Wall Autolysin of Lactobacillus acidophilus Strain 63 AM Gasser

Jacques Coyette and Jean-Marie Ghuysen

ABSTRACT: The autolysin of Lactobacillus acidophilus strain 63 AM Gasser has the specificity of an endo-N-acetylmuramidase. It hydrolyzes both N-acetylmuramic acid and N,G-diacylormuramic acid linkages. It does not exhibit any amidase or endopeptidase action. It is present in both log-phase and stationary-phase cells.

In stationary-phase cells its action upon the wall peptidoglycan is inhibited.

Experiments carried out with several Gram-positive bacteria (Cole, 1965) and with Escherichia coli (Schwarz et al., 1969) strongly suggest a zonal growth of the wall peptidoglycan at least during cell division. Biochemical study of the biosynthesis of the peptidoglycan also strongly suggests that the insertion of newly synthesized β-1,4-N-acetamidoaminyl-N-acetylmuramyl peptide fragments requires the creation of nonreducing N-acetamidoglycaminyl receptor sites in the glycans strands (for a review, see Ghuysen, 1968). Wall lytic endo-N-acetylmuramidases could thus play the role of providing those receptor sites and it may be that the safe enlargement of the peptidoglycan sacculus is due, in fact, to a strict coordination between the biosynthetic and the hydrolytic processes. So far it is known, Streptococcus faecalis appears to be one of the simplest model with regard to the study of wall growth at the cellular level. Indeed, the active wall-bound autolytic system consists of a single enzyme that has the specificity of an endo-N-acetylmuramidase (Shockman et al., 1967b). Moreover, it has been shown that the cell autolysin is the site where the active autolysin is located and the region where new wall material is inserted (Shockman et al., 1967a; Shockman and Martin, 1968; Shockman and Cheney, 1969; Pooley and Shockman, 1969). In contrast to S. faecalis which exhibits a single plane of division, other spherical microorganisms that are characteristically arranged in clusters such as Staphylococcus aureus (Tipper, 1969), and rod-shaped bacteria such as E. coli (Weidel and Pelzer, 1964) and Bacillus subtilis (Young, 1966a,b) have complex autolytic systems. These systems contain enzymes such as amidases, endopeptidases, and endo-N-acetylmuramidases that do not appear to be consistent with a role in wall biosynthesis but that might be involved in other phenomena such as competence, excretion, and permeation of large molecules.

The experiments hereby presented show that L. acidophilus 63 AM Gasser is another simple model that may be useful for the study of wall expansion and cell division in a rod-shaped microorganism.

Materials and Methods

Growth conditions, analytic techniques (measurement of reducing groups, acetamido sugars, and N-terminal groups), and walls preparation and structure have been described (Coyette and Ghuysen, 1970).

Enzymes. Streptomyces F1, endo-N-acetylmuramidase was used (Ghuysen, 1968).

Experimental Section

Autolysis and Bacterial Growth. Strains of Lactobacilli are known to autolyse (Knox and Brandsen, 1962). Preliminary experiments carried out with L. acidophilus 63 AM Gasser showed that the rate of autolysis of log-phase cells suspension was maximal in a 0.05 M citrate buffer, pH 5. The specific autolytic activity during growth of L. acidophilus was determined as follows: cells were harvested at various times and washed by centrifugations with cold distilled water. The pellets were resuspended in 0.05 M citrate buffer, pH 5, and the turbidity of each cell suspension was adjusted to an optical
anisms may not be identical in these two microorganisms.

References