

Measurement of Bacteriolytic Enzymes

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The most widely used assays for lysozyme (see P. Jollès, *Angew. Chem. Intern. Ed. Engl.* 3:28, 1964) as well as assays for more recently discovered bacteriolytic agents (J. H. Hash, *Arch. Biochem. Biophys.* 102:379, 1963; C. A. Schindler and V. T. Schuhardt, *Proc. Natl. Acad. Sci. U.S.* 54:414, 1964; J. C. Ensign and R. S. Wolfe, *J. Bacteriol.* 90:395, 1965; J. W. Zyskind, P. A. Pattee, and M. Lache, *Science* 147:1458, 1965) have been based on the reduction in turbidity of suspensions of susceptible bacterial cells. The assay for the *Chalaropsis N*-acetylhexosaminidase (Hash, *Arch. Biochem. Biophys.* 102:379, 1963), which was based on the reduction in turbidity of suspensions of *Staphylococcus aureus* cells, was complicated by buffer effects, and it was difficult to obtain different preparations of cells with the same susceptibility to lysis. It was observed that isolated cell walls from different preparations of cells were uniformly susceptible to lysis. An assay was developed that was based on reduction in turbidity of isolated cell walls rather than intact cells. This assay was independent of buffer concentration over the range 0.01 to 0.2 M, and different preparations of cell walls showed the same sensitivity. Other bacteriolytic enzymes have been tested, and the method appears to be applicable to all enzymes that solubilize the cell wall murein. In the present study, these enzymes include: lysozyme, *Chalaropsis B* enzyme, lysostaphin (courtesy of Mead Johnson Research Center and M. Glenn Koenig), *Pseudomonas* enzyme (courtesy of P. A. Pattee), and *Myxobacter AL-1* enzyme (courtesy of R. S. Wolfe). Lysozyme is a β -1,4-*N*-acetylmuramidase and *Chalaropsis B* is a β -1,4-*N*,*O*-diacetylmuramidase. The *Pseudomonas* and *Myxobacter* enzymes are peptidases. Lysostaphin contains both peptidase and β -1,4-*N*-acetylglucosaminidase activities.

Bacterial cell walls were prepared by standard procedures (M. R. J. Salton, *The Bacterial Cell Wall*, American Elsevier Publishing Co., New York, 1964) and were extracted with cold 10% trichloroacetic acid to remove teichoic acid and other polyelectrolytes. *Micrococcus lysodeikticus* walls were used for lysozyme and *S. aureus* walls were used for the other enzymes.

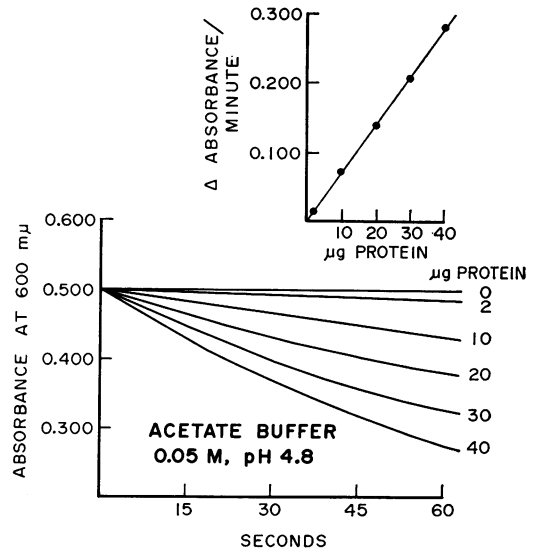


FIG. 1. Relationship between *Chalaropsis B* protein concentration and changes in absorbance of *Staphylococcus aureus* cell wall suspensions.

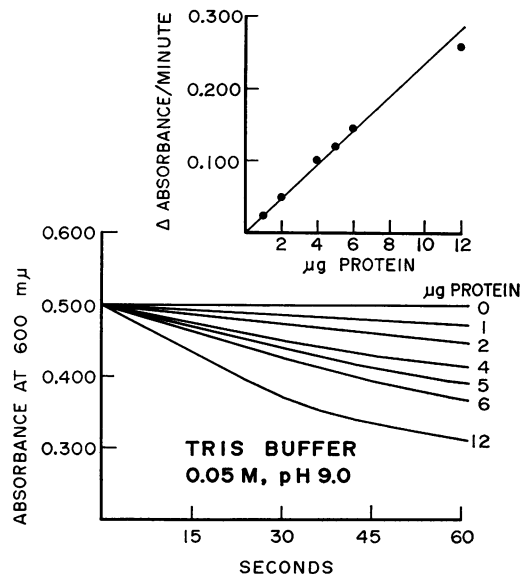


FIG. 2. Relationship between *Myxobacter AL-1* protein concentration and changes in absorbance of *Staphylococcus aureus* cell wall suspensions.

Cell walls, uniformly suspended in 0.05 M buffer of suitable pH, were diluted so that the initial absorbance at 600 m μ with a 1-cm light path was approximately 0.50. A recording spectrophotometer with the cuvette chamber maintained at 25 C was used. Enzyme was added (1.0-ml total reaction volume), and the decrease in absorbance was measured for 1 to 2 min.

The relationship between protein concentration and decrease in absorbance of *S. aureus* cell wall suspensions for *Chalaropsis* B and *Myxobacter* AL-1 enzymes is shown in Fig. 1 and 2. Lysozyme, lysostaphin, and *Pseudomonas* enzyme gave similar results, but *Chalaropsis* and *Myxobacter* are shown as representative of enzymes that cleave glycosidic and peptide bonds,

respectively, in the cell wall murein. In all cases, the initial rates of reaction (first 10 to 20% reduction in turbidity) were linear with time and as shown in each inset were directly proportional to enzyme concentration. The addition of neutral salts, such as sodium chloride, was not necessary and, in fact, was inhibitory for some of the enzymes. For the *Chalaropsis* B enzyme, one unit was defined as the amount of enzyme necessary to give an initial reduction in absorbance of *S. aureus* cell wall suspensions of 0.001 per minute under the conditions described. By varying the buffer and perhaps the cell walls, this unit would appear to be suitable for any bacteriolytic enzyme that solubilizes the cell wall murein.