SUGAR UPTAKE BY GERMINATING *BACILLUS SUBTILIS* SPORES

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Received 19 May 1971

1. Introduction

The phosphoenolpyruvate (PEP) phosphotransferase system first described by Kundig, Ghosh and Roseman [1], which has been shown to play an important role in the transport of sugars by a number of bacteria [2], has been detected in a number of species of *Bacillus* [3, 4]. This investigation was carried out to determine whether this system was present in dormant spores, or whether it was synthesized during or following the germination process, and to determine the temporal relationship between germination and the functioning of an active glucose transport system. It was found that the complete transport system was present in dormant spores of *Bacillus subtilis*, and that spores began actively to transport the non-metabolizable glucose analog 2-deoxy-D-glucose a short time after the commencement of germination.

2. Materials and methods

Dormant spores of *B. subtilis* ATCC 6633 were prepared as described previously [5].

Spores were activated by heating at 60° for 60 min, while suspended in 33 mM phosphate buffer (pH 7.2) containing 0.3 mM $MgCl_2$; germination was brought about by the addition of L-alanine (Sigma) at a final concentration of 5.6 mM. Germination was followed by measuring decrease in absorbance at 625 nm, as described by Vary and McCormick [6].

The ability of spores to take up glucose was followed during the germination process by measuring uptake of the non-metabolizable analog, 2-deoxy-D-[U–^{14}C] glucose (International Chemical and Nuclear Corp., Burbank, Calif.). The isotopic sugar was added to an activated spore suspension (0.0625 mg dry wt per ml) in 33 mM phosphate buffer (pH 7.2) containing 0.3 mM $MgCl_2$, at a final concentration of 0.5 mM labelled 2-deoxy-D-glucose (0.02 μCi per μmole). Samples of 1 ml were removed at appropriate time intervals, filtered through membrane filters (25 mm diameter, 0.45 μm porosity), and washed with cold buffer. Filte with spores thereon were transferred to vials containing Bray's scintillation fluid [7] and counted with a Packard Liquid Scintillation Spectrometer.

PEP phosphotransferase activity of spores was determined by a modification of the method of Ghosl and Ghosh [8] as follows: spores were washed with 0.15 M KCl, and resuspended in 0.1 M phosphate buffer, pH 7.0, at a density of 40 mg dry wt per ml. The suspension was frozen overnight at –20°, then thawed at room temperature, washed and resuspende in buffer as above at the same density. The reaction mixture contained: labelled 2-deoxy-D-glucose (0.05 M, 0.02 μCi/μmole) 0.1 ml; PEP (0.1 M), 0.44 ml; $MgCl_2$ (0.1 M), 0.09 ml; phosphate buffer (0.1 M, pH 7.0), 0.35 ml; frozen-thawed cell suspension, 1.0 ml; water to final volume 3.0 ml. After incubation at 37° for 1 hr, spores were removed from the reaction mixture by centrifugation, and the amount of phosphorylated 2-deoxy-D-glucose was determined in the supernatant by a modification of the chromatographi method of Winkler [9] described previously [4]. Results are expressed as the percentage of total coun eluted from the column which appeared in the 2-deoxy-D-glucose phosphate peak.

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3. Results and discussion

The relationship between the uptake of 2-deoxy-D-glucose and the germination of *B. subtilis* spores, in the absence and presence of actinomycin D, is shown in fig. 1. It is clear that rapid uptake of the isotopic sugar began a short time after the commencement of spore germination; a linear rate of sugar transport was achieved by the time 40% germination had taken place. At the point of 8% germination, the spores had taken up approximately 400 nmoles of the sugar per mg dry wt. Using the figure of 73% water in germinated spores, as reported by Black and Gerhardt [10], and assuming that all of the water is available to the sugar, and that the non-metabolizable analog is not incorporated into cellular material, this would represent 400 nmoles per 3.7 μl cell water, or an intracellular concentration of over 100 μmoles per ml, 200 times in excess of the concentration in the external medium.

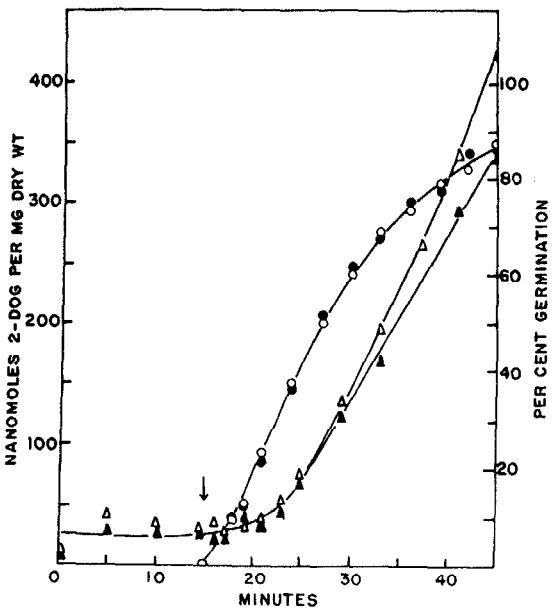

Fig. 1. Relationship between germination and 2-deoxy-D-[^{14}C]-glucose (2-DOG) uptake in *B. subtilis*. Spores suspended in buffer, activated, and germinated by the addition of L-alanine at the time indicated by the arrow, as described in Materials and methods. Germination (○) and 2-deoxy-D-glucose uptake (△) in the absence if actinomycin D; germination (●) and 2-deoxy-D-glucose uptake (▲) in the presence of actinomycin D (33 μg/ml).

Table 1
Phosphoenolpyruvate phosphotransferase activity[a] of *Bacillus subtilis* spores.

Preparation	Per cent 2-deoxy-D-[^{14}C]glucose phosphorylated
Dormant spores	36
Activated spores	40
Germinated spores	70
Spores germinated in presence of:	
Actinomycin D (30 μg/ml)	70
Chloramphenicol (100 μg/ml)	70

[a] Reaction mixture as described in Materials and methods.

Fig. 1 also shows that the onset of sugar transport activity during germination was not inhibited significantly by actinomycin D. It is known that the synthesis of macromolecules is not essential during germination, and that there is a complete absence of protein synthesis in spores germinated in the presence of actinomycin D [11]. Thus, it is indicated that the components of the transport system were not synthesized *de novo* during germination, but pre-existed in the dormant spore. This notion was confirmed by assays of the PEP phosphotransferase system in spores at various stages of development, as is shown in table 1. It is clear that the complete system was present in dormant and activated spores, as well as in germinated spores, and that there was no diminution in activity occasioned by germination in the presence of the RNA and protein synthesis inhibitors, actinomycin D and chloramphenicol, respectively. The reduced activity measured in ungerminated spores is in all likelihood due to the limited efficiency of the freezing and thawing technique in reducing crypticity, as compared with germinated spores and vegetative cells.

Roseman [2] has emphasized the importance of the PEP phosphotransferase system in the physiology of micro-organisms carrying out anaerobic glycolysis. The importance of this system in sugar transport by germinating spores is consistent with the findings of Goldman and Blumenthal [12] that germinating spores metabolize glucose primarily by the Embden-Meyerhof pathway, where PEP is a key intermediate, and

with the findings of Nelson and Kornberg [13, 14] that spores contain significant amounts of 3-phosphoglyceric acid, which is a direct precursor of PEP, and that phosphorylated sugars are accumulated by spores very early in the germination process.

Acknowledgement

This work was carried out during the tenure by G.F. of a World Health Organization Research Training Grant.

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