Some Properties of Staphylococcal α-Toxoid

A. W. BERNHEIMER, J. H. FREER, I. LOMINSKI, AND G. SESSA

Department of Microbiology, New York University School of Medicine, New York, New York 10016

Received for publication 15 July 1968

Staphylococcal α-toxin is a protein having a sedimentation coefficient of about 3S. It can exist as a nontoxic soluble aggregate having a sedimentation coefficient of 12S and also as a nontoxic insoluble polymer (1). In addition to causing diverse cytolytic effects, this toxin is capable of disrupting artificial lipid spherules (5). The toxin appears to undergo polymerization to the 12S form in the presence of either natural or artificial membranes (3). We wished to know whether conversion of α-toxin to toxoid in the presence of formaldehyde involves polymerization and whether toxoid retains the capacity to disrupt artificial lipid spherules.

A sample of α-toxin purified through stage 5 of the procedure described by A. W. Bernheimer and L. L. Schwartz (2), containing 5.6 mg of protein per ml of phosphate-buffered saline (pH 7.0), was divided into two portions: one portion received 5% formaldehyde to a final concentration of 0.2% (w/v) and was allowed to stand at room temperature for 96 hr; nothing was added to the other portion, which was refrigerated for the same period of time. Titration of hemolytic activity (2) revealed that the two solutions contained 24,000 and 200,000 hemolytic units per ml, respectively; i.e., “toxoiding” had occurred to the extent of 88%. Each solution was passed through a column of cross-linked dextran beads equilibrated with 0.03 M borate buffer (pH 8.3) containing 0.1 M KCl. The effluents each showed two peaks (Fig. 1), the larger of which represents 3S α-toxin and the smaller 12S. The points for toxoid fell essentially on the curve drawn through the points for toxin, indicating that toxoiding is not accompanied by conversion of toxin to the 12S form and that the molecular size of toxoid does not differ substantially from that of toxin.

In a similar experiment in which 12S-free toxin (2) was converted to toxoid to the extent of 97%, toxoid was found to possess very slight capacity to release trapped chromate ions from artificial lipid spherules (5) as compared to active toxin. Electron microscopic examination of toxoid-treated spherules revealed neither the morphological disruption of spherules observable when toxin is used nor the numerous ring structures (apparently 12S aggregates) which are a characteristic effect of toxin on spherules (3).

The results indicate that, with the concentrations of protein and formaldehyde used, the conversion of staphylococcal α-toxin to toxoid is not accompanied by an increase in molecular size and therefore does not involve polymerization. This finding contrasts with the behavior of tetanus toxin (sedimentation coefficient = 6.4S), which gives rise on toxoiding to a 7.15 monomer and to polymers about 35 times as large as the monomer (4). In addition, our results show that toxoiding of α-toxin results not only in loss of toxic (hemolytic) and spherule-disrupting activities but also in loss of ability to polymerize on contact with spherules. It is therefore tempting to speculate that polymerization of 3S toxin to the 12S form may occur through the same groups that are responsible for toxicity; alternatively, formaldehyde may affect the toxic groups as well as the groups responsible for polymerization.
This investigation was supported by a grant from The Life Insurance Medical Research Fund and by Public Health Service grant AI-02874 from the National Institute of Allergy and Infectious Diseases. A. W. Bernheimer was the recipient of Public Health Career Program Award 5K6-AI-14-198.

LITERATURE CITED