Infrared Spectroscopy of Single Eucaryotic Cells

Peter Lasch [a], Luis Chiriboga [b], Anthony Pacifico [a] and Max Diem [a]

[a] Department of Chemistry and Biochemistry, City University of New York, Hunter College, 695 Park Avenue, New York, NY 10021, USA

[b] Department of Pathology, Bellevue Hospital & New York University Medical Center, 27th Street and First Avenue, New York, N.Y. 10016

In an effort to understand the spectral changes observed between healthy and diseased tissues and cells, we have undertaken studies that correlate these spectral changes with other indicators of cell biology. Three different experiments will be described that illustrate the amazing sensitivity of IR microspectroscopy (IR-MSP) toward the overall architecture of eucaryotic cells, toward the stages within of cell's division cycle, and toward the response of cells to biochemical signals.

The overall biochemical architecture of large, healthy oral mucosa cells was studied by IR-MSP, using both single detector instrumentation and a focal plane array detector equipped prototype instrument (IRScope II, Bruker Optik, Germany). Spatial resolution of these mapping experiments was *ca.* 12 and 5 μm, respectively. These experiments revealed that the cytoplasmic region of a cell, and the cell nucleus, exhibit very similar spectra which are totally dominated by protein spectral contribution.[1] Membrane components and nucleic acids (both nuclear DNA and nuclear/cytoplasmic RNA) contribute very little to the overall spectra in terminally differentiated and quiescent cells.

When cultured cells (myeloid leukemia) are synchronized according to their cell division cycle stages, one finds that in the quiescent stage (G0), and also during most of the Gap1 (G1) and Gap2 (G2) phases, the overall spectrum is dominated by protein spectral contribution. However, starting at the G1/S interphase, during the S phase and until about the S/G2 point, the spectra of individual cells, as well as those collected by averaging methods, exhibit very strong DNA signals. These signals were correlated to a variety of cell biological methods, such as fluorescence activated cell sorting (FACS), and DNA digestion studies. Similar results have been reported for synchronized fibroblasts.[3]

Finally, in order to establish differences between normal and cancerous cells, we report spectra of wild type fibroblasts (rat liver, 3Y1 cell line) and a transformed cell line that overexpresses the epidermal growth factor (EGF) receptor (3Y1R). FACS analysis, thymidine uptake and Western blots of both the 3Y1 and 3Y1R were used to establish the exact growth patterns of these cell cultures. Upon treatment with EGF, a significant spectral change is observed for the 3Y1R, but not the 3Y1 cells; furthermore, a different spectral response was observed depending on whether or not the cells were starved before EGF treatment. These observed spectral changes are interpreted in terms of cells progressing toward cancer or apoptosis, respectively.

1. P. Lasch, M. Boese and M. Diem, "FT-IR Spectroscopic Investigations of Single Cells on the Subcellular Level", *Vibrational Spectroscopy* (Special Issue), submitted, July 2000
2. S. Boydston-White, T. Gopen, S.Houser, J.Bargonetti, and M. Diem, "Infrared Spectroscopy of Human Tissue: V. Infrared spectroscopic studies of Myeloid Leukemia (ML-1) cells at different phases of the cell cycle", (1999) *Biospectroscopy*, **5**, 219-227
3. Holman, H.N, Martin, M.C., Blakely, E.A., Bjornstad, K. and McKinney, W.R., "IR spectroscopic characteristics of cell cycle and cell death probed by synchroton-based FTIR spectromicroscopy", *Biopolymers: Biospectroscopy*, in press, 2000