Calcium Release and Germination of Bacterial Spores

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During germination and subsequent outgrowth of bacterial spores a number of well documented physical and biochemical changes occur (Gould & Dring, 1972). A wide variety of chemical and physical stimuli will initiate these changes, including potential metabolites such as amino acids, sugars and purine ribosides, non-nutrients such as EDTA and surfactants and physical treatments such as hydrostatic pressure and abrasion. The extreme diversity of these germination initiators renders it difficult to imagine how they might act through a common mechanism. We have been examining the possibility that many of these germinants might act by displacing calcium from spore structures. Dormant bacterial spores are characterized by an extremely high calcium content, probably in excess of 200 mM, which is thought to occur as a chelate with dipicolinic acid (pyridine-2,6-dicarboxylic acid). The bulk of this calcium is rapidly lost from the spore in the first minutes of germination.

Rode & Foster (1961) observed that several n-alkyl primary amines would germinate Bacillus spores. Further, with respect to a large number of the changes typically observed during germination, these non-nutrient germinants produced results virtually identical with those produced by nutrient initiators. Provided the amines were removed rapidly after germination had been initiated, the germinated spores remained viable and capable of outgrowth into normal vegetative cells. The potency of these alkylamines as germinants increased with carbon chain length above seven carbon atoms.

In an unrelated study of calcium displacement from phospholipid monolayers by pharmacologically active and other organic bases, Hauser & Dawson (1968) observed that the ability of a series of straight-chain aliphatic amines to displace calcium from phosphatidylinositol monolayers increased with the number of carbon atoms up to C12.

Since a large number of other nutrient and non-nutrient spore germinants clearly possess a potential protonated amino group or alternatively have the ability to chelate calcium, we decided to investigate the effect of alkyl amines of various chain lengths and other germinants on calcium release from spores and spore lipid monolayers.

Hauser & Dawson (1968) showed that when the logarithm of the concentration of amine required to displace 50% of calcium bound to the phospholipid monolayers was plotted against the number of carbon atoms in the amine, a constant slope was obtained. Using the decrease in absorbance of spore suspensions as an index of germination, we repeated this experiment, plotting the logarithm of amine concentration required to give a fixed rate of absorbance decrease against carbon chain length. This gave a line of constant slope from C3 to C13. In a second series of experiments we examined the ability of the amines to displace 50% of the total calcium from spores produced in medium containing 45Ca2+. Once again a linear relationship was found between the logarithm of amine concentration required to release 50% of spore calcium and carbon chain length from C3 to C13.

Spore lipids were then extracted and purified (Ellar & Posgate, 1974) and used to prepare 45Ca2+-containing monolayers. Measurements of the ability of the amines to displace calcium from these monolayers again revealed a linear relationship between release of 50% bound calcium and carbon chain length.

As noted by Hauser & Dawson (1968) such linear relationships are also often found in any homologous series between the logarithm of the distribution coefficients between two immiscible phases and the number of carbon atoms in the molecule. At the present time the distribution of the high concentrations of endogenous calcium within spore structures is not known. Calcium is required for maintenance of the integrity of spore membranes (Fitz-James, 1971) and the relatively high concentrations of cardiolipin in spore membranes (Ellar & Posgate, 1974) might result in a greater affinity for this cation.

Both our results and those of Hauser & Dawson (1968) demonstrating the ability of
alkyl amines to penetrate a monomolecular phospholipid layer and displace calcium and our observations on the effectiveness of these compounds in releasing calcium from intact spores together might suggest that calcium displacement from a lipid-rich spore structure is an early event in germination. A number of biological events are initiated by the binding or release of calcium to/from membranes (Williams, 1969). This need not imply that the whole of spore calcium is associated with spore membranes, but rather that a small proportion of spore calcium which is membrane-bound plays a crucial role in maintaining spore dormancy. For example, calcium binding could exert a significant effect on such membrane properties as degree of hydration, permeability etc. Nor does this suggestion necessarily imply that the whole spore calcium is responsible for the heat resistance of bacterial spores. An ability to displace this fraction of spore calcium from spore membranes might then represent a common factor linking a variety of known germinants. With this in mind, we are currently examining the ability of a wide range of nutrient and non-nutrient germinants to displace calcium from spore lipid monolayers and comparing this with their efficiency as germination initiators for intact spores. Preliminary results for a diverse range of compounds such as pyridoxine, dipicolinic acid and glutamic acid are encouraging. However, two of the most effective germinants, L-alanine and glucose do not appear to be efficient in displacing calcium from the monolayers when examined separately. Since in many cases optimal germination rates are obtained by a combination of two or more compounds, we are also examining the effect of such combinations on calcium displacement. Nevertheless, the possibility exists that a number of effective germinants exert their effect by initiating an endogenous mechanism for calcium displacement and that these compounds may be distinguished from others such as the alkyl amines, which are themselves efficient exogenous displacers of calcium.

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Possible Model for the Origin of Spore Protein in Bacterial Sporulation
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An examination of the biochemistry of bacterial sporulation reveals several problems which have remained unsolved because of the lack of methods for separating the developing forespore from the mother cell at all stages in its development (Kornberg et al., 1968). Only when the two cellular compartments are analysed separately is it possible to distinguish their contribution to the overall morphogenesis which is taking place. One outstanding question concerns the location of the extensive protein turnover which has been observed during sporulation (Mandelstam & Waites, 1968; Spudich & Kornberg, 1968b). Thus it is not known whether this turnover is confined to the mother cell, the forespore, or is a feature of metabolism in both cell compartments. In an attempt to answer this question, Spudich & Kornberg (1968a) undertook a careful analysis of proteins found in the mature spore. They considered two models to explain the origin...