Spores of Microorganisms

XII. Non-participation of the Preexisting Sporangial Cell Wall in the Formation of Spore Envelopes and the Gradual Synthesis of DAP-containing Structures during Sporogenesis of Bacilli

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ABSTRACT

Preexisting $^{14}\text{C}$-DAP in vegetative cells of *Bacillus cereus* is not incorporated into the spores, but is released into the medium after sporogenesis is complete. Exogenous $^{14}\text{C}$-DAP added to the medium before sporulation is incorporated intensively into the sporangia and practically all of it is taken up by the spores. During sporogenesis, two periods of increased incorporation of $^{14}\text{C}$ into hot TCA-precipitate of cells are found after adding $^{14}\text{C}$-DAP — one before formation of the spores, when $^{14}\text{C}$-lysine formed by decarboxylation is incorporated together with $^{14}\text{C}$-DAP, and one during the “whitening” phase, when any $^{14}\text{C}$-lysine is no longer incorporated. The incorporation of exogenous $^{14}\text{C}$-lysine into the sporangial proteins is also markedly elevated during the presporulation phase and at the outset of sporogenesis.

One of the protective systems of spores of bacilli and clostridia is their highly complex envelope structure. They form a permeability barrier, artificial loss of whose specific permeability for exogenous substances as a result of mechanical injury (Rode & Foster, 1960a, b) or chemical injury (Rode & Foster, 1960c, d) leads to germination. In clostridia, the sporangial wall can form part of this envelope system; in bacilli, it is degraded and new structures are formed. The main part of the envelope structure of the mature spore is the rigid coat (or coats), which is composed chiefly of polymerized non-dialyzable peptides containing glutamic acid, diaminopimelic acid (DAP) and alanine, together with muramic acid and glucosamine, which are released into the medium during germination by an intracellular lytic system (Strange & Powell, 1954; Powell & Strange, 1956; Strange & Dark, 1956, 1957a, b; Strange, 1959). The same or similar peptides are often part of the cell wall of bacteria (Cummins & Harris, 1956; Salton, 1956; Strange & Dark, 1957a, b). On studying the fractions of lysed cells it was found that peptides of this structure were transferred from the insoluble to the soluble fraction during sporogenesis proper (Powell & Strange, 1956) and that some at least were released into the medium (Strange & Dark, 1957b).

Spore formation takes place in a culture when multiplication of the cells is almost complete and the stock of exogenous substrates is already depleted. According to some findings in the literature, sporulation appears to be an endotrophic process, in which material already present in the vegetative cell is reorganized and reutilized in building the spore structure (Hardwick & Foster, 1952; Foster & Perry, 1954; Perry & Foster, 1954).

The above non-dialyzable peptides,
with a typical structure, are present not only in free spores, but also in the presporulation phase of vegetative cells. In the experiments described below the author attempted to detect whether preexisting DAP, a typical component of these peptides, could be used in formation of the spores and whether exogenous DAP could be utilized during sporogenesis, and to study the correlation of these processes in relation to the individual stages of spore formation.

MATERIALS AND METHODS

The model was *Bacillus cereus* (strain NCIB 8122), cultivated on liquid medium containing 0.3% Bacto-Peptone, 0.1% glucose, phosphates and trace elements. To obtain large volumes of culture or for dividing the culture, 1-litre flasks containing 300 ml. medium were cultivated on a shaker (240 3-cm. deflections/min.). In experiments in which the culture was left in contact with radioactive amino acids, it was incubated in 25 ml. nutrient medium on a shaker (120 4.5-cm. deflections/min.) A spore suspension was always used as the inoculum. The culture was incubated at 30°C. The method of successive contacts of the culture with radioactive amino acids was used in previous experiments (Vinter, 1960), the interval in all experiments being one hour. In experiments with radioactive amino acids, samples taken from the culture were precipitated with 5% trichloroacetic acid (TCA), extracted with fat solvents, washed with 5% TCA and heated at 90°C for 30 minutes, as in previous experiments (Vinter, 1959). The radioactivity of the washed and treated protein suspension was measured with a methane 2π flow counter and was expressed as the number of counts per minute. Radioactivity was related either to the dry weight of the cells in a given volume of culture or to the amount of nitrogen in the TCA precipitate. All measurements were done with a Friessecke-Hoepfner counter.

The following radioactive amino acids were used: 2-¹⁴C-α, α'-diaminopimelic acid (DAP) labelled with ¹⁴C at the C₂ site. This was prepared in the Isotope Laboratory of the Czechoslovak Academy of Sciences by condensation of γ-bromo-propylphthalimidonate with ethylacetic-aminomalonate-²-¹⁴C. L-lysine-¹⁴C (The Radiochemical Centre, Amersham, England) and ¹⁴C-aspartic acid (both uniformly labelled) were also used. The nitrogen in TCA precipitate was determined by the usual Kjeldahl technique. In all the experiments parallel samples of TCA precipitate were hydrolysed in 6 N-HCl for 20 hours at 100—105°C and after evacuating the HCl they were chromatographed on Whatman No. 1 paper in a butanol-acetic acid-water system (4 : 1 : 1). After measuring the radioactivity of the chromatograms they were autoradiographed on roentgen film (Agfa Laue). Exposure lasted 5—7 weeks. Dipicolinic acid was determined in the sporangia and spores by the method of Janssen, Lund and Anderson (1958). ⁴⁵CaCl₂ was used to determine the phase of maximum incorporation of calcium into the sporulating cells, by the method of one hour's contact with the culture. The ⁴⁵Ca concentration in the cells was determined by a thin-window tube counter after washing the cells in 0.001 N-HCl (Vinter, 1960, 1962). The percentage of prespores and spores in the culture was determined by direct microscopic observation and counting (Vinter, 1955).

RESULTS

Distribution of ¹⁴C-diaminopimelic acid in sporulating cells and free spores of *Bacillus cereus*

The culture was incubated from the time of inoculation in the presence of ¹⁴C-DAP (0.02 μc/ml., total DAP concentration 4 μg/ml.); ¹²C-Lysine was also added, in 1×10⁻³M concentration, to reduce decarboxylation of DAP and utilization of any ¹⁴C lysine formed.