Quantitative Determination of Na^+- and K^+-Content of Single Mycobacterial Cells Using LAMMA

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With the aim of finding out whether the newly developed Laser Microprobe Mass Analyser (LAMMA) is suitable for quantitative measurements on very small biological samples and of checking the homogeneity of elemental distribution within the individual cells of a mycobacterial culture we measured the Na^+- and K^+-contents of single mycobacterial cells.
The LAMMA-instrument is a combination of a laser microscope with a time-of-flight mass spectrometer. Very small sample volumes - down to approximately 1 μm^3 of thin sections or of bacterial cells - are evaporated and partially ionized by a laser pulse and analyzed according to mass.
The mycobacteria (H37 Ra) were grown in Dubos medium,harvested after 14 days, washed four times in Tris-buffer or destilled water at 4 °C, and prepared on Formvar foil fixed to a standard copper grid. In most instances single bacteria were distributed on the film far enough apart to allow the analysis of one single cell at a shot. The laser beam was focussed in a way guaranteeing the analysis of a complete cell. To get satisfying statistics more than 100 cells were sampled.
Quantitative data were obtained from a comparison of the Na^+- and K^+-peak heights of the sample with those from standard specimens prepared as Epon thin sections. The thickness of the standard specimen was controlled by measuring in a Ge(Li)-detector the γ-activity of Fe-59 added to the Epon.
The values for a single H37 Ra cell as measured by us are

$$Na^+: (9.3\pm2.3)\cdot10^{-16}\ g \text{ and } K^+: (1.3\pm0.3)\cdot10^{-15}\ g.$$

The nearly identical contents for the two elements may be explained by the washing procedure at a temperature of 4 °C which is expected to influence the active transport mechanism. Under certain assumptions on the density of air-dried cells the above results point to a satisfying agreement with those obtained by us from more integral methods e.g. the atomic absorption spectroscopy, the neutron activation analysis or the application of the tracer technique. For this comparison, however, it has to be considered that for quantitative LAMMA-measurements still more information on the probably different interactions between the laser beam and the various specimens is needed.