Differential Light Scattering Measurements of Heat-Treated Bacteria

RICHARD M. BERKMAN AND PHILIP J. WYATT

Science Spectrum, Inc., Santa Barbara, California 93105

Received for publication 6 May 1970

Effects of heat on diameter, size distribution, and refractive index of Staphylococcus epidermidis suspensions were determined accurately by computer analysis of differential light scattering data.

Differential light scattering measurements have been shown (3-5) to be potentially powerful tools for studying bacterial morphology. Such measurements consist of recording the angular distribution of light scattered from a suspension of bacteria which has been illuminated by a plane parallel beam of monochromatic light. These so-called differential scattered intensities (DSI) depend upon mean size, shape, and structure of the scattering particles (3). We have applied DSI measurements to study dynamic morphological changes in cultures of Staphylococcus epidermidis during the course of heat treatment.

In preparing autologous staphylococcal vaccines, many laboratories use heat as a sterilization procedure. Such a treatment supposedly does not destroy the immunogenic properties of vaccines and would be expected, therefore, to have little or no effect on cell walls. A recent study of thermally induced changes in staphylococci (1) demonstrated that optical density and light scattering properties of cells are directly influenced by heat. However, the observed optical density and light scattering changes did not correlate well with other data such as those obtained by electronic cell counter, hematocrit, and electron microscope. Results of our DSI studies will clarify the interpretation of these previously obtained nephelometric measurements. In addition, DSI measurements can detail very accurately the size and refractive index changes which occur upon heating.

For the experiments described here, the culture of S. epidermidis was grown for 24 hr at 37°C on nutrient agar (Difco). Washed cells were either diluted directly into distilled water (control) or they were heated at 60°C in Heart Infusion Broth (Difco) for various periods before diluting. The heating and cooling procedures were designed to achieve rapid equilibrium. All samples were diluted to about 2 x 10^8 bacteria per ml for the light scattering measurements. The DSI measurements were made with a Differential I light scattering photometer (Science Spectrum, Inc., Santa Barbara, Calif.) with a vertically polarized He-Ne light source (632.8 nm). The instrument records the relative intensity of scattered light as a function of scattering angle, from 5° to 150° measured with respect to the incident beam.

Figure 1 presents the DSI of a washed suspension of S. epidermidis recorded immediately before heating. Figure 2 shows details of qualitative changes in light scattering in the vicinity of the two maxima after various periods of heating. In Fig. 2, we have shifted the curves with respect to scattered intensity to simplify comparisons. Since thermally induced morphological changes manifest themselves by changes in DSI, the evolution of the light scattering curves, as evidenced in Fig. 2, is a vivid indication that some morphological changes did occur.

To determine quantitatively just how heating affected cell morphology, an extensive numerical analysis was performed. Data from Fig. 1 and 2 were replotted to correct for volumetric shortening (2, 4). The corrected curves were interpreted quantitatively on the basis of Rayleigh-Gans scattering theory (3, 5). By using a high-speed digital computer (CDC 6400), the scattering data were analyzed (3, 5) and the analysis showed that the heat treatment (i) caused a reduction in mean cell size and (ii) resulted in an increased cytoplasmic optical density relative to cell wall. This latter change is a manifestation of the decrease in free water within the cytoplasm. In quantitative terms, the average radius derived for the control cells on the basis of the corrected curves was 432 nm (±10 nm). After 30 min of heating, this value had decreased to 403 nm (±10 nm). The refractive index ratio (5) changed from about...
VOL. 20, 1970
NOTES

Fig. 1. Differential scattered intensity of vertically polarized 632.8-nm light from S. epidermidis. The culture was grown on Heart Infusion Agar at 37°C for 24 hr and resuspended in distilled water. Final concentration was approximately $2 \times 10^8$ cells per ml.

Fig. 2. Differential scattered intensity of vertically polarized 632.8-nm light from heated suspensions of S. epidermidis. Cell suspensions were heated at 60°C in Heart Infusion Broth for the time periods shown. Final concentration was approximately $2 \times 10^8$ cells per ml for each sample. Curves were shifted to facilitate comparison.

Webelieve that electron micrographs taken of heated cells (1) have limited value in this type of experiment since distortion or damage may occur during preparation of specimens already weakened by heat. Furthermore, nephelometric devices which measure light scattering at a single fixed angle, such as 45° or 90°, cannot be used to measure changes in cell size because such changes manifest themselves by shifts in angular location of the scattering peaks. By measuring the angular variation of scattered light, instruments such as the Differential I light scattering photometer clarify the dynamics of these structural changes.

The light scattering techniques and instruments under development (5) show great promise as tools to study cell physiology and morphology under conditions where the ordinary light microscope is inadequate. Differential light scattering can bridge the gap between the light microscope and the electron microscope in the degree of resolution possible, in the elimination of the need for fixation, and in the rela-

-0.45 (±0.1) for the control cells to -0.22 (±0.1) for the 30-min heat-treated cells. The average cell-wall thickness remained nearly constant at 108 nm (±20 nm) despite the heating. Finally, we deduced that the standard deviation of the cell radius increased slightly from about 106 nm (±8 nm; control) to 120 nm (±8 nm) after treatment at 60°C for 30 min.

Our results show, as expected, that moderate heat treatment, although having little or no effect on cell wall, caused a measurable reduction in cell size. Some of these changes, indicated by a shift in the primary peak, occurred after only 3 min of heating. Although the data indicated an approximately linear cell shrinkage over the 30-min heating period, colony counts indicated that more than 99% of the cell population was killed after 3 min, presumably as a result of enzyme inactivation. The gross changes observed in the secondary scattering peak between 10 and 30 min of heating most probably arose, as would be expected, from protein denaturation and precipitation of internal solutes.

We believe that electron micrographs taken of
tive speed at which these measurements can be obtained.

We thank David G. Gorbet for his competent technical assistance.

This investigation was supported by the U.S. Army Medical Research and Development Command, Office of the Surgeon General.

LITERATURE CITED

1. Allwood, M. C., and A. D. Russell. 1969. Thermally induced changes in the physical properties of Staphylococcus aureus. J. Appl. Bacteriol. 32:68-78.
2. Kratochvil, J. P., and C. Smart. 1965. Calibration of light scattering instruments. III. Absolute angular intensity measurement on Mie scatterers. J. Colloid. Sci. 20:875-892.
3. Wyatt, P. J. 1969. Differential light scattering: a physical method for identifying living bacterial cells. Appl. Optics 7:1879-1896.
4. Wyatt, P. J. 1969. Identification of bacteria by differential light scattering. Nature (London) 221:1257-1258.
5. Wyatt, P. J. 1970. Cell wall thickness, size distribution, refractive index ratio and dry weight content of living bacteria (Staphylococcus aureus). Nature (London) 226:277-279.