Fructose 2,6-bisphosphate and germination of fungal spores

(fungi/*Phycomyces*/dormancy)

ANDRÉ VAN LAERE*, EMILE VAN SCHAFTINGEN†, AND HENRI-GÉRY HERS†‡

*Laboratorium voor Plantenbiochemie, Katholieke Universiteit Leuven, Kardinaal Mercierlaan, 92 B-3030 Heverlee, Belgium; and †Laboratoire de Chimie Physiologique, Université de Louvain, and International Institute of Cellular and Molecular Pathology, UCL 75.39, 75 Avenue Hippocrate, B-1200 Brussels, Belgium

ABSTRACT   Induction of germination of *Phycomyces blakesleeanus* spores by a heat shock and subsequent incubation at 25°C in a glucose- or 6-deoxyglucose-containing culture medium resulted in an intense (20–40 times the initial value) rise in the concentration of fructose 2,6-bisphosphate and hexose 6-phosphates. The increase in the concentration of fructose 2,6-bisphosphate but not that of hexose 6-phosphates was restricted to a 25-min period during which the spores acquired an irreversible capacity to germinate. Incubation of the spores in water for any period of time during this critical period resulted in a parallel decrease in their ability to form hexose phosphates and to germinate. A similar rise in hexose phosphate concentration was also observed when *P. blakesleeanus* spores were activated by incubation in the presence of acetate and also after induction of germination of other dormant (*Neurospora tetrasperma*) or nondormant (*Mucor racemosus*) fungal spores. Extracts of dormant and heat-activated *P. blakesleeanus* spores contain a fructose 1,6-bisphosphatase that is inhibited by fructose 2,6-bisphosphate and AMP in a synergistic manner. They also contain a 6-phosphofructo-2-kinase and a fructose-2,6-bisphosphatase.

Dormancy is an arrest in development of fungal or bacterial spores or of seed embryos under conditions otherwise suited for growth. The breaking of dormancy and the subsequent germination of spores or seeds constitute a model system in the study of cell development and differentiation and, for this reason, have attracted much attention. Germination can be initiated by a variety of physical or chemical means. Heating of *Phycomyces blakesleeanus* spores in water for 5 min at 50°C (these conditions may vary slightly from one batch of spores to another) induces in about 90% of the spores an irreversible capacity to germinate upon incubation in an appropriate culture medium at 25°C. If the heat shock is performed at a slightly lower temperature or for a shorter duration, the percentage of germination is reduced; furthermore, the spores can be deactivated within 10–30 min upon incubation in water although not if they are immediately transferred in the culture medium. The presence of glucose in the culture medium seems to be the main factor that prevents this deactivation (1). Spores of *P. blakesleeanus* can also be activated by a variety of other means, including incubation in the presence of acetate or propionate (2) or pretreatment with sodium pyrosulfite (3). Ascospores of *Neurospora tetrasperma* are heat-activated with no subsequent effect of glucose (4). Spores of *Mucor racemosus* are not dormant and germinate when incubated at 20°C in a culture medium containing glucose or a glucose analog (5).

The role of glucose in the germination of *P. blakesleeanus* spores has drawn our attention to the potential participation of fructose 2,6-bisphosphate in this process. Fructose 2,6-bis-

phosphate is a stimulator of phosphofructo-1-kinase (PFK-1), initially discovered in the liver (6, 7) but also present in numerous eukaryotic organisms, including fungi and higher plants (reviewed in refs. 8 and 9). PFK-1 from yeast (10, 11) and from *P. blakesleeanus* spores (12) is highly sensitive to stimulation by fructose 2,6-bisphosphate. Fructose 2,6-bisphosphate is also a potent inhibitor of fructose-1,6-bisphosphatase from various sources, including yeast (13). However, the presence of fructose-1,6-bisphosphatase in *P. blakesleeanus* has not been reported previously. In the liver, fructose 2,6-bisphosphate is formed from fructose 6-phosphate and ATP by a 6-phosphofructo-2-kinase (PFK-2) and converted to fructose 6-phosphate and P_i by a fructose-2,6-bisphosphatase. The activity of these two enzymes is modified by phosphorylation under the action of cyclic AMP-dependent protein kinase (8). In the liver and in yeast, fructose 2,6-bisphosphate is rapidly synthesized when glucose is abundant and its most obvious function is to stimulate glycolysis. However, it is suspected that fructose 2,6-bisphosphate may play other biological roles, particularly in the expression of several "glucose effects" (8, 9), among which the germination of fungal spores is an interesting example.

The present paper describes a very large and transient increase in fructose 2,6-bisphosphate concentration that characterizes the first irreversible step of germination of fungal spores. Large changes in the concentration of other hexose phosphates were also observed.

MATERIALS AND METHODS

Materials. 6-Deoxyglucose was from Koch–Light Laboratories (Colnbrook, England); fructose 2,6-bisphosphate (14) and [2-^{32}P]fructose 2,6-bisphosphate (15) were prepared as described earlier. Beef heart cyclic AMP-dependent protein kinase was from Sigma. Auxiliary enzymes and biochemicals were from Boehringer Mannheim. All other chemicals were from Merck (Darmstadt, F.R.G.) or Serva (Heidelberg).

Activation and Incubation of Spores. Sporangiospores of *P. blakesleeanus* (strain K1$^+$) were heat-activated by shaking in a water bath for the time and at the temperature indicated. They were then incubated at 25°C under continuous shaking in either water, 83 mM glucose, 83 mM 6-deoxyglucose, or standard culture medium containing 83 mM glucose, 10 mM KH_2PO_4, 6.7 mM asparagine, 1 mM $MgSO_4$, 0.5 mM $Ca(NO_3)_2$, 0.1 mM $FeCl_3$, and 3 μM thiamine. Alternatively, spores were activated by incubation in the presence of 0.1 M ammonium acetate. Mucor spores (*M. racemosus*, CBS 277.49) were incubated in a culture medium containing 2% glucose, 1% peptone, and 0.3% yeast extract. Ascospores of *N. tetrasperma* (CBS 377.74)

Abbreviations: PFK-1, phosphofructo-1-kinase; PFK-2, 6-phosphofructo-2-kinase.
‡ To whom reprint requests should be addressed.

were heat-activated and incubated in water, because germination of these spores occurs without addition of any chemicals.

The capacity to germinate was measured by the percentage of spores germinated after 8 hr of incubation at 25°C in the culture medium. Swollen and vacuolated spores and spores with a germ tube were counted as germinated.

Extraction of Spores. Two milliliters of a spore suspension containing the equivalent of 2 mg (for the determination of fructose 2,6-bisphosphate) or 10 mg (when also hexose 6-phosphates were assayed) of dry spores was poured in 10 ml of ice-cold water. After a 30-sec centrifugation, the pellet of spores was resuspended in 1 ml of ice-cold 0.1 M KOH and homogenized as described (16). The extracts were frozen in liquid N_2 and stored at $-15°C$. For the measurement of enzyme activities, the same procedure was used, except that the equivalent of 100 mg of dry spores was resuspended in 1.5 ml of a solution containing 25 mM Hepes, 50 mM KCl, 1 mM dithiothreitol, and 0.5 mM phenylmethylsulfonyl fluoride (pH 7). Insoluble material was eliminated by a 10-min centrifugation at $10,000 \times g$.

Assay Methods. Fructose 2,6-bisphosphate was determined in alkaline extracts according to ref. 17; glucose 6-phosphate, fructose 6-phosphate (18), and fructose 1,6-bisphosphate (19) were assayed in perchloric acid extracts prepared by mixing 1 vol of alkaline extract with 0.5 vol of 10% perchloric acid. Under all conditions tested, the concentration of fructose 6-phosphate amounted to about one-fourth of that of glucose 6-phosphate. PFK-2 was assayed by the production of fructose 2,6-bisphosphate in a mixture containing 50 mM Hepes (pH 7.1), 100 mM KCl, as well as phosphate, ATP, and fructose 6-phosphate at the indicated concentrations and $MgCl_2$ in a 2 mM excess over ATP. Glucose 6-phosphate was also present at a concentration 3.5 times that of fructose 6-phosphate. Fructose-2,6-bisphosphatase was assayed by the production of $[^{32}P]P_i$ from $[2\text{-}^{32}P]$fructose 2,6-bisphosphate (15) in a mixture containing 50 mM Hepes (pH 7.1), 100 mM KCl, and 2 mM $MgCl_2$. Fructose-1,6-bisphosphatase was assayed spectrophotometrically in the presence of 100 mM KCl, 50 mM Hepes (pH 7.1), 2 mM magnesium acetate, 1 mM NH_4Cl, 0.5 mM EDTA, 0.3 mM NADP, and 5 μg of glucose-6-phosphate dehydrogenase per ml, 10 μg of phosphoglucose isomerase per ml, and various concentrations of substrate and inhibitors. All enzymic assays were performed at 25°C. One unit of enzyme is the amount that catalyzes the conversion of 1 μmol of substrate per min under the conditions described above.

RESULTS

The Concentration of Fructose 2,6-Bisphosphate and of Hexose 6-Phosphates in Germinating Spores in Relation to Their Capacity to Germinate. The content of dormant *P. blakesleeanus* spores in fructose 2,6-bisphosphate is close to 5 nmol/g of dry weight. This concentration was not increased when dormant spores were incubated in the presence of glucose, despite the fact that they can metabolize this sugar (16); it was not significantly modified during the heat treatment (Fig. 1), even when heating was prolonged to 30 min or was performed in the presence of 83 mM glucose, the culture medium, or 0.1 M acetate (not shown). In contrast, the concentration of fructose 2,6-bisphosphate increased during the subsequent incubation at 25°C in the culture medium, reaching 20–40 times its initial value after 10 min; it then decreased equally rapidly, to reach almost its basal value after 30–60 min (Fig. 1A). The concentration of hexose 6-phosphates was also greatly increased subsequent to the heat treatment, and this increase as well as the subsequent decrease preceded slightly those in fructose 2,6-bisphosphate.
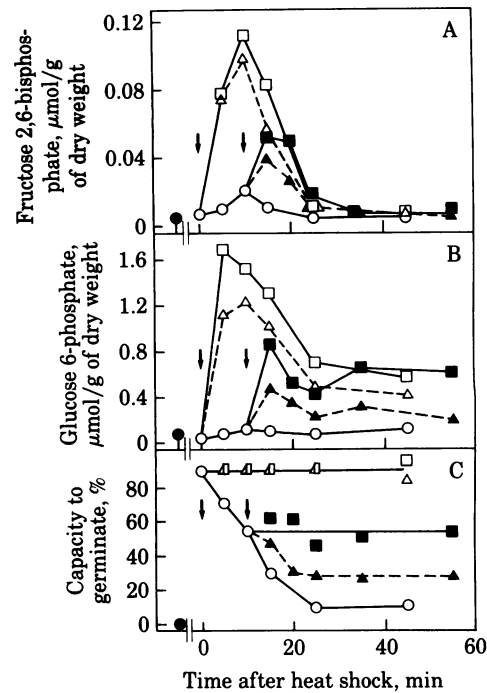

FIG. 1. Fructose 2,6-bisphosphate (A) and glucose 6-phosphate (B) content and capacity to germinate (C) of *P. blakesleeanus* spores activated by heat. Spores heated for 5 min at 45°C were incubated at 25°C in the culture medium ($\triangle$, $\blacktriangle$), 83 mM glucose ($\square$, $\blacksquare$), or water ($\bigcirc$). Arrows indicate times (0 min, open symbols; 10 min, closed symbols) at which spores previously incubated in water were centrifuged and resuspended in the culture medium or the glucose solution. $\bullet$, Dormant spores.

However, their disappearance was far less complete because the concentration of glucose 6-phosphate was still about 10 times its initial value when that of fructose 2,6-bisphosphate had returned to nearly the basal level (Fig. 1B).

The magnitude of these effects and also the percentage of activation of the spores were greatly dependent upon the composition of the 25°C incubation medium and on the time at which the spores were exposed to glucose or to the culture medium. In the experiment shown in Fig. 1, part of the spores was also incubated at 25°C in the presence of either 83 mM glucose or 6-deoxyglucose (not shown) rather than in the culture medium, with similar results. Under these various conditions, the spores acquired progressively an apparently irreversible capacity to germinate because, if after 15 min (at the peak of hexose phosphate concentration) they were transferred to water, they remained fully activated for at least 1 hr. Transfer to water at an earlier time caused partial deactivation (not shown). If the spores were incubated in water from the beginning, only small amounts of hexose phosphates (Fig. 1 A and B) were formed and 90–95% of the spores rapidly lost their capacity to germinate (Fig. 1C). If, after 10 min of deactivation in water, the spores were transferred either to the culture medium or to a glucose or 6-deoxyglucose solution (not shown), the concentration of hexose phosphates rose markedly, but only to reach, and then follow in its decline, the already declining level observed in spores not subjected to partial deactivation (Fig. 1). The rise in hexose phosphates in the presence of the glucose medium was then about half that in the culture medium. As shown in Fig. 1C, the transfer to the culture medium stabilized, as expected, the germination capacity of the spores at the level reached at the time of the transfer, whereas the transfer to the glucose medium was less efficient in this respect. Transfer of the spores from water to the culture medium at a time later than 10 min
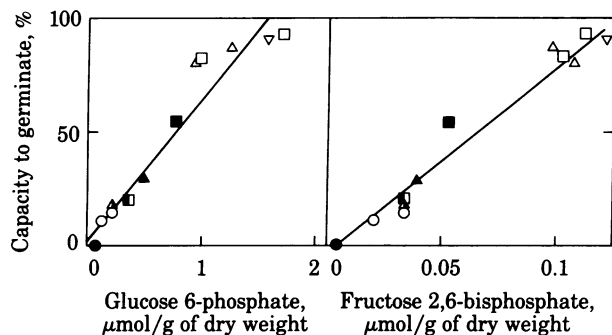
FIG. 2. Correlation between the maximal concentration of glucose 6-phosphate or fructose 2,6-bisphosphate in spores of *P. blakesleeanus* reached after the heat shock and their capacity to germinate. Data are taken from Fig. 1 and another similar experiment. Same symbols as in Fig. 1, except 83 mM 6-deoxyglucose (▽) added at 0 min; glucose (▲) or culture medium (■) added at 20 min. The correlation coefficients were 0.960 and 0.985, respectively.

was characterized by a progressive reduction in the formation of hexose phosphates and in the percentage of germination, with a complete disappearance of these effects at 30 min (not shown). As shown in Fig. 2, the capacity of the spores to germinate under these various conditions correlated with the maximal concentration of fructose 2,6-bisphosphate and of glucose 6-phosphate in the spores.

When the heat shock was performed at different temperatures, it appeared that fructose 2,6-bisphosphate was formed in the presence of culture medium only after a heat shock that induced spore germination (Fig. 3). After heating at 46°C, some fructose 2,6-bisphosphate was also formed upon incubation of the spores in water and the rate of deactivation was then markedly slower than after heating at lower temperature (see also Fig. 1). Under these conditions, about 5% of the spores had acquired an irreversible capacity to germinate (not shown). As a rule, when the temperature was increased, one observed a greater and more prolonged formation of fructose 2,6-bisphosphate even in water, a higher percentage of germination, and a slower rate of deactivation in water.
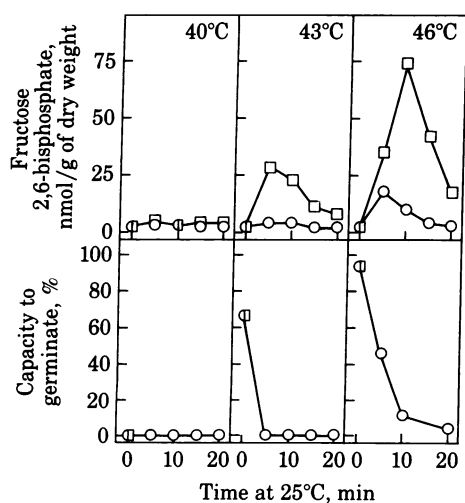

FIG. 3. Effect of the temperature at which the heat shock was performed on the fructose 2,6-bisphosphate content of *P. blakesleeanus* spores and on their capacity to germinate. Spores were heated for 5 min at the indicated temperature and subsequently incubated at 25°C in water (○) or culture medium (□). Note that, by definition, the capacity to germinate of spores incubated for zero time in water is the same as that of spores incubated for any period of time in the culture medium.
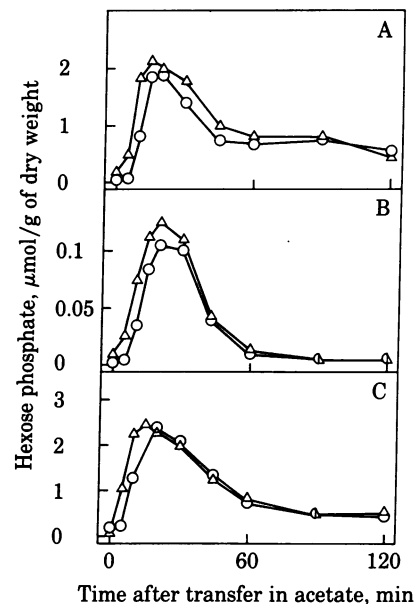

FIG. 4. Concentration of glucose 6-phosphate (*A*), fructose 2,6-bisphosphate (*B*), and fructose 1,6-bisphosphate (*C*) in *P. blakesleeanus* spores incubated in the presence of acetate. Spores were incubated with 0.1 M acetate in the presence (△) or absence (○) of 1.5% glucose. Both treatments caused 95% germination of the spores upon subsequent incubation in the culture medium.

A transient formation of fructose 2,6-bisphosphate and of hexose 6-phosphates was also observed when the spores were activated by incubation in the presence of acetate, with the major difference being that the time scale was about doubled (Fig. 4). Glucose was not required but had, nevertheless, a positive effect—namely, to cancel the short lag that precedes the formation of hexose phosphates in the absence of glucose. In this experiment, fructose 1,6-bisphosphate was also measured; its formation, like that of glucose 6-phosphate, slightly preceded that of fructose 2,6-bisphosphate. A glucose- or 6-deoxyglucose-dependent formation of fructose 2,6-bisphosphate was also observed when the spores were activated by pretreatment in sodium pyrosulfite (not shown).

Finally, we have found that the formation of fructose 2,6-bisphosphate in germinating fungal spores was not limited to *P. blakesleeanus* but could also be observed with other dormant or nondormant spores. We show in Fig. 5 the transient increase in fructose 2,6-bisphosphate that occurred in *N. tetrasperma* ascospores previously heated for 5 min at 62°C. Glucose was not required for the subsequent germination of these spores or for the formation of fructose 2,6-bisphosphate. Similar results were
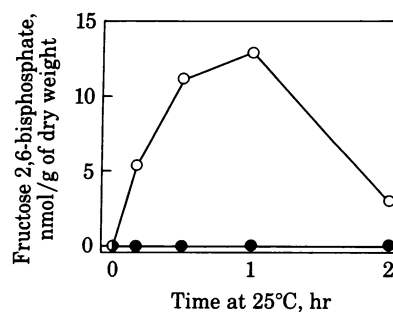

FIG. 5. Concentration of fructose 2,6-bisphosphate in spores of *N. tetrasperma* after a heat treatment. Spores were heated (activated) (○) or not heated (control) (●) for 3 min at 62°C in water and then were incubated at 25°C in water.
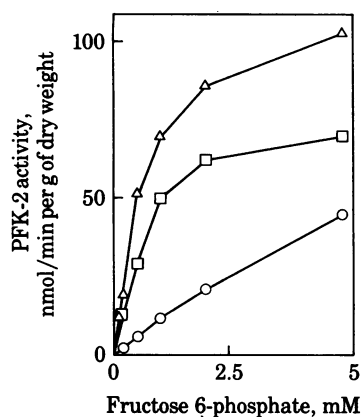
FIG. 6. Saturation of PFK-2 by fructose 6-phosphate. The enzyme activity was measured in an extract of dormant spores that had been filtered through 20 vol of Sephadex G-25. The concentration of ATP was 2 mM; the P_i concentrations were 0 (o), 1 (□), and 5 (△) mM.

obtained with the spores of *M. racemosus* when incubated at 25°C in a glucose-containing culture medium, without preliminary heat activation (not shown).

Biosynthesis and Biodegradation of Fructose 2,6-Bisphosphate in a Cell-Free Extract. As illustrated by Fig. 6, a cell-free extract of *P. blakesleeanus* spores catalyzed the formation of fructose 2,6-bisphosphate when incubated in the presence of ATP,Mg and fructose 6-phosphate, indicating the presence of a PFK-2. As described for the rat liver enzyme (20), the fungal PFK-2 was greatly stimulated by P_i. Its K_m for fructose 6-phosphate was then ≈ 1 mM. The K_m for ATP,Mg was close to 1 mM (not shown). We have calculated that 1 g of dry spores contains enough enzyme to form 100 nmol of fructose 2,6-bisphosphate per min at 25°C at substrate saturation and about 10 nmol at 0.1 mM fructose 6-phosphate (actual concentration in the spores 5 min after heat activation), accounting, therefore, for the rise in fructose 2,6-bisphosphate concentration shown in Fig. 1. PFK-2 activity was the same in dormant and heat-activated spores and also at the time of rapid disappearance of fructose 2,6-bisphosphate. The PFK-2 activity was not modified upon prolonged incubation of the cell-free extract in the presence of NaF, ATP,Mg, and cyclic AMP with or without beef heart cyclic AMP-dependent protein kinase.

When incubated in the presence of 10 μM [2-^{32}P]fructose 2,6-bisphosphate, a cell-free extract of heat-activated *P. blakesleeanus* spores liberated ≈ 10 nmol of [^{32}P]P_i per min/g of dry weight and a half-maximal rate of this reaction was observed at 1 μM fructose 2,6-bisphosphate. This reaction was 70–80% inhibited in the presence of 5 mM P_i (results not shown). The rate of reaction was not modified by an incubation of the spores at 25°C for as long as 40 min.

Inhibition of *P. blakesleeanus* Fructose-1,6-Bisphosphatase by Fructose 2,6-Bisphosphate. We have observed that a crude extract of *P. blakesleeanus* catalyzes the Mg-dependent hydrolysis of fructose 1,6-bisphosphate and that this reaction is inhibited by fructose 2,6-bisphosphate and also by AMP. Dormant and activated spores contained ≈ 4 units of fructose-1,6-bisphosphatase per g of dry weight. In the presence of 100 μM fructose 1,6-bisphosphate, a half-maximal inhibition was obtained with 0.2 μM fructose 2,6-bisphosphate or 0.2 mM AMP and the action of these two inhibitors was synergistic (not shown).

DISCUSSION

The dramatic but transient burst in fructose 2,6-bisphosphate concentration that follows the breaking of dormancy in fungal spores and accompanies an increase in the concentration of other

hexose phosphates is an unexpected observation that may open new perspectives in the biochemistry of cell differentiation. It raises numerous questions that are discussed below.

Role of Glucose. Although this investigation was started because of the reported role of glucose in the activation of fungal spores, several facts indicate that exogenous glucose is not required for the irreversible activation of the spores as well as for the formation of fructose 2,6-bisphosphate and of other hexose phosphates. First, when heating of *P. blakesleeanus* spores was sufficient or when acetate was used as an activation agent, the addition of glucose was not required. It was also not required for the germination of the ascospores of *N. tetrasperma*. Second, even after suboptimal heating of *P. blakesleeanus* spores, glucose can be replaced by its nonmetabolizable analog 6-deoxyglucose, indicating that its ability to stimulate germination is unrelated to its further metabolism and that exogenous glucose is not itself the precursor of either glucose 6-phosphate or fructose 2,6-bisphosphate. These phosphoric esters are presumably formed from endogenous glucose originating from the hydrolysis of trehalose, at least during the first 30 min after the heat shock. Activation of trehalase is, indeed, an early although transient event in *P. blakesleeanus* spore germination (21).

Mechanism of Fructose 2,6-Bisphosphate Formation. Assuming that the biosynthesis of fructose 2,6-bisphosphate occurs in fungi as in mammalian liver by transphosphorylation from ATP on fructose 6-phosphate, it requires the presence of an active form of PFK-2 and of its two substrates, fructose 6-phosphate and ATP. We found that PFK-2 was as active in extracts of dormant spores as in extracts of activated spores and that this enzyme remained active even when the concentration of fructose 2,6-bisphosphate went back to a low value. Furthermore, we have obtained no indication that fungal PFK-2 could be, as the mammalian liver enzyme, an interconvertible enzyme, the activity of which is modulated by phosphorylation and dephosphorylation. Fructose 6-phosphate was barely detectable in dormant spores and during the heating step, but its concentration increased rapidly immediately after the heat shock. It reached a maximal value slightly before that of fructose 2,6-bisphosphate but later on decreased to ≈ 30–40% of the maximal value, maintaining then a value quite sufficient to insure an important activity of PFK-2. Similar variations in fructose 6-phosphate concentration were previously observed after heat activation of *Neurospora* ascospores (22). ATP concentration was not measured but is known not to be different in dormant and activated spores (1). Therefore, it appears that the increase in fructose 2,6-bisphosphate concentration could be simply a consequence of the rise in fructose 6-phosphate concentration, but it is not clear why the biosynthesis of fructose 2,6-bisphosphate is apparently restricted to the first 15 min of germination.

Mechanism of Fructose 2,6-Bisphosphate Degradation. In the liver, fructose 2,6-bisphosphate is converted back to fructose 6-phosphate by hydrolysis under the action of a specific fructose-2,6-bisphosphatase, the activity of which is increased by phosphorylation by cyclic AMP-dependent protein kinase (8, 15). The very low K_m of the phosphatase, which in an extract of *P. blakesleeanus* catalyzes the same reaction, is in favor of the presence of a similar specific enzyme in fungi. However, this activity was not modified in relation to the rate of synthesis and degradation of fructose 2,6-bisphosphate in the activated spores. Furthermore, if one takes into account the inhibitory action of physiological concentrations of P_i, the recorded activity was too low to account for the extremely rapid rate of breakdown observed between 15 and 30 min. This indicates either that the enzymic activity was measured under inappropriate conditions in the cell-free extracts or that another mechanism is responsible for the consumption of fructose 2,6-bisphosphate. It has

Developmental Biology: Van Laere *et al.*

Proc. Natl. Acad. Sci. USA 80 (1983) 6605

been proposed elsewhere (9) that fructose 2,6-bisphosphate could act as a donor of a 6-phosphofructosyl group in various biosynthetic processes. According to this view, the possibility exists that fructose 2,6-bisphosphate continued to be formed after 15 min but that its concentration decreased because it was rapidly used for biosynthetic purposes.

Role of Fructose 2,6-Bisphosphate and Other Hexose Phosphates in the Breaking of Dormancy and Subsequent Germination. It must first be emphasized that hexose phosphates are not formed during the heat step that causes the activation of *P. blakesleeanus* spores and therefore are not involved in the breaking of dormancy itself. However, a rapid synthesis of fructose 2,6-bisphosphate, as well as of other hexose phosphates, is an early biochemical event in the germination of dormant and nondormant fungal spores. Contrary to the other hexose phosphates, fructose 2,6-bisphosphate is not a glycolytic intermediate but a metabolic signal, formed and degraded by specific mechanisms; its best established function is to control glycolysis and gluconeogenesis at the level of PFK-1 and of fructose 1,6-bisphosphatase, causing a shift in the fructose 1,6-bisphosphate/fructose 6-phosphate ratio (6). It has also been established that 1 μM fructose 2,6-bisphosphate fully activates PFK-1 from *P. blakesleeanus* spores (12) and we have shown that it would also greatly inhibit fructose-1,6-bisphosphatase from the same origin. Therefore, one could postulate that the burst of fructose 2,6-bisphosphate that follows the breaking of dormancy is a way of stimulating glycolysis, which is known to operate at a high rate during early germination (23), at the expense of glucose formed from trehalose.

Several facts argue against this simple hypothesis: first, unless a great part of it were bound to proteins, the concentration of fructose 2,6-bisphosphate in dormant spores was saturating with regard to PFK-1, and therefore the up to 40-fold increase in its concentration, which occurred later on, was disproportionate to the requirement of the enzyme. Furthermore, the high concentration of fructose 1,6-bisphosphate (in the millimolar range versus micromolar in mammalian liver) indicates that PFK-1 became rapidly non-rate-limiting. Second, the concentrations of hexose 6-phosphates and of fructose 1,6-bisphosphate increased before that of fructose 2,6-bisphosphate and remained elevated after that of the latter had returned to its basal level. Therefore, one reaches the conclusion that the activation of glycolysis probably results from the availability of glucose formed by hydrolysis of trehalose more than from the formation of fructose 2,6-bisphosphate.

The most remarkable aspect of the synthesis and breakdown of fructose 2,6-bisphosphate (and, less strikingly, of other hexose phosphates) in *P. blakesleeanus* spores is their time course. Indeed, these changes occur only during a critical period that directly follows the heat shock and then does not exceed 30 min. Whereas all spores have initially the capacity to germinate upon transfer into the culture medium, it appears that those that were incubated in water lost in a parallel manner the capacity to form hexose phosphates and to germinate (see Fig. 2). By contrast, the spores that were incubated for 15 min in the presence of glucose or 6-deoxyglucose and had formed a maximal amount of hexose phosphates had acquired an irreversible capacity to germinate. It is apparent from Fig. 1 that 15–20 min after the heat shock, fructose 2,6-bisphosphate can only be degraded, no matter the time at which its synthesis was initiated, indicating that its role is terminated. This allows the general conclusion that a series of irreversible events related to the capacity to germinate occur during this critical period. The role of fructose 2,6-bisphosphate and of glycolytic intermediates in these events remains to be clarified.

1. Van Laere, A. J., Van Assche, J. A. & Carlier, A. R. (1980) *Exp. Mycol.* **4**, 260–268.
2. Borchert, R. (1962) *Beitr. Biol. Pflanz.* **38**, 31–61.
3. Van Assche, J. A., Van Laere, A. J. & Carlier, A. R. (1978) *Planta* **139**, 171–176.
4. Sussman, A. S. (1954) *J. Gen. Physiol.* **38**, 59–77.
5. Tripp, M. L. & Paznokas, J. L. (1982) *J. Gen. Microbiol.* **128**, 477–483.
6. Van Schaftingen, E., Hue, L. & Hers, H. G. (1980) *Biochem. J.* **192**, 887–895.
7. Van Schaftingen, E., Hue, L. & Hers, H. G. (1980) *Biochem. J.* **192**, 897–901.
8. Hers, H. G. & Van Schaftingen, E. (1982) *Biochem. J.* **206**, 1–12.
9. Hers, H. G., Hue, L. & Van Schaftingen, E. (1982) *Trends Biochem. Sci.* **7**, 329–331.
10. Bartrons, R., Van Schaftingen, E., Vissers, S. & Hers, H. G. (1982) *FEBS Lett.* **143**, 137–140.
11. Nissler, K., Otto, A., Schellenberger, W. & Hofmann, E. (1983) *Biochem. Biophys. Res. Commun.* **111**, 294–300.
12. Van Laere, A. J. (1983) *J. Gen. Microbiol.* **129**, in press.
13. Van Schaftingen, E. & Hers, H. G. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 2861–2863.
14. Van Schaftingen, E. & Hers, H. G. (1981) *Eur. J. Biochem.* **117**, 319–323.
15. Van Schaftingen, E., Davies, D. R. & Hers, H. G. (1982) *Eur. J. Biochem.* **124**, 143–149.
16. Van Laere, A. J. & Carlier, A. R. (1975) *Planta* **125**, 217–225.
17. Van Schaftingen, E., Lederer, B., Bartrons, R. & Hers, H. G. (1982) *Eur. J. Biochem.* **129**, 191–195.
18. Hohorst, H. J. (1963) in *Methods of Enzymatic Analysis*, ed. Bergmeyer, H. U. (Academic, New York), pp. 134–138.
19. Czok, R. & Lamprecht, W. (1974) in *Methods of Enzymatic Analysis*, ed. Bergmeyer, H. U. (Academic, New York), pp. 1446–1451.
20. Van Schaftingen, E. & Hers, H. G. (1981) *Biochem. Biophys. Res. Commun.* **101**, 1078–1084.
21. Van Assche, A. J., Carlier, A. R. & De Keersmaker, H. I. (1972) *Planta* **103**, 327–333.
22. Eilers, F. I., Ikuma, H. & Sussman, A. (1970) *Can. J. Microbiol.* **16**, 1351–1352.
23. Furch, B., Poltz, J. & Rudolph, H. (1976) *Biochem. Physiol. Pflanz.* **169**, 249–256.