JOURNAL OF BACTERIOLOGY, July 1970, p. 270-271
Vol. 103, No. 1

Activation Energy for Glucose-Induced Germination of *Bacillus megaterium* Spores

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Received for publication 30 March 1970

The maximum germination rate of *Bacillus megaterium* QM B1551 spores in glucose increased, and the lag before its attainment decreased, with increasing germination temperature. The activation energy for germination (μ = approximately 20 kcal/mole), based on rate or on lag, was consistent with an enzymatic mechanism.

It has been shown that the germination rate and lag of *Bacillus cereus* spores on L-alanine (12) and of *B. megaterium* spores on glucose (6) depended on the germination temperature. On the basis of the more detailed measurements reported here, we now find that the activation energy (μ) for *B. megaterium* spore germination, like that for outgrowth (6), is in the range typical for enzymatic reactions, whereas that for spore activation (5) is consistent with protein denaturation.

Spores of *B. megaterium* QM B1551, produced on the medium of Arret and Kirshbaum (1) without agar, were activated as previously described (5) by heating at 60 C for 10 min in aqueous suspension (0.5 mg of spores/ml). The heat-activated spores were germinated in Klett tubes (final spore concentration, 0.4 mg/ml) in 25 mM glucose, buffered with 50 mM potassium phosphate (*p*H 7.0), at temperatures from 15 to the optimum at 30 C (2.5 C increments). Germination was followed by decrease in the optical density (OD) of the spore suspensions at 560 nm (Klett-Summerson colorimeter). Germination is expressed as per cent OD loss, $[(K_i - K_t)/K_i] \times 100$, where K_i is the initial Klett reading and K_t is that after incubation for t min. An OD loss of 30% was equivalent to approximately 50% germination, as determined by phase darkening or stainability.

The rate and lag of germination were estimated from plots (Fig. 1) of the OD loss of spore suspensions during incubation with buffered glucose at various temperatures. The germination rate, estimated as the per cent OD loss per min of incubation during the period of maximum rate of OD decrease; and the germination lag, estimated as the time of incubation required before the initiation of the maximum rate, were temperature-dependent (Table 1). The germination rate and the reciprocal of the lag were used as the rate functions in Arrhenius plots (Fig. 2). The activation energy (μ) for germination was calculated (2) from the slopes of these plots and, whether based on rate or lag (μ_{rate} = 19.0 kcal/mole; μ_{lag} = 20.7 kcal/mole), was of a magnitude consistent with an enzymatic reaction. Arrhenius plots based on other estimates of rate, such as reciprocal time for a definite percentage loss in OD (e.g., 12.5% OD loss), slope of a plot of logarithm residual ungerminated spores versus time (7), or slope of a plot of logarithm of residual OD versus time (8), all indicated activation energies (μ = 18 to 21 kcal/mole) similar to those estimated from Fig. 2. The lower activation energy calculated by Mehl and Wynne (7) for *Clostridium sporogenes* (PA 3679) spore germination (μ = 10.3 kcal/mole) may have been a species characteristic or may have been due to the germination criterion selected (loss of heat resistance). Indeed, the activation energies, which we have calculated from the data of Riemann (9) for PA 3679 spore germination (based on rate of loss of refractility) in calcium-dipicolinic acid and in ethylenediaminetetraacetate, were approximately 20 to 30 kcal/mole. Furthermore, the magnitude of μ for *B. megaterium* spore germination did not differ substantially from that calculated for *B. cereus* on the basis of germination rate in L-alanine (8) or in a complex medium (13), or on the basis of germination lag (but not of rate) on calcium-dipicolinic acid (3).

The high activation energy (μ = 72.4 kcal/mole) reported previously (5) for activation of *B. megaterium* spores is in the range typical for macromolecular denaturation (11) and is thus consistent with the postulate (4) that spore activation may involve denaturation of a protein

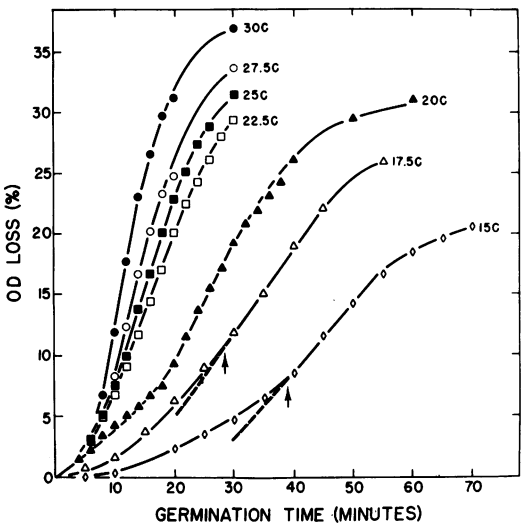

FIG. 1. *Germination of Bacillus megaterium spores at various temperatures. Heat-activated spores (60 C, 10 min) were germinated at the indicated temperature in 25 mM glucose, buffered with 50 mM potassium phosphate (pH 7.0). Germination rate was estimated from the curves as the per cent OD loss per min during the period of maximum rate of OD decrease. Germination lag was estimated as the time required before initiation of the maximum rate, this time being indicated by arrows for germination at 15 and 17.5 C. The dashed lines are extensions of the linear portions of the curves representing maximum rate at these temperatures.*

TABLE 1. *Rate and lag of germination of Bacillus megaterium spores at various temperatures*

Temperature (C)	Germination[a]	
	Rate (per cent OD loss per min)	Lag (min)
15	0.56	39
17.5	0.70	29.2
20	1.0	18.2
22.5	1.35	12.6
25	1.70	11.6
27.5	2.1	9.0
30	2.9	6.4

[a] Germination rate and germination lag were estimated from Fig. 1.

responsible for maintenance of the dormant state of a spore. On the other hand, the activation energies for outgrowth (6) and for germination (this paper)—25 kcal/mol and 20 kcal/mole, respectively—are in a range consistent with enzymatic reactions (10).

We thank G. R. Mandels and E. T. Reese for their critical reviews of the manuscript.

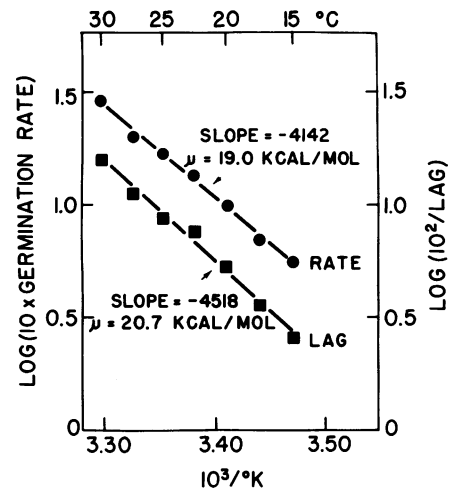

FIG. 2. *Effect of temperature on the germination of Bacillus megaterium spores (Arrhenius plots). Germination rate (●) and lag (■), estimated from Fig. 1, were used as the rate functions. Slopes of the straight lines with best fit were computer-calculated by the method of the sum of least squares. Activation energies (μ) were calculated from the slopes (2).*

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