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GLUCOSE METABOLISM VIA THE EMBDEN-MEYERHOF PATHWAY IS NOT INVOLVED IN ATP PRODUCTION DURING SPORE GERMINATION OF BACILLUS MEGATERIUM QM B1551. A STUDY WITH A MUTANT LACKING HEXOKINASE

KEIJI SANO, MIEKO OTANI, and CHISAE UMEZAWA

Laboratory of Biochemistry, School of Pharmacy, Kobe-Gakuin University, Igawadani-cho, Nishi-ku, Kobe 673, Japan

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In order to investigate contributions by glucose metabolism via the Embden-Meyerhof pathway and that via the direct oxidation route to gluconate to initial ATP production during spore germination, respiratory activity and RNA synthesis were compared between the mutant lacking hexokinase and the parent spores of Bacillus megaterium QM B1551. We found that time courses of those metabolic events were almost identical between those spores, thus clearly indicating that NADH formed by a spore-specific enzyme glucose dehydrogenase (EC 1.1.1.47) is solely responsible for aerobic production of ATP at this stage.

ATP is almost depleted in the dormant spores (1, 2), therefore, it has been long assumed that, upon spore germination, glucose metabolism is exclusively initiated by phosphorylation with ATP that is derived from anaerobic breakdown of spore 3PGA pool (1). Once the first molecules of ATP are thus generated, the EM pathway becomes operative as the central route for glucose metabolism, whereby NADH, the most effective ATP source, would be subsequently provided, as proposed by Setlow and Kornberg (1). If the metabolic sequences described above were true, spore respiration and RNA synthesis, the latter depends on active ATP generation, should begin only after the onset of 3PGA breakdown, which is observed only 3 min after triggering germination (1, 3).

On the other hand, spores possess another unique route for glucose oxidation (3, 5) that is catalyzed by NAD(P)-dependent GDH (EC 1.1.1.47). This route is highly specific to spores and entirely independent from the

Abbreviations: GDH, glucose dehydrogenase (EC 1.1.1.47); 3PGA, 3-phosphoglycerate; EM pathway, Embden-Meyerhof pathway.

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phosphorylation by ATP. Occurrence of a functional respiratory system is well recognized in spores (6, 7, 8), therefore, it seems extremely probable that initial ATP in germinating spores can be efficiently generated via aerobic oxidation of NADH that is formed by GDH reaction (2, 3).

In order to examine possible contributions by those two pathways to aerobic ATP production during germination, in this study, we isolated mutant spores lacking hexokinase, and found that GDH is solely responsible for the aerobic ATP production.

MATERIALS AND METHODS

Isolation of a mutant: Bacillus megaterium QM B1551 was mutagenized, and colonies unable to grow on glucose but able to grow on gluconate were isolated by methods similar to those described previously for the isolation of other mutants (4).

Spore germination: Purified spores were prepared as described previously (3). Spores were activated by heating at 65 C for 15 min, then germinated at 37 C, in a minimal medium (9) containing glucose as a sole carbon source. Oxygen uptake was monitored using an oxygen electrode (Yellow Spring Instument, type 5331). Where indicated, 25 μM [2-^{14}C]uracil (5.9 Ci/mol) was further added (10), and at indicated times, 0.3 ml of the culture was transferred to a tube containing cold 7 % trichloracetic acid. The acid-insoluble materials thus formed were collected on glass fiber filters (GC 50, Toyo Roshi Co. Ltd., Japan) presoaked with the same acid containing unlabeled uracil. The filters were then washed 3 times with this solution and twice with ethanol. Radioactivities retained on the filters were counted to determine [^{14}C]uracil incorporated into RNA. Glucose, gluconate and enzyme activities were determined as described previously (3, 4).

RESULTS AND DISCUSSION

Characterization of the mutant: Since GDH is not present in growing cells, but is found only in spores (3), we attempted to isolate mutants lacking hexokinase by selecting colonies unable to grow on glucose as a sole carbon source. Enzyme activities (nmol/min per mg spores) of the mutant spores thus obtained were (activities of parent spores in parentheses): hexokinase (EC 2.7.1.1), <1.0 (31.0); GDH, 513 (820); glucose 6-phosphate dehydrogenase (EC 1.1.1.49), 26.3 (57); gluconokinase (EC 2.7.1.1.12), 46.2 (31.0); 6-phospho-gluconate dehydrogenase (EC 1.1.1.44), 66 (68). Those activities, together with response to germinants and 3PGA content, were like those of the parent spores (3, 11), except a defect of hexokinase in the mutant. To confirm that glucose can be metabolized solely by GDH in the mutant, the spores were

germinated in the presence of [1-^{14}C]glucose, and we found that 483 nmol glucose/mg spores was consumed during 30 min of germination. Among them, 387 nmol was found to be accumulated as gluconate and the remaining 75 nmol was specifically released as $^{14}CO_2$ by 6-phosphogluconate dehydrogenase. In the latter case, 6-phosphogluconate must be formed by gluconate phosphorylation by gluconokinase, as described previously (4). Thus, a sum of these two metabolites represents 96 % of glucose consumed in the mutant (for parent spores, see ref. 3), which clearly indicates that the other pathways, including the EM pathway, were not operative in the mutant spores.

Respiration: Notwithstanding a defect of the key enzyme needed for the metabolism via the EM pathway, Fig. 1 clearly shows that respiratory activity of the mutant spores was almost identical with that of the parent spores. Since respiration brought by endogenous nutrient sources was only marginal, it is almost certain that, in the mutant, the NADH formed by GDH was exclusively responsible for the marked enhancement of spore respiration observed during germination. Thus, our finding strongly indicates that glucose metabolism via the EM pathway, which represents ca. 20 % of the total glucose consumption in the parent (3), is not necessarily involved in NADH provision even in the parent spores. As described previously (3, 4), the Krebs cycle did not function at this stage. Fig. 1 also shows that time

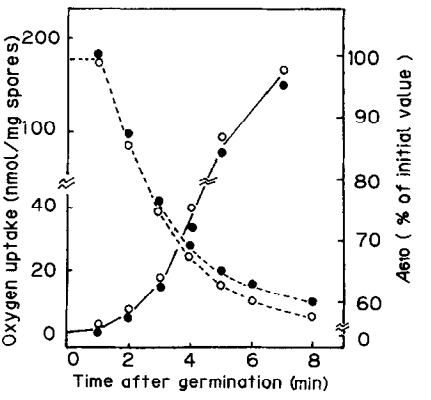

Fig. 1. Oxygen uptake during germination of the parent and the mutant spores lacking hexokinase. Heat-activated spores (2 mg/ml) of the parent (o) and the mutant (•) were germinated in a minimal medium containing 5 mM glucose as a sole carbon source. Germination was monitored by % reduction of initial O.D.. Solid lines, respiration; dotted lines, germination.

courses of germination were identical between the parent and the mutant.

RNA synthesis: Another strong evidence for our proposal was obtained by comparing RNA synthesis between those spores. As shown in Fig. 2, the timing and rate of [^{14}C]uracil incorporation into acid-insoluble materials were perfectly identical between the mutant and parent spores. This incorporation was totally abolished by omitting glucose, thus indicating that a minor amount of ATP derived from endogenous sources (e.g. 3PGA) did not substantially contribute to RNA synthesis. Rifampicin also abolished the synthesis. ATP forming ability estimated from the net amount of uracil incorporation seems to be significantly lower than that expected from the oxygen uptake shown in Fig. 1. This difference, however, is not surprising as explained below: (i) RNA synthesis requires much more amount of ATP than the actual amount of uracil incorporated (10), (ii) substantial amount of ATP must be utilized to activate other metabolic events already operative by this stage (2), and (iii) P/O ratio in spores might be considerably lower than that reported in animal mitochondria.

Our previous studies (2, 3) showed that GDH is certainly involved in aerobic ATP production during spore germination. However the EM pathway is also operative at this stage, therefore, its possible contribution to ATP production could not be precluded before. Our present study using the mutant

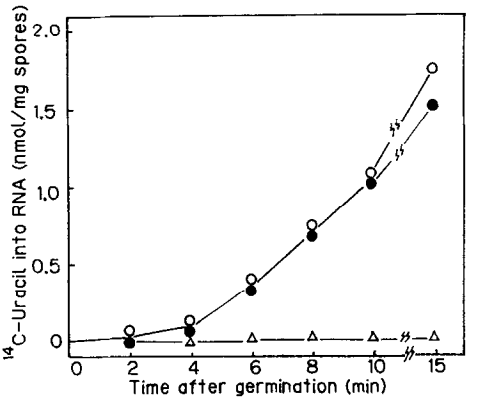

Fig. 2. RNA synthesis during germination of the parent and the mutant spores. Heat-activated spores were germinated in the medium containing 5 mM glucose and 25 μM [2-^{14}C]uracil as described in Materials and Methods. △, without glucose or with 10 μg/ml rifampicin. Other conditions and symbols are as for Fig. 1.

spores clearly indicates that glucose metabolism *via* the EM pathway is not essential to satisfy ATP demand at the early stage of spore germination. Our proposed pathway for glucose metabolism, that is entirely independent from phosphorylation by ATP, seems to be extremely preferable for the spores, since they contain virtually no ATP. However, it seems to be also probable that, after completion of germination, the EM pathway may play a central role in glucose metabolism, since the mutant could not grow on glucose; GDH is not found in vegetative cells (3).

Strauss (12) suggested a primary role of GDH in spore germination, based on a finding that mutant spores (*B. subtilis*) lacking GDH could not germinate well. In *B. megaterium*, however, spore germination, which is defined as a process of breakdown of dormancy, is triggered even in the absence of glucose. Therefore, we would rather propose that GDH may not play a key role in triggering germination, but is mainly responsible for the marked activation of spore metabolic events, by providing the most efficient source for ATP, *i.e.* NADH. In addition to this, NADH might be involved in cleavage of certain disulfide compounds that is observed during germination (13).

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