Synchrotron Vibrational Linear Dichroism of Retinal Rod Cells

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Introduction

Fourier Transform Infrared Spectroscopy (FTIR) is a powerful technique for the study of biological molecules, providing insight into both structure and mechanism. To date, most studies are performed using partially or fully purified biomolecules, often reconstituted under conditions that mimic some features of their native cellular environment. To reduce artifacts due to purification and reconstitution it is highly desirable to be able to perform these measurements directly *in vivo*, with biomolecules still in their cellular surroundings. We now demonstrate the viability of this option using synchrotron FTIR spectromicroscopy.

Science

Retinal rod cells are responsible for vision under conditions of weak illumination. They are composed of an inner segment (RIS: Rod Inner Segment) portion, which is the site of cellular metabolism, and an outer segment (ROS: Rod Outer Segment) containing the molecular machinery involved in phototransduction [1].

We show that synchrotron Fourier Transform Infrared Spectroscopy (FTIR) spectromicroscopy can be used to measure vibrational linear dichroism (VLD) within individual vertebrate photoreceptor rod cells and to quantify the orientation of major molecular components. The diameter of a ROS is 4-7 µm, close to the diffraction limit for radiation in the mid-infrared spectral region, and an infrared synchrotron source is necessary to perform spectromicroscopy measurements on individual ROS with high signal-to-noise ratio. We used the Mid-IR beamline 01B1-1 at the Canadian Light Source to perform transmission VLD FTIR measurements of the ROS of intact rod cells from the toad *Bufo americanus*.

Discussion

Spectra recorded with polarized infrared light are shown in Figure 1.

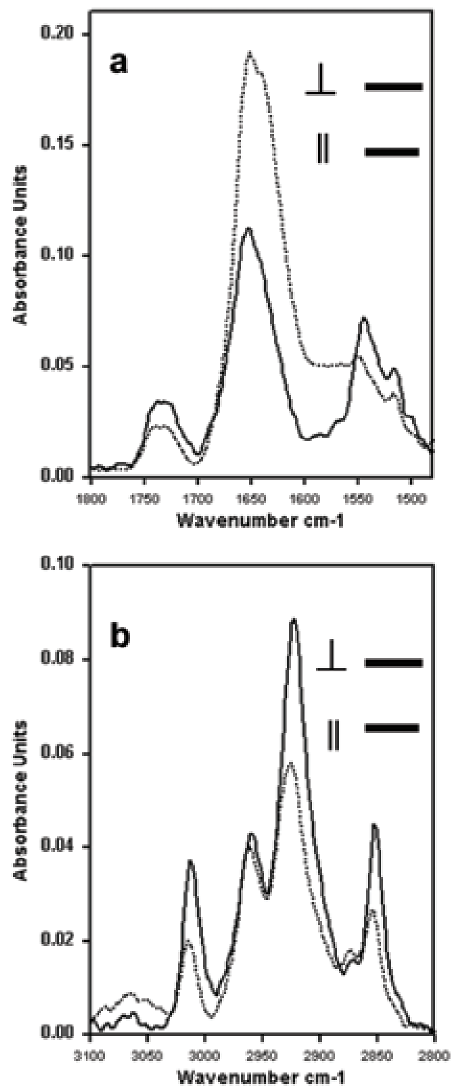

Figure 1: a) ROS spectra recorded in the carbonyl absorption region with light polarized perpendicular (dotted line) and parallel (continuous line) to the ROS axis, b) ROS spectra recorded in the methyl absorption region with light polarized perpendicular (dotted line) and parallel (continuous line) to the ROS axis.

Spectra in the 1500-1800 cm^{-1} region show marked dichroism as expected from a strongly anisotropic system like the ROS. The band corresponding to the Amide I transition is polarized along the ROS axis, perpendicular to the flat surface of disks, whereas the band corresponding to the Amide II transition shows stronger polarization in the orthogonal direction, within the plane of the disks.

Measurement of polarization ratios provides a measure of the average angle, γ, between the axis of α-helical structures and the axis of the ROS [2]. The orientation of α-helices within the ROS can be described by a value of $\gamma = 30°$. Rhodopsin is a predominantly α-helical protein and its abundance within the ROS makes it the dominant contributor to Amide I linear dichroism in the α-helical region. Therefore we propose that γ be interpreted as a quantitative measure of the average angle formed specifically by the α-helices of rhodopsin with the ROS longitudinal axis. For comparison and to validate the measurement, we derived γ from the crystal structure of rhodopsin (PDB entry 1F88) [3], providing a value of $\gamma = 24°$.

The orientation of rhodopsin α-helices has been previously measured by FTIR VLD using reconstituted disks or ensembles of fractured ROS. These works reported values for γ ranging from 38º to 51° [4, 5]. To date our results, obtained *in vivo*, provide the closest agreement between a spectroscopic measurement of γ for rhodopsin α-helices and the crystallographic value.

Spectra in the 2700-3100 cm^{-1} region also show remarkable dichroism, in particular for methylene symmetric and antisymmetric stretching bands, at 2852 cm^{-1} and 2924 cm^{-1} respectively, and for the stretching vibration of C-H bonds on trigonal carbons at 3012 cm^{-1}.

These results show ROS disk membranes as relatively ordered systems on the molecular scale, more than previously reported from FTIR studies on purified disks and membrane fragments [5]. The ROS is known to be a highly anisotropic system at the nanometre scale, thanks to the ordered stacking of disk membranes. Our results indicate that an ordered structure with axial symmetry extends down to the molecular level, and characterizes both the orientation of rhodopsin molecules and the acyl chains of phospholipids.

Conclusion

We have shown the feasibility of a VLD study on an intact cell using synchrotron FTIR spectromicroscopy. The type of spectroscopic study reported in this work is traditionally carried out on samples of purified proteins and lipids, or fragments of biological membranes. This involves extraction, purification and reconstitution of sample components, making sample preparation lengthy and often difficult and expensive, in particular for membrane proteins. In addition, the condition and stability of reconstituted biomolecules is always a major issue in interpreting spectroscopic results and extending conclusions to the situation *in vivo*. By performing FTIR spectromicroscopy directly on the intact cell we bypass all these issues, providing a simpler and cheaper way to probe molecular structure and function in a native environment

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