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DIFFERENTIAL SCANNING CALORIMETRIC OBSERVATIONS CONCERNING THE ACTIVATION MECHANISM OF SPORES OF PHYCOMYCES BLAKESLEEANUS

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Summary

Spores of Phycomyces were scanned in a Differential Scanning Calorimeter. The spectrum obtained was clearly influenced by previous activation of spores by heat or by acetate.
When spores were allowed to return to dormancy the original spectrum of dormant spores was restored. The high temperature at which the difference in the spectrum between activated and dormant spores was found points to a protein denaturation. It is suggested therefore that the activation of spores is obtained through a conformational change of a protein.

Introduction

Dormant sporangiospores of Phycomyces blakesleeanus can be activated by heat shock (1), by treatment with some chemicals e.g. acetate (2), or by gamma irradiation (3). Rewetting of spores seems to be essential for the activating mechanism while activating treatments like heat (1) or gamma irradiation (3) are almost ineffective with dry spores.
The relationship between the different activating treatments, and the mechanism which yields the onset of germination are still unclear. The fact that simple physical treatments like heat shock can activate spores suggests that some transitions in the spores could be involved in activation. Membranes e.g. are known to display characteristic transitions (4). Further, spores of Phycomyces contain oil globules which "disappear" after activation (5), a process which could involve some lipid "melting". Thermal analysis by differential scanning calorimetry has been proven very useful in tracing transition phenomena (4) and therefore experiments

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were performed, in which the influence of wetting and activating treatment on the D.S.C.-curves are studied.

Methods

Phycomyces blakesleeanus (strain 1+) was grown and the spores were harvested as described by Van Assche et.al. (1972) and the initial culture was kindly provided by this author.
Differential scanning calorimetric measurements were performed using a Perkin Elmer DSC apparatus, cooled by a Lauda Ultra-Kryostat UK 60 SW. Large Volume Capsules (LVC, 75 µl) were used as sample containers; they suppress solvent vaporization thereby eliminating interfering effects of the vaporization heat. The LVC were filled with ± 35 mg of dry spores. The spores then were wetted with amounts of water as indicated in the figure legends. Hermetically closed capsules were transferred to the sample holder and scanned. An appropriate heating rate was 2.5°/min. All scans were made at range 2 and at paper speed 160 mm/h.
In all figures the distance between two scale divisions on the ordinate corresponds to 1 milliwatt.

Results and discussion

From Figure 1 it can be seen that the spectrum obtained in the differential scanning calorimeter (DSC) depends on the hydratation of the spores. Adding increasing amounts of water to the spores, results in an increasingly more detailed scan. The major endotherm peak with its maximum near 67 °C probably represents protein denaturation, as generally found with living cells.

The irreversibility of that endotherm process, demonstrated in Figure 2, confirms that conclusion. Spores scanned in DSC to 67 °C indeed are "denaturated", and no longer germinate when they are brought into culture medium. More surprising however is, that with spores heated in DSC to 50 °C, also only about 14% germination is obtained when they are sown on culture medium. Such spores however can germinate after an additional heat-shock, which means that in DSC-pans activation did not succeed. This obviously is due to the poor hydration, which is only about 50% in DSC capsules (35 mg spores with 30 µl water).

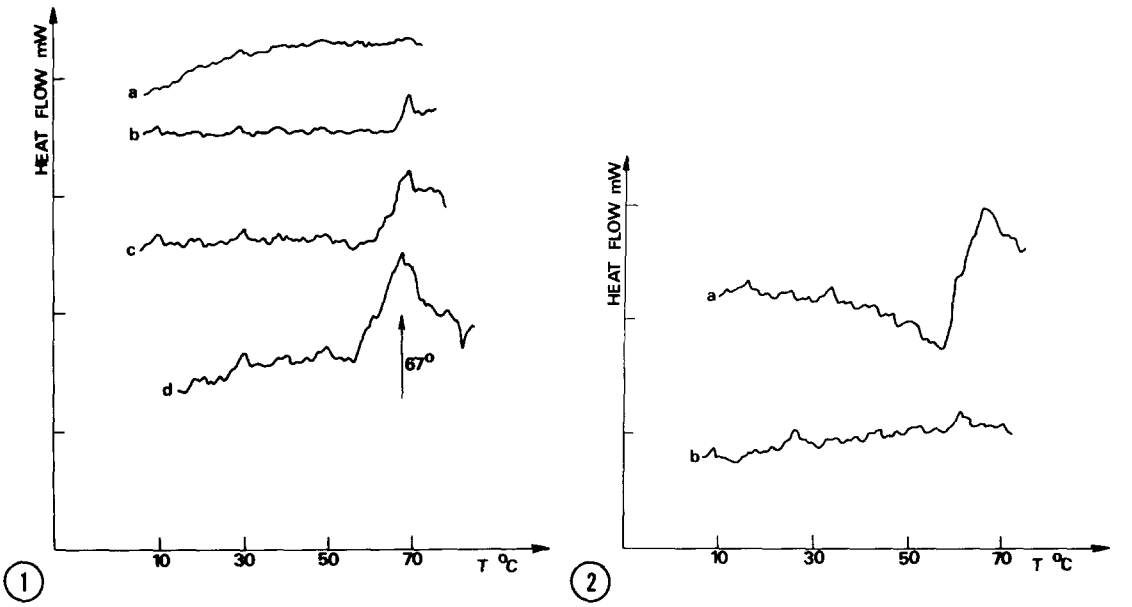
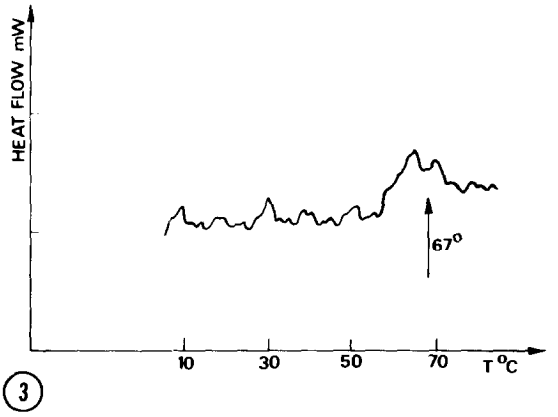


Fig. 1. Dormant spores were scanned with increasing amounts of water : a : no water added; b : with 7 µl water; c and d : with 15 µl and 30 µl of water respectively.

Fig. 2. Dormant spores, two successive scans with the same spores : 32.8 mg of dormant spores with 30 µl water were scanned until 70 °C (line a), cooled rapidly and scanned again (line b).

The small peaks obtained in the activating temperature range (40 °C - 50 °C) thus do not represent the activation process. Unfortunately further hydration of spores in this DSC-pans cannot be reached without decreasing the already small amount of spores used in the scans. Another attack therefore was to compare calorimeter scans from dormant and activated spores. For this purpose spores were heated in exces of water in a waterbath; then they were isolated by centrifugation and dried immediately by a cold air stream. This was done in order to prevent spores to return to the dormant condition (see 1). Figure 3 shows a spectrum obtained with such activated spores. It can be seen that previous activation influences the "protein-denaturation" peak, its top at 67 °C being strongly reduced by the heat-shock. This decrease in protein denaturation near 67 °C is most probably related to the activation mechanism : previous heating of dry spores at



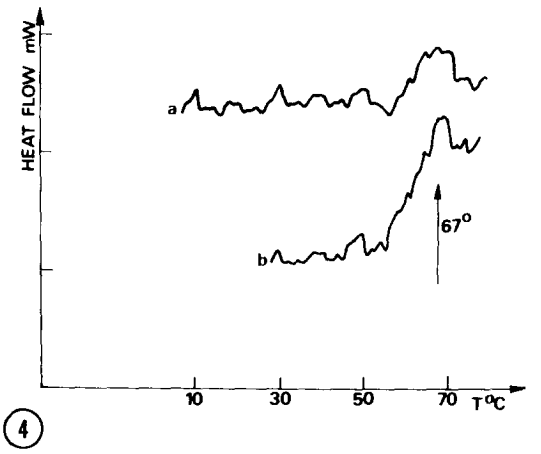


Fig. 3. Activated spores scanned in DSC :
Spores were activated in water (3 min, 50 °C), centrifugated and dried by a cold air stream. 53 mg of such superficially dried spores were scanned with 30 µl of water.

Fig. 4. Spores activated previously in water are compared with spores that after such activation became dormant again by keeping them in cold water :
line a : 31.2 mg spores were heated in water (41 °C, 5 min), isolated and dried by air stream. The spores were scanned with 30 µl water.
line b : 31.4 mg spores, activated as in a, were left in water for 20 min after cooling and then scanned in DSC.

50 °C e.g., a non-activating treatment, does not influence the subsequent scan in water, since the picture of dormant spores as shown in Figure 2a is obtained. Also heating spores at 38 °C does influence nor the protein denaturation peak, nor the germination (results not shown in the text). At the other hand heating spores for 3 min. at 41 °C, a treatment resulting in about 30% activation, caused a marked decrease of the calorimetric peak (see Figure 4,a). Samples of the spores activated at 41 °C were left in water at room temperature for various periods before scanning them in DSC. According to Halbsguth and Rudolph (1) return to dormancy is a slow process, taking a few hours. The reappearence of the protein denaturation peak at 67 °C then apparantly preceedes the reoccurence of germination inhibition, since we found that keeping spores for 20 minutes in water before starting

the scan is sufficient in order to restore the initial dormancy-picture (see Figure 4,b).

Another important result is that a completely different activating treatment, like keeping spores for 10 min. at 30 °C in 0,1 M NH_4-acetate (6), also causes the decrease of the peak at 67 °C (picture like in Figure 3, not shown in the text). The fact that activation procedures influence the subsequent protein denaturation pattern suggest that in the activation mechanism a protein change is involved. A conformational change of a protein during activation e.g. could cause the decrease of the endotherm peak on denaturation.

Much work will be needed in order to confirm that interpretation, and to find the meaning of the conformational change involved. From our results it seems that comparing protein fractions isolated from dormant and activated spores could be helpfull in order to situate the activation. The existence of a calorimetric peak characteristic for the dormant condition could be used in order to test which factors do interfere with the activation mechanism or with the reestablishment of dormancy.

The use of larger calorimeter capsules probably would offer the possibility of measuring directly the change which yield the onset of the germination, and to trace the proteins involved using isolated spore fractions.

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