*Biochem. J.* (1983) **210**, 411–417
*Printed in Great Britain*



# Precursor processing during the maturation of a spore-coat protein in *Bacillus megaterium* KM

Gordon S. A. B. STEWART and David J. ELLAR
*Department of Biochemistry, University of Cambridge, Tennis Court Road, Cambridge CB2 1QW, U.K.*

(*Received 11 August 1982/Accepted 29 October 1982*)

A protein of apparent mol.wt. 35000 that is extractable from the purified coat fraction of *Bacillus megaterium* KM spores is synthesized during sporulation as a precursor protein from which a 12–13 amino acid peptide is removed. Cleavage of this small peptide is delayed until 60–90min after precursor synthesis and is concomitant with the morphological appearance of stage VI. The addition of chloramphenicol, subsequent to precursor synthesis, prevents the appearance of this late processing event. Two-dimensional non-equilibrium pH-gradient gel electrophoresis of the integument extract of forespores isolated at stage V from sporangia pulse-labelled with L-[$^{35}$S]methionine 1h before isolation, revealed both unprocessed and processed components. Similar analysis of total protein from the corresponding mother cells revealed only the unprocessed component in relatively small amounts, suggesting that, although the protein may be synthesized in the mother-cell compartment, processing may be restricted to the forespore. Peptide analysis by limited proteolysis was used to examine the relationship between the 35000- and a 17500-mol.wt. coat protein. The possible implications of limited proteolytic processing to maturation of the spore coat are discussed.

The coats that envelop bacterial endospores contain one or more sporulation-specific proteins (Walker *et al.*, 1967). Genetic and biochemical analysis of the synthesis and assembly of these proteins presents challenges in three important areas. First, it requires understanding of translational and/or transcriptional gene control during morphogenesis in a prokaryote. Secondly, it requires an understanding of the mechanisms by which these bacteria ensure that coat proteins are specifically assembled on only one of the three membrane surfaces that face the cytoplasm in a sporulating cell. Thus if the forespore compartment is the site of coat protein synthesis, the proteins must be guided across a double membrane of mutually opposed polarity (Wilkinson *et al.*, 1975) to the site of coat assembly. Alternatively if coat proteins are synthesized in the mother-cell compartment, some specific receptor/docking mechanism must exist to ensure that coat-protein deposition occurs only on the cytoplasmic face of the forespore outer membrane and not on the equivalent face of the mother-cell plasma membrane. Finally the coat proteins present an additional challenge because of the indications that their structure and chemistry may play a major role in imposing and/or maintaining the extreme dormancy and resistance that characterize bacterial spores.

A number of previous reports have suggested that spore-coat proteins may be synthesized as high-molecular-weight precursors, which are proteolytically cleaved to produce smaller mature proteins (Cheng & Aronson, 1977; Aronson & Pandey, 1978; Munoz *et al.*, 1978*a*,*b*; Stelma *et al.*, 1978; Pandey & Aronson, 1979; Aronson, 1981; Goldman & Tipper, 1981). These precursors can be identified immunologically in both *Bacillus cereus* and *Bacillus subtilis* as proteins ranging from mol.wt. 21000 to mol.wt. 65000, which give rise to polypeptides of mol.wt. 12000–13000. There do, however, exist conflicting data, as Jenkinson *et al.* (1981) could find no evidence to suggest that the 12000-mol.wt. coat peptide in *Bacillus subtilis* has a higher-molecular-weight precursor.

The spore coat of *Bacillus megaterium* KM has recently been characterized and the timing of synthesis of coat-specific proteins determined (Stewart & Ellar, 1982). Concomitant with the morphological appearance of the spore coat is the synthesis of three coat proteins, two of which are

Abbreviations used: SDS, sodium dodecyl sulphate.



0306-3283/83/020411-07$2.00 © 1983 The Biochemical Society

molecular-weight multiples of the smallest protein of apparent mol.wt. 17500. The present paper examines the putative precursor–product relationship of coat proteins in this organism. Although after characterization of the coat isolated from stage V forespores and pulse–chase experiments, no evidence was found for the type of proteolytic cleavage described above, the results do show that at 60–90 min after synthesis a precursor of the 35000-mol.wt. coat protein undergoes a limited post-translational proteolytic modification to remove 12–13 amino acids (1500 Da). The experiments also suggest that this precursor is synthesized in the mother-cell compartment.

## Materials and methods

### *Bacterial strain and growth*

The organism used in these studies was a sporogenic strain of *Bacillus megaterium* KM. The conditions for growth and sporulation were as previously described (Ellar & Posgate, 1974), with the exception of a modified sporulation medium (Stewart *et al.*, 1981).

### *Chemicals*

Acrylamide and *NN'*-methylenebisacrylamide were obtained from BDH. *Staphylococcus aureus* V8 proteinase was obtained from Miles Laboratories, Slough, Berks., U.K. L-[$^{35}$S]-Methionine and [$^{14}$C]methylated molecular-weight standards were obtained from The Radiochemical Centre, Amersham, Bucks., U.K. All other chemicals were from Sigma Chemical Co., St. Louis, MO, U.S.A.

### *Electrophoresis*

SDS/polyacrylamide-gel electrophoresis, non-equilibrium pH-gradient electrophoresis and fluorography were carried out as described previously (Stewart & Ellar, 1982).

### L-[$^{35}$*S*]*Methionine pulse of a sporangial culture between 5 and 6 h and the preparation of spore, forespore and mother-cell fractions*

L-[$^{35}$S]Methionine (200 $\mu$Ci) was added aseptically to 1 litre of a sporulating culture 5 h ($t_5$) after the end of exponential growth (late stage IV/early stage V). After 1 h ($t_6$), 400 ml of labelled culture was harvested aseptically by centrifugation (10000 $g$ for 3 min at 30°C) and resuspended to its original volume in the supernatant from a parallel culture incubated without radioactive label and containing 500 $\mu$g of L-methionine/ml. The culture was transferred to a 2-litre flask for continued incubation until spore release at $t_{12}$. Spores were harvested as described previously (Stewart & Ellar, 1982).

In parallel with the above, 500 ml of labelled culture was cooled on ice, harvested by centrifugation and the cell pellet was resuspended in 30 ml of ice-cold SPM buffer [0.6 M-sucrose/0.1 M-potassium phosphate (pH 6.3)/0.016 M-$MgSO_4$/0.001 M-L-methionine]. The cells were re-centrifuged once and resuspended in 30 ml of SPM buffer. Lysozyme (4 mg; three times crystallized from egg white) (lysozyme/cell dry wt. ratio 1:100) was added and the cells protoplasted by incubation at 37°C for 35 min. A 100 $\mu$l sample was removed for the determination of radioactivity incorporated into trichloroacetic acid-precipitable material. Forespores were released from the protoplasted sporangia by using 6 × 3 s pulses with a 1.2 cm sonic probe (Dawe Instruments, London W.3, U.K.) (Ellar & Posgate, 1974). The temperature was maintained below 5°C by periodic immersion of the culture tube in an ethanol/solid $CO_2$ bath. Isolated forespores were separated from the mother-cell material by centrifugation at 10000 $g$ for 3 min at 4°C. A portion (100 $\mu$l) of the supernatant was removed for the determination of radioactivity incorporated into trichloroacetic acid-precipitable material. The forespore pellet was washed twice with SPM buffer by repeated centrifugation and finally resuspended in a small volume for dry-weight determination.

Mature spores and forespores were extracted at 100 mg dry wt./ml in SDS/sample buffer [50 mM-Tris/HCl (pH 7.5)/1% (w/v) SDS/25 mM-dithiothreitol/2 mM-phenylmethanesulphonyl fluoride/1 mM-EDTA/10% (w/v) glycerol/0.0025% (w/v) Bromophenol Blue] by heating for 5 min at 100°C. The resulting insoluble material was removed by centrifugation at 30000 $g$ for 10 min. Mother-cell protein concentration was determined by the method of Lowry *et al.* (1951) and samples were solubilized in SDS/sample buffer at a protein concentration of 10 mg/ml by heating for 5 min at 100°C.

### *Peptide mapping by limited proteolysis*

The method used was based on that of Cleveland *et al.* (1977). Protein (50 $\mu$g) from whole-spore and forespore SDS/sample buffer extracts was electrophoretically separated on 6 cm/15% acrylamide gels. A 20% acrylamide gel was prepared with a 4 cm/6% acrylamide stacking gel. A 2 cm space was left above the stacking gel to accommodate the one-dimensional tracks, which were sealed into place with stacking-gel mixture, leaving a 0.5 cm space between the gel and the top of the plates after polymerization. A small volume of stacking gel containing 10 $\mu$g of V8 proteinase from *S. aureus* was evenly distributed over the top 0.5 cm of gel and allowed to polymerize. The gel was electrophoresed as described previously (Stewart & Ellar, 1982) with

the exception that the current was switched off for 30min just before the Bromophenol Blue dye entered the 20% acrylamide separating gel. Duplicate tracks from the first-dimension gel were stained for inclusion as markers at the top of the proteinase gel.

*Pulse for 5 min with L-[$^{35}S$]methionine at stage V and subsequent chase*

Two 100ml portions from a 1 litre stage V sporulating culture were harvested by centrifugation (10000**g** for 3min at 30°C). The supernatants were transferred to two 250ml flasks and maintained aerated at 30°C. The cell pellets were each resuspended in 10ml of sporulation medium (Stewart *et al.*, 1981), pre-aerated and equilibrated to 30°C and prepared without acid casein hydrolysate, enzymic casein hydrolysate or enzymic yeast extract. The resuspended cultures were transferred to 25ml flasks in an orbital incubator maintained at 30°C and operating at 200 cycles/min. L-[$^{35}$S]Methionine (100$\mu$Ci) was added to each flask and after 5min 10mg of L-methionine was added to culture 1 and 10mg of L-methionine plus 1mg of chloramphenicol was added to culture 2. The cultures were harvested by centrifugation and the supernatants discarded. The cell pellets were resuspended in the 100ml of sporulation media from which they had initially been removed, supplemented with 100mg of L-methionine for culture 1 and 100mg of L-methionine plus 10mg of chloramphenicol for culture 2. During continued orbital incubation at 30°C, 10ml samples of both cultures were withdrawn at specified time intervals, transferred to 40ml of boiling propan-2-ol and refluxed for 15min to destroy all enzyme activity. The cells were harvested by centrifugation in 15ml Corex glass tubes and washed three times with ice-cold PEP buffer [10mM-potassium phosphate (pH7.4)/5mM-EDTA/1mM-phenylmethanesulphonyl fluoride/10mM-NaCl]. Each pellet was resuspended in 0.7ml of PEP buffer and 4.1–4.2g of glass beads (0.10–0.11mm diameter) added. The cells were disrupted by vortex-mixing each tube on a Whirlimixer (Fisons Scientific Apparatus, Loughborough, Leics., U.K.) for up to 4min (Johnstone *et al.*, 1982). The extent of mother cell and forespore disruption was determined by phase-contrast microscopy and it should be noted that the ratio of cell suspension volume to the weight of glass beads is critical for efficient breakage. The glass beads were removed from each sample by filtration with ice-cold PEP buffer by using a 4ml sintered base glass column. Filtrates were collected in 15ml Corex glass tubes and the particulate material, which included the cell walls and forespore integuments (comprising spore coat, cortex and outer membrane), was pelleted by centrifugation (30000**g** for 10min at 4°C). The pellets were washed once by re-centrifugation before extraction in 200$\mu$l of SDS/sample buffer at 100°C for 5min. Insoluble cortex and peptidoglycan were removed by centrifugation at 30000**g** for 10min. A portion (5$\mu$l) of each sample was diluted with a further 35$\mu$l of SDS/sample buffer before electrophoresis. An L-[$^{35}$S]methionine steady-state-labelled spore extract, used as an integument protein standard, was prepared as previously described (Stewart & Ellar, 1982).

## Results and discussion

*Two-dimensional gel electrophoresis of spore and forespore integuments and mother-cell cytoplasm from $t_5$–$t_6$ L-[$^{35}S$]methionine-labelled cells*

A culture of sporulating cells was pulse-labelled with L-[$^{35}$S]methionine between $t_5$ and $t_6$ and spores were harvested after allowing sporulation to continue until 12h ($t_{12}$). Forespore and mother-cell fractions were prepared from a portion of the culture harvested immediately after pulse-labelling. The distribution of radioactivity incorporated into the mother-cell and forespore compartment was 41.3% and 58.7% respectively. This is a slightly higher mother-cell incorporation than that previously described for L-[U-$^{14}$C]phenylalanine incorporation by Eaton & Ellar (1974), but is in agreement with that recently described for *Bacillus subtilis* by Watabe *et al.* (1981). The specific radioactivity of the forespore SDS/sample buffer extract was five times that of the mother-cell extract, suggesting that, although total protein synthesis is relatively evenly distributed at this time, the mother cell may be actively synthesizing only about 20% of its total protein complement. Furthermore, since the forespore radioactivity itself may reflect synthesis of only 20% of total forespore protein, the mother-cell value may well be closer to 4%.

The distribution of radioactivity from whole-spore extracts, forespore extracts and total mother-cell protein was analysed by non-equilibrium pH-gradient electrophoresis as shown in Fig. 1. Fig. 1(*a*) shows the whole-spore extract, which is equivalent to the profile previously published for pulse-labelling between $t_5$ and $t_6$ (Stewart & Ellar, 1982). Figs. 1(*b*) and 1(*c*) show the corresponding forespore and mother-cell extracts respectively. Several significant differences can be seen between the mature-spore and forespore profiles. The larger number of proteins in the forespore extract reflects a 30% increase in the efficiency of extraction at this immature stage of development. Of greater interest, however, are first, the presence in the mature spore of labelled proteins (filled arrowhead), which cannot be identified in the forespore and which must therefore reflect post-translational modification or assembly during forespore maturation. Secondly, although the 17500-mol.wt. mature coat protein (small open arrowhead) is present as a protein of identical molecular weight

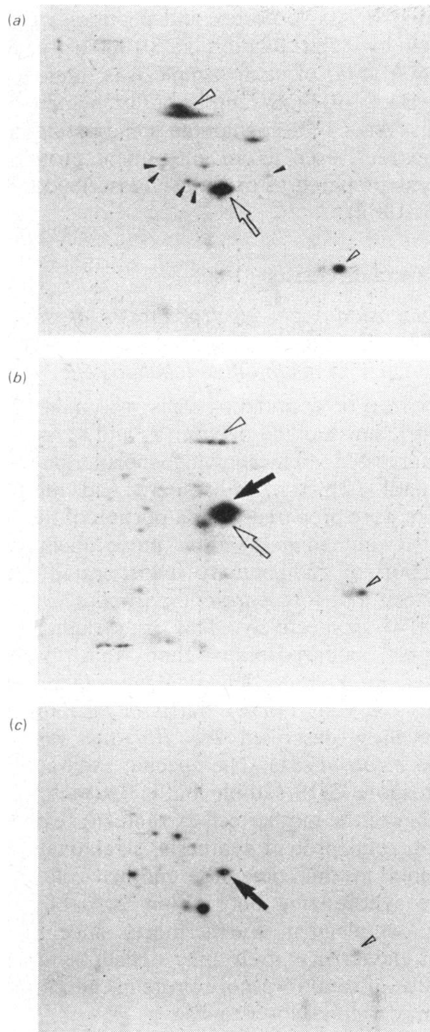


Fig. 1. *Fluorographs of non-equilibrium-pH-gradient-electrophoresis-separated proteins obtained from (a) mature-spore integuments. (b) isolated forespore integuments and (c) mother-cell cytoplasm, pulse-labelled with* L[$^{35}S$]*methionine between $t_5$ and $t_6$ as described in the text*
Filled arrowheads, proteins in the spore integuments that cannot be identified in the forespore; large open arrowhead, 65000-mol.wt. protein; small open arrowhead, 17500-mol.wt. coat protein; open arrow, 35000-mol.wt. coat protein; filled arrow, 35000-mol.wt. coat protein precursor. (*a*) and (*b*) were fluorographed for 8 days and (*c*) for 21 days.

in the forespore, the charge distribution is slightly more basic, as reflected by a positional shift to the right. Thirdly, the 65000-mol.wt. putative glycoprotein (large open arrowhead) (Stewart & Ellar, 1982), which shows multiple charge heterogeneity in the mature spore, is less heterogeneous in the forespore extract, suggesting that most of the maturation of this protein must occur after stage V. Finally, the most significant difference between the mature-spore and forespore patterns is the presence in the latter of a higher-molecular-weight component (filled arrow) of the 35000-mol.wt. coat protein (open arrow) of apparently identical pI.

Comparison of Figs. 1(*b*) and 1(*c*) shows that the 17500-mol.wt. coat protein is just detectable in the mother-cell fraction after extended fluorography (small open arrowhead). The 35000-mol.wt. protein, however, is clearly detectable (filled arrow) and appears as only a single spot in the mother-cell, equivalent in position to the high-molecular-weight component in the forespore gel. The presence of a single component excludes the possibility that its detection within the mother cell reflects forespore contamination. The above observation strongly suggests that the mother cell is the site of synthesis of a precursor to the 35000-mol.wt. coat protein and that, in addition, it may also be the site of synthesis of the 17500-mol.wt. coat protein. Nevertheless, a number of alternative possibilities cannot as yet be ruled out. For example, by comparison with the mature 35000-mol.wt. protein, the precursor may be very easily extracted from the forespores during isolation procedures. The only previously published evidence for the site of spore-coat synthesis reported immunocytochemical localization of purified coat-specific antigen within the forespore compartment (Short *et al.*, 1977). The spore-coat material used for antibody preparation in this latter study still contained the spore outer membrane, which forms part of the integument fraction (Crafts-Lighty & Ellar, 1980), and the possibility exists therefore that the antiserum was preferentially directed towards forespore-specific membrane proteins rather than the structural components of the spore coat that are under examination in the present paper.

*Peptide mapping by limited proteolysis*

It has been previously reported that three of the major components identified in pulse-labelled spore integuments relate to a 17500-mol.wt. coat protein as molecular-weight multiples, namely monomer, dimer and trimer (Stewart & Ellar, 1982). The observation that monomer synthesis appears to be *de novo* but that the putative dimer exhibits potential precursor processing prompted a further study of the relationships between these three proteins. Peptide mapping, by using either enzymic or chemical techniques, has proven a valuable tool for establishing stuctural similarities beween polypeptides and this technique was therefore applied to integument-protein analysis. Preliminary investigations to characterize the proteinase sensitivity of the purified 17500-mol.wt. coat protein [prepared as previously

described (Stewart & Ellar, 1982)] were performed by a one-dimensional SDS/polyacrylamide-gel-electrophoretic system essentially as described by Cleveland *et al.* (1977) (results not shown). The coat protein was totally resistant to trypsin and chymotrypsin at protein/proteinase ratios of up to 10:1, a ratio that resulted in complete proteolytic fragmentation of ovalbumin used as a control. This observation is consistent with the previously reported resistance of lysozyme-treated coats of *Bacillus cereus* PX to tryptic and chymotryptic digestion (Gould & King, 1969). *S. aureus* V8 proteinase cleaves at the *C*-terminal side of aspartic acid and glutamic acid residues. Although most of the acidic residues in the 17500-mol.wt. protein are probably amidated (Stewart & Ellar, 1982), the high percentage of the total amino acid composition accounted for by these residues (20%) prompted an investigation of the sensitivity of this protein to V8 proteinase. Preliminary experiments gave incomplete digestion of the coat protein over a 10-fold range of V8 proteinase concentration. However, a single proteolytic peptide was reproducibly obtained.

Fig. 2 shows an extension of the peptide mapping procedure of Cleveland *et al.* (1977) to provide a comparative map after SDS/polyacrylamide-gel-electrophoretic separation of a complex protein mixture. Fig. 2(*a*) shows the peptide map of spore (left) and forespore (right) extracts revealed by Coomassie Blue staining. The V8 proteinase band (large open arrowhead) (mol.wt. 27700) can be seen extending across the entire gel. In this system, native proteins that are not proteolysed form a diagonal line. Fig. 2(*b*) is a fluorograph of the Coomassie Blue-stained gel showing the SDS/sample buffer extracts from mature spores and forespores obtained from sporangia pulse-labelled with L-[$^{35}$S]-methionine between $t_5$ and $t_6$. The one-dimensional gels in Fig. 2 correspond to the starting material for the two-dimensional gels shown in Figs. 1(*a*) and 1(*b*) respectively. Two spots representing the native 17500-mol.wt. coat protein and its proteolytic product are labelled in both maps with filled arrowheads. The 35000-mol.wt. protein from the whole-spore extract produces a single spot (small open arrowhead), which is extremely close to half the molecular weight of the mature protein. This unusual cleavage pattern suggests that the native protein is split precisely in two by V8 proteinase action and this brings it extremely close to the molecular weight of the 17500-mol.wt. coat protein. The absence of the single proteolytic fragment, which is characteristic of the 17500-mol.wt. protein, must, however, suggest that the two proteins are not necessarily structurally similar. Comparison of the peptide patterns from the mature spore and forespore reveals two further interesting features. The peptide profiles of the 65000-mol.wt. protein

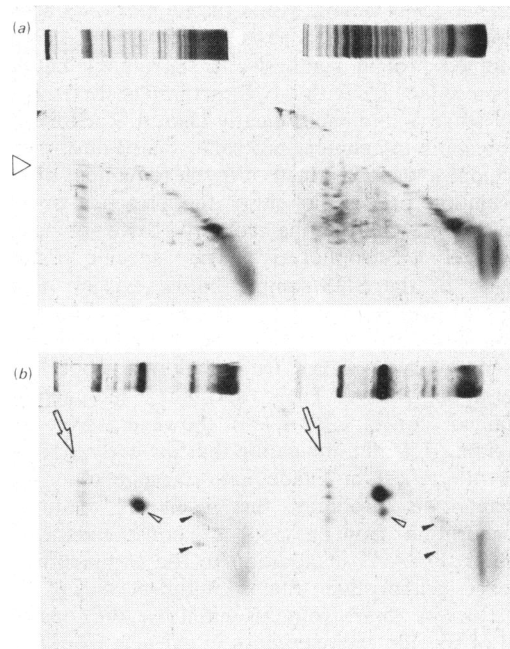


Fig. 2. *Peptide mapping by limited proteolysis with S. aureus V8 proteinase of electrophoretically separated SDS/sample buffer extracts from mature spores and forespores obtained from sporangia pulse-labelled with* L-[$^{35}$*S*]*methionine between* $t_5$ *and* $t_6$

(*a*) Coomassie Blue-stained gel; (*b*) 14 day fluorograph. Filled arrowhead, the native and proteolytic fragment from the 17500-mol.wt. coat protein; small open arrowhead, the proteolytic fragment from the 35000-mol.wt. coat protein; open arrow, the series of proteolytic fragments from the 65000-mol.wt. protein; large open arrowhead, the V8 proteinase.

(open arrow) are slightly different, again suggesting late modification of this protein between stage V and mature-spore release. Of particular significance is the presence of two spots in the region of the 35000-mol.wt. protein in the forespore extract (Fig. 2*b*). The lower spot (small open arrowhead). exactly correlates with the equivalent proteolytic fragment from the mature spore. The upper spot, however, presumably reflects a similar 'mid-point' cleavage of the higher-molecular-weight species and therefore further supports its identity as a precursor protein.

### *Definitive identification of a precursor–product relationship for the 35000-mol.wt. coat protein by the pulse–chase technique*

Precursor processing of the 35000-mol.wt. coat protein was further investigated with specific

reference to its timing within the temporal sequence of sporangial morphogenesis and its dependence on continued protein synthesis. A sporulating culture was pulse-labelled with L-[$^{35}$S]methionine for 5 min at early stage V and subsequently chased. The identity of forespore integument proteins labelled during this short pulse was examined over the remainder of the sporulation process in either the presence or the absence of chloramphenicol by SDS/polyacrylamide-gel electrophoresis. The specific radioactivity of the SDS/sample buffer extracts from cultures incubated in the absence of chloramphenicol showed a 3-fold increase in the first 30 min of chase over those from cultures containing chloramphenicol. The specific radioactivity remained constant (±4.7%), however, over the remaining 150 min, indicating that the early increase does not reflect an inadequate chase regime. The increment is probably the result of continued incorporation from a pool of non-exchangeable labelled tRNA$^{Met}$ in addition to the completion of nascent polypeptide chains initiated during the pulse period. Apart from this initial rise, the constant level of specific radioactivities in extracts from both cultures must reflect a negligible turnover of the forespore integument proteins after synthesis at stage V.

Fig. 3 shows the fluorograph obtained after SDS/polyacrylamide-gel electrophoresis of all the SDS/sample buffer extracts. Processing of the 35000-mol.wt. coat protein (filled arrowhead) by proteolytic cleavage of an estimated 1500-mol.wt. fragment (12–13 amino acids) is clearly demonstrated (tracks 3–10). The processing event does not occur until 60–90 min after precursor labelling (stage VI) and furthermore, specifically requires continued protein synthesis, as demonstrated by the absence of processing in extracts from cultures incubated with chloramphenicol (tracks 11–18). In addition to proteolytic processing of the 35000-mol.wt. protein, confirmation of the late maturation of the 65000-mol.wt. protein (open arrowhead) can also be obtained from Fig. 3. This protein is initially synthesized as a homogeneous-molecular-weight species. Heterogeneous higher-molecular-weight species, possibly associated with glycosylation, become evident about 60–90 min after synthesis, again concomitant with stage VI.

The time-dependent limited proteolysis of 12–13 amino acids from a spore-coat protein is a unique observation in sporulation. The association of processing with stage VI suggests a possible link with the marked structural changes in the spore coat occurring between stage VI and final release. These

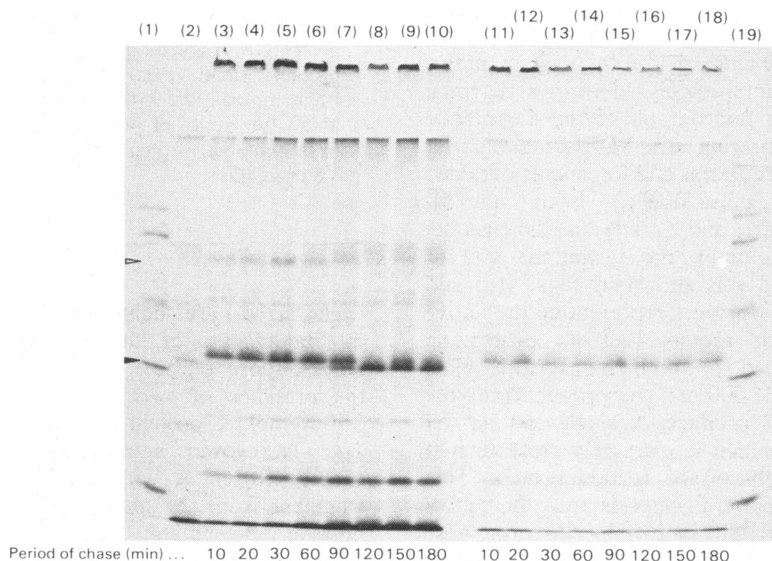


Fig. 3. *A 20-day fluorograph of forespore integument proteins isolated from sporangia after a 5 min pulse with* L-[$^{35}$*S*]*methionine at stage V and subsequent chase*

Tracks (1) and (19), Amersham [$^{14}$C]methylated molecular-weight standards: myosin (mol.wt. 200000), phosphorylase *b* (92500), albumin (69000), ovalbumin (46000), carbonic anhydrase (30000) and lysozyme (14300); track (2), L-[$^{35}$S]methionine steady-state labelled spore extract; tracks (3)–(10), extracts obtained after 10, 20, 30, 60, 90, 120, 150 and 180 min of chase respectively; tracks (11)–(18), extracts obtained after 10, 20, 30, 60, 90, 120, 150 and 180 min of chase in the presence of 100 $\mu$g of chloramphenicol/ml. Filled arrowhead, 35000-mol.wt. coat protein; open arrowhead, 65000-mol.wt. protein.

changes render the coat resistant to SDS extraction in the absence of thiol-reducing reagents (Stewart & Ellar, 1982).

A role for a proteolytic enzyme in *Bacillus subtilis* coat maturation has previously been indicated from studies relating the dependence of the acquisition of lysozyme resistance, a spore property intimately associated with the spore coat (Roberts & Hitchins, 1969), to the serine proteinase inhibitor phenylmethanesulphonyl fluoride (Jenkinson *et al.*, 1980). In these studies, the inclusion of phenylmethanesulphonyl fluoride and/or chloramphenicol at $t_{4.5}$ resulted in only 50% of the final spore population acquiring lysozyme resistance. This suggests that a proteinase needed for developing this property has still to be synthesized. Cheng & Aronson (1977) have reported a direct role for an intracellular serine proteinase in *Bacillus cereus* coat processing. Mutation of this proteinase results in the phenotypic expression of lysozyme sensitivity, which can be related to a defect in the packing of coat monomer. The isolation from *Bacillus cereus* of a unique coat-associated serine proteinase (Srivastava & Fitz-James, 1981), further supports a significant role for proteolysis in spore-coat maturation. For example, the late processing of a coat protein, which, through associated conformational changes, modifies the potential for disulphide formation, could result in a maturation process leading to the acquisition of lysozyme resistance and the maintenance of spore dormancy. Processing of the 35000-mol.wt. coat protein in *Bacillus megaterium* KM may reflect such a maturation process.

We thank the Science and Engineering Research Council for financial support.

## References


Aronson, A. I. (1981) *J. Bacteriol.* **145**, 541–547

Aronson, A. I. & Pandey, N. K. (1978) in *Spores VII* (Chambliss, G. & Vary, J. C., eds.), pp. 54–61, American Society for Microbiology, Washington, DC

Cheng, H. M. & Aronson, A. I. (1977) *Proc. Natl. Acad. Sci. U.S.A.* **74**, 1254–1258

Cleveland, D. W., Fischer, S. G., Kirschner, M. W. & Laemli, U. K. (1977) *J. Biol. Chem.* **252**, 1102–1106

Crafts-Lighty, A. & Ellar, D. J. (1980) *J. Appl. Bacteriol.* **48**, 135–145

Eaton, M. W. & Ellar, D. J. (1974) *Biochem. J.* **144**, 327–337

Ellar, D. J. & Posgate, J. A. (1974) in *Spore Research 1973* (Barker, A. N., Gould, G. W. & Wolf, J., eds.), pp. 21–40, Academic Press, London

Goldman, R. C. & Tipper, D. J. (1981) *J. Bacteriol.* **147**, 1040–1048

Gould, G. W. & King, W. L. (1969) in *Spores IV* (Campbell, L. L., ed.), pp. 276–286, American Society for Microbiology, Washington, DC

Jenkinson, H. F., Kay, D. & Mandelstam, J. (1980) *J. Bacteriol.* **141**,793–805

Jenkinson, H. F., Sawyer, W. D. & Mandelstam, J. (1981) *J. Gen. Microbiol.* **123**, 1–16

Johnstone, K., Simion, A. F. & Ellar, D. J. (1982) *Biochem. J.* **202**, 459–467

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275

Munoz, L. E., Sadaie, Y. & Doi, R. H. (1978*a*) *J. Biol. Chem.* **253**, 6694–6701

Munoz, L. E., Nakayama, T. & Doi, R. H. (1978*b*) in *Spores VII* (Chambliss, G. & Vary, J. C., eds.), pp. 213–219, American Society for Microbiology, Washington, DC

Pandey, N. K. & Aronson, A. I. (1979) *J. Bacteriol.* **137**, 1208–1218

Roberts, T. A. & Hitchins, A. D. (1969) in *The Bacterial Spore* (Gould, G. W. & Hurst, A., eds.), pp. 611–670, Academic Press, London

Short, J. A., Walker, P. D., Hine, P. & Thomson, R. O. (1977) *J. Appl. Bacteriol.* **43**, 75–82

Srivastava, O. P. & Fitz-James, P. (1981) *J. Gen. Microbiol.* **126**, 463–475

Stelma, G. N., Aronson, A. I. & Fitz-James, P. (1978) *J. Bacteriol.* **134**, 1157–1170

Stewart, G. S. A. B. & Ellar, D. J. (1982) *Biochem. J.* **202**, 231–241

Stewart, G. S. A. B., Johnstone, K., Hagelberg, E. & Ellar, D. J. (1981) *Biochem. J.* **198**, 101–106

Walker, P. D., Thompson, R. O. & Baillie, A. (1967) *J. Appl. Bacteriol.* **30**, 317–320

Watabe, K., Iida, S., Nakamura, K., Ichikawa, T. & Kondo, M. (1981) *Microbiol. Immunol.* **25**, 545–556

Wilkinson, B. J., Deans, J. A. & Ellar, D. J. (1975) *Biochem. J.* **152**, 561–569