EFFECT OF NUTRITIONAL AND PHYSIOLOGICAL FACTORS ON THE REACTION BETWEEN LACTOBACILLUS PLANTARUM AND MURAMIDASE*

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While investigating the effects of nutritional deficiencies on cell wall composition in L. plantarum, we were unable to detect major compositional changes in several nutritional types in which an enhanced sensitivity of the amino acid transport process to osmotic forces had suggested the existence of structural abnormalities in the cell (Holden and van Balgooy, 1964). Since alterations in the tertiary structure and surface charge of the complex wall matrix might occur despite the apparent absence of major changes in gross composition, the reaction between such cells and polyelectrolytes was studied. This report summarizes experiments showing that the lytic and binding reactions between L. plantarum and muramidase are markedly dependent on the physiological age of the cell and are enhanced by a pantothenic acid deficiency. Previous studies have indicated that this organism is relatively muramidase-resistant (e.g., Salton and Pavlik, 1960).

METHODS

L. plantarum was grown as described previously (Holden, 1959). Freshly-harvested, water-washed cells were used to determine sensitivity to lysis by muramidase and the ability to bind this protein. The lytic reaction was measured at 37° in 0.03 M tris pH 7.0 using muramidase at 10 µg/ml, cells at 0.2 mg/ml (dry weight), and observing the

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decline in optical density at 640 mμ. The binding of muramidase was observed turbidimetrically at 9° using higher concentrations of the enzyme (50-1000 μg/ml), all other conditions remaining unchanged. Purified cell walls were isolated from cell sonicates using a procedure based on the method of Cummins and Harris (1956). The reaction between purified cell walls and muramidase also was monitored turbidimetrically, in this case at 450 mμ. Muramidase (egg white) was a 3X crystalline preparation purchased from Sigma Chemical Company. The O-acyl content of cell walls was estimated using the procedure of Hestrin (1949).

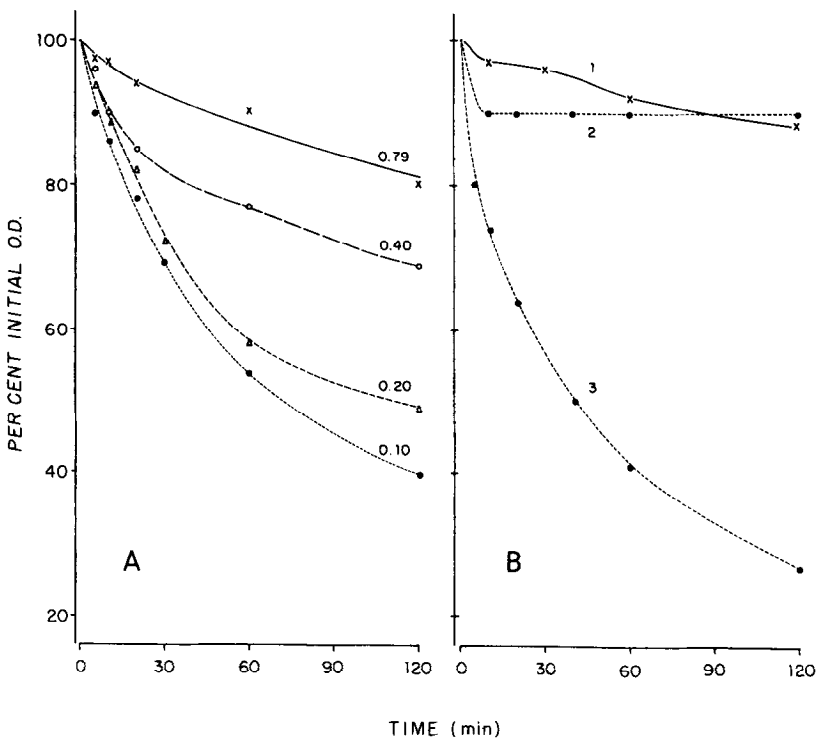

FIG. 1A. Dependence of muramidase sensitivity in nutritionally-normal cultures on growth phase. The numbers on the curves refer to cell density at harvest (mg dry weight per ml). The decrease in O.D. shown is that attributable to muramidase.
FIG. 1B. Effect of vitamin deficiencies on muramidase sensitivity. Curve 1, vitamin B_6-deficient cells, grown with 10 mμg/l pyridoxamine, harvested at 0.16 mg/ml; curve 2, biotin-deficient cells, grown with 50 mμg/l biotin, harvested at 0.16 mg/ml; curve 3, pantothenate-deficient cells, grown with 5.3 μg/l pantothenic acid, harvested at 0.21 mg/ml. All cultures were harvested near the end of the period of active growth.

The wall material analyzed was obtained from freshly-harvested cells by sonication and extensive washing with cold water.

RESULTS AND DISCUSSION

The susceptibility of L. plantarum to lysis by muramidase depends on the age of the source culture. Cells taken during the early exponential growth phase (0.1 and 0.2 mg/ml, Fig. 1A) were readily attacked by the enzyme. Sensitivity declined steadily thereafter to the low level observed with stationary-phase cells. The muramidase sensitivity of B. megaterium appears also to vary with culture age (Chaloupka et al., 1962). Curtailment of growth at relatively low cell densities by restricting the availability of biotin or vitamin B_6 resulted in cell populations which were muramidase-resistant (Fig. 1B). The resistant state seems, therefore, to be associated with the stage of growth rather than with the population density; however, when growth was restricted using suboptimal levels of pantothenic acid, the resultant cells were unusually sensitive to lysis (Fig. 1B).

A number of chemical properties of the cell wall, including a reduced O-acetyl content, have been associated with muramidase sensitivity (Brumfitt, Wardlaw and Park, 1958). The O-acyl content of cell wall fractions from L. plantarum, cultivated under the conditions described above, were measured (Table I). Although early exponential phase cell walls generally contained 15% fewer O-ester groups, the period during which these increased to the maximum level did not coincide with the period in which muramidase sensitivity declined markedly. In contrast, walls from pantothenic acid-deficient cells had a markedly diminished O-ester content. The muramidase sensitivity of L. arabinosus (plantarum) also can be increased by base treatment (Salton and Pavlik, 1960), presumably because the wall O-acyl content is thereby reduced. The effect of a pantothenate deficiency on muramidase sensitivity supports the view that the wall O-acetyl content influences muramidase

TABLE I

CELL WALL O-ACYL CONTENT OF L. PLANTARUM NUTRITIONAL VARIANTS

Cell Type	Density at Harvest	Wall O-Acyl Content	Cell O.D. Reduction by Muramidase*
	mg/ml	µmole/mg	%
Control	0.10	0.62	51
	0.30	0.72	40
	0.50	0.68	12
Pantothenate-deficient	0.15	0.38	70
Biotin-deficient	0.11	0.68	10
Vitamin B_6-deficient	0.14	0.41	13

* 120-min incubation.

sensitivity. The large decline in O-acyl content of vitamin B_6-deficient cell walls without a corresponding increase in muramidase sensitivity undoubtedly reflects the markedly reduced mucopeptide content of these walls (Holden and van Balgooy, 1964). The mucopeptide which is present probably has a normal O-acyl content.

When whole cells were exposed to muramidase at levels much in excess of those required to observe lysis (50-1000 µg/ml), the optical density of the cell suspension increased greatly. This phenomenon has been encountered previously (cf. Salton, 1957) and has been attributed to muramidase binding at the cell surface. At pH 7 (Fig. 2) the extent of this reaction correlated with muramidase sensitivity. Specifically, pantothenate-deficient and early exponential phase cells reacted more intensely than did the other cell types. At pH 10, all cell types showed a marked response of similar magnitude. Microscopic examination established that the increase in optical density was not accompanied by cell clumping. The large increase in muramidase binding by pantothenate-deficient cells suggests an increased availa-

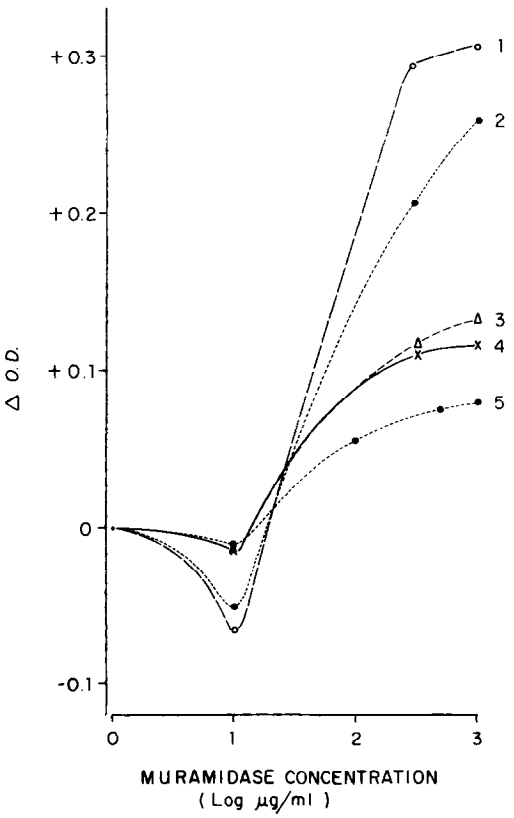

FIG. 2. Optical density changes of cell suspensions incubated with high concentrations of muramidase. Incubation for 60 min at pH 7.0. Curve 1, pantothenate-deficient cells; curve 2, nutritionally-normal, early exponential phase cells; curve 3, vitamin B_6-deficient cells; curve 4, biotin-deficient cells; curve 5, nutritionally-normal, late exponential phase cells.

bility of negatively-charged groups in addition to the reduced wall O-acyl content.

The optical density increases illustrated in Fig. 2 have been shown to be accompanied by removal of muramidase activity from the buffer (measured, using dried M. lysodeikticus cells as substrate). At any muramidase concentration there was a linear relation between muramidase removal and optical density increase; however, for any given optical density increase, different amounts of muramidase were removed from the buffer depending on the enzyme concentration and pH. Other basic proteins such as ribonuclease and cytochrome c also are bound by pantothenate-deficient and early exponential phase cells.

The lytic and binding reactions observed with whole cells also

were studied using purified cell wall preparations. There were several differences in response. Walls from pantothenate-deficient cells were by far the most sensitive to lysis. Walls from control, early exponential phase cells, although more sensitive than the remaining types, were distinctly less sensitive than those from pantothenate-deficient cells. In contrast, they were by far the most reactive in the binding reaction; however, the apparent reduction in binding activity of walls from pantothenate-deficient cells might be caused by their extreme sensitivity to lysis.

SUMMARY

The muramidase sensitivity of L. plantarum varies markedly with physiological age and is enhanced by a pantothenic acid deficiency. The sensitivity to lysis of pantothenate-deficient cells, but not of early exponential phase cells, correlates with a marked decline in cell wall O-acyl content. Muramidase-sensitive cells also bind this enzyme when exposed to high concentrations of muramidase, suggesting that the distribution of charges at the cell surface can be altered by these nutritional and physiological factors.

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