Surface Charges of Two Types of *Bacillus megaterium* Spores Differing in Their Response to *n*-Butane

By K. WATANABE AND S. TAKESUE

*Institute of Microbiology, Faculty of Pharmaceutical Sciences,
Fukuoka University, Fukuoka, Japan*

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Rode & Foster (1965) showed that the germination of spores of *Bacillus megaterium* is sometimes affected by hydrocarbons such as *n*-butane: one type of spore (AL), which responds germinatively to L-alanine and inosine, is inhibited by *n*-butane; a second type of spore (GN), which responds to glucose and KNO₃, is not inhibited, but even accelerated a little by the same compound. The study of the biological activity of these kinds of compounds in respect of bacterial spores is useful in elucidating the mechanism of germination. In this communication, the effect of *n*-butane on the superficial charges of the spores was investigated; differences in electron microscopical morphology have already been shown by Rode (1968): the alanine type spores have a veined surface with a superficial beaded ultrastructure pattern, the glucose type spores have the well-known prominent equatorial ridge and polar knob appearance.

METHODS

Bacillus megaterium ATCC 19213 (designated AL strain) and QM B 1551 (designated GN strain) were used. Spores were produced on an agar medium according to the methods described by Rode & Foster (1960). *n*-Butane (98 mole % purity) was obtained from Tokyo Kasei Co.

RESULTS AND DISCUSSION

n-Butane was bubbled continuously into 5 ml. water suspensions of the two types of spores (O.D. = 0.5, about 10⁸/ml.) in a 20 ml. beaker, for the time tested, through a tapering glass tube at 2 to 3 bubbles/sec., and at every min. the pH value of the suspensions was determined by a Hitachi model M5 pH-meter. The test was at room temperature, and no special precautions were taken to prevent loss of gas from the suspensions during measurement. Fig. 1*a* shows that both spore suspensions usually started at about pH 6.2. The pH increased rapidly to a maximum value; the rate of increase depended on the speed of gassing. After that, the pH values did not vary, unless the gas bubbling stopped, when they rose slightly. Bubbling *n*-butane into a water control caused no changes in pH. The pH value of the AL spore suspension was more alkaline than that of GN spores. Flushing with air for a few seconds to remove the gas resulted in prompt falls in the pH values of both spore suspensions to the initial levels. A second bubbling of *n*-butane again caused a rise of the pH values of both spore suspensions, showing that the change in pH values of the suspensions caused by *n*-butane was reversible. Some kind of change, therefore, in superficial charge located on some special parts of the spores might have been induced by *n*-butane through a structural change of surrounding water. The spore coats which enclose the central

core of dormant spores consist primarily of macromolecules such as proteins or polysaccharides. The changes of physiochemical characteristics of macromolecules by non-polar compounds have recently received the attention of many investigators. Wetlaufer & Lovrien (1964) found that action of hydrocarbons in an aqueous solution resulted in changes in viscosity, pH value, optical rotation and ultraviolet absorption spectra of proteins such as bovine serum albumin or β -lactoglobulin.

Fig. 1. Change in the pH values (a) and the amounts of titratable HCl (b) of spore suspensions induced by continuous bubbling of *n*-butane. Spores of *Bacillus megaterium* ATCC 19213 (—●—) and of QM B1551 (—○—) were suspended in water at a concentration of O.D. = 0.5 (Hitachi electric colorimeter, model EPO-B, red filter).

The change in the concentration of intrinsic hydrogen ions of *n*-butane-treated spore suspensions were examined by means of a Radiometer model TTT 1 c pH-stat. Five ml. of spore suspension or control spore-free water were introduced into the pH-stat cell covered with a fitting Teflon disc, which was bored to receive the electrodes, magnetic stirring bar, and the tube for flushing gas and air. While being bubbled with *n*-butane at room temperature, the sample solutions were titrated automatically with 0.001N-HCl ($f = 1.004$) to bring the pH values back to the initial pH 6.2. As shown in Fig. 1b, the amounts of HCl solution needed for the back titration increased with time and finally reached constant values for the respective spore suspensions. However, while the Δ pH was smaller, the Δ H⁺ (about 0.22 ml.) was larger in the case of GN spores, and the reverse was the case with AL spores (Δ H⁺, about 0.12 ml.), which suggests that the buffer action of the former was stronger than that of the latter, and suggests further that there existed more ionizable residues on the surface of the former.

The next feature examined was whether such a buffer action (presence of ionizable groups on the surface) of spores was of a substantive nature or one induced by *n*-butane. After the pH values of the suspensions were adjusted to about 3 with a few drops of dilute HCl, a pH-titration curve of 10 ml. spore suspensions (O.D. = 0.9) was constructed

by means of the pH-stat using 0.05 N-NaOH ($f = 0.997$). A large quantity of alkali was consumed, due to negative charges such as COO^- , between pH 3 and 4.5 in both cases. Both titration values increased gradually in parallel with the pH values. The titration values of GN spores, however, were always higher than those of AL spores; for example, at pH 7, GN spores = 0.035 ml., AL spores = 0.010 ml., when the initial value of both suspensions was pH 4.5. No noticeable inflexion point in the curve showing the pKa value was observed in either case (i.e. if there were an inflexion point, less than 1.8×10^{-4} N-NaOH would have been needed to detect it). These results suggest that most parts of the surface of both types of spores are occupied by non-polar regions. When *n*-butane is introduced into the spore suspensions, microconfigurations of spores are easily altered, possibly through the structural changes of water covering the surfaces; consequently the pK values of Brönstedt's acid are increased. These reversible changes of H^+ equilibrium might have been induced by configurational changes unfolding, stabilization or other changes in macromolecular fractions probably existing in some special sites of the spore surfaces. GN Spores have more ionizable groups as compared with AL spores and the buffer action against the change of H^+ equilibrium (ex. $\text{RCOO}^- + \text{H}^+ \rightarrow \text{RCOOH}$, $\text{RNH}_2 + \text{H}^+ \rightarrow \text{RNH}_3^+$) caused by an interaction with *n*-butane is stronger, resulting in the smaller amount of change of net H^+ .

The evidence presented here agrees with the view of Rode (1968) that GN spores may have a greater number of available binding sites for H^+ than AL spores. Furthermore, the differences in the charges of two types of spores seem to have some connexion with the fact that at germination each responds quite differently to *n*-butane.

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