Investigating single living cells with synchrotron infrared microspectroscopy

David Moss

Forschungszentrum Karlsruhe, Synchrotron ANKA, Postfach 3640,
D-76021 Karlsruhe, Germany

It has been convincingly demonstrated in many studies that infrared spectroscopy of cells and tissues can deliver powerful classification information - for example for identifying types of tissues, recognizing and mapping disease states, taxonomical classification of microorgansims, etc. However, the sheer chemical complexity of such systems generally precludes any specific molecular interpretation of the spectral information obtained. This is regrettable, because if the power of infrared spectroscopy to characterize essentially any molecule in any environment could be harnessed for the study of the behaviour and reactions of biological molecules in situ in the cell, this would help to bridge the century-old gap between the holistic biological and reductionist biochemical approaches, which is one of the grand challenges of biological science and very much on the topical agenda through today's focus on such research tools as functional genomics, proteomics and metabolomics.

Similar information surfeit problems were already encountered when infrared spectroscopists first began to work with purified biological macromolecules, where the very large number of chemical bonds leads to widespread overlap of individual infrared bands, so that only a few broad bands and regions of continuum absorbance are observed. Some coarse structural information such as secondary structure motifs can be seen in such spectra, but in general not the individual atomic bonds. One successful solution was found in this latter case through the introduction of reaction-induced difference spectroscopy, in which only the spectral changes accompanying a biologically relevant change of state (conformational changes, ligand binding, etc.) are recorded. This yields a drastically simplified spectrum in which bands arising from individual atomic bonds and functional groups can readily be identified and interpreted in terms of their roles in the molecular mechanism.

One easily comes to the thought that the previous solution might also work for the newer problem. To see the biomolecules in a cell is a problem on roughly the same scale as seeing the atoms in proteins, so perhaps a solution could be found by applying reaction-induced difference spectroscopy to cells. There would clearly be a number of technical problems to be overcome, not least of which is that the cells would have to be alive in order to react to biologically relevant stimuli for the purpose of difference spectroscopy, implying in turn that the measurements would have to be performed through aqueous media - the exact opposite of the drying and fixation approaches that have been employed in the vast majority of vibrational spectroscopy studies of cells and tissues to date. Nevertheless, with the high brilliance advantage provided by a synchrotron infrared beam, it seemed reasonable to try out the idea at the synchrotron light source ANKA.

This experiment has now been carried out with a number of users groups at ANKA tackling various biological questions, and while the ultimate goal of this approach cannot be said to have been achieved yet, experience to date has demonstrated its fundamental feasibility and helped to identify the problems that still need to be overcome. This presentation will describe the current state of play in IR spectroscopy of single living cells and consider how further progress could be made in future. Since similar experiments were carried out simultaneously and independently by Max Diem and his coworkers using conventional benchtop instrumentation, the talk will also examine the question of what benefits, if any, are gained by using synchrotron light sources for this type of study.