.1 μmol/min per mg of protein in log-phase cells and 0.2 μmol/min per mg of protein in dormant spores and sporulating cells (Table 1, Fig. 1) and an average dipeptide molecular weight of 200.

[f] Calculated by assuming average values for tripeptide hydrolysis of 0.075 μmol of bonds split/min per mg of protein in log-phase cells and 0.24 μmol of bonds split/min per mg of protein in dormant spores and sporulating cells (Table 2, Fig. 2) and an average value of 150 g for the weight of amino acid solubilized on cleaving one bond in a tripeptide.

[g] Calculated from data in Table 4 by averaging values for all six substrates tested.

[h] Calculated from data in Table 5.

at about the same rate as protein degradation during spore germination (Table 6).

Enzymes involved in protein and peptide hydrolysis. Clearly, one would like to identify the individual enzymes involved in the protein and peptide hydrolyses reported here. For instance, is peptide hydrolysis carried out by one or several enzymes? Several active aminopeptidases, similar to those described in *B. stearothermophilus* by Roncari and Zuber (13), are present in all stages of growth of *B. megaterium*, and these enzymes may be responsible for most of the peptidase activity in cell and spore extracts (Setlow, unpublished data). However, firm identification of these enzymes as those responsible for peptide hydrolysis must await their purification.

It is known that one endoprotease accounts for most of the intracellular proteolytic activity in sporulating *B. megaterium* (10) but little is known about proteases in log-phase cells or dormant spores. However, the fact that the A and B proteins are degraded more rapidly by extracts of dormant spores than sporulating cells (Table 5) suggests that the enzyme responsible is not a small amount of sporulating protease trapped in the dormant spore, but rather some different enzyme(s) capable of attacking low-molecular-weight labile proteins, but not other more typical proteins. The rapid degradation of proteins A and B by extracts of log-phase cells also suggests that the enzyme degrading these proteins may not be unique to the dormant spore, although further work is needed to prove this point. Whatever the nature of the dormant spore protease(s) which attacks proteins A and B, clearly an intriguing question about this enzyme(s) is the mechanism whereby it remains inactive in the dormant spore despite the presence of its substrate, yet rapidly becomes active in the first minutes of spore germination.

ACKNOWLEDGMENTS

I am grateful for the excellent technical assistance of Cynthia Janovicz and Scott Wood.

This work was supported by Public Health Research grant GM-19698 from the National Institute of General Medical Sciences.

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