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Infrared Microspectroscopy of Individual Human Cervical Cancer (HeLa) Cells

Melissa Romeo, Christian Matthäus, Milos Miljkovic, and Max Diem

Departments of Chemistry and Biochemistry, Graduate School and University Center, City University of New York, 365 Fifth Avenue, New York, NY 10036

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

Abstract

We report infrared (IR) spectra observed for individual, cultured human cervical cancer (HeLa) cells. Spectra were collected microscopically, in reflection/absorption modes, from cells deposited and dried on microscope slides or from cells grown directly on slides. Within the spectra of the dried cells, significant spectral heterogeneity exists that was previously attributed to different stages of the cell cycle [Boydston-White. S., et al., *Biospectroscopy* 1999, 5, 219–227; Holman, H. Y., et al., *Biopolymers Biospectrosc* 2000, 57, 329–335; Tobin, M. J., et al., in *First BASIE Workshop*, Karlsruhe, Germany, 2003]. The results reported here confirm earlier findings and present the possibility of determining the abundance of cells within each stage of the cycle from the IR spectra. In an accompanying paper, we show that the spectra of cells in suspension exhibit spectral intensity distributions that are different from that of the dried cells. This result has far-reaching implications for the use of infrared microspectroscopy to screen dried cell preparations for the presence of abnormal cells.

Keywords

infrared microspectroscopy; cultured cervical cancer cells; cell cycle

INTRODUCTION

Fourier transform infrared microspectroscopy (FTIR-MSP) has emerged as a powerful tool for the identification of pathogenic microorganisms¹ and for the detection of tissue architecture and/or malignant abnormalities.² For the former application, databases of well over 1000 microorganisms have been collected. The sensitivity of FTIR-MSP is sufficiently high to distinguish drug-resistant from drug-sensitive bacterial strains and to differentiate different bacteria to the strain level.

The analysis of eukaryotic cells, however, is more difficult. Early reports of differentiation between normal and dysplastic cervical cell exfoliates, using IR spectra averaged over thousands of cells, were based on a fortuitous correlation³ and were subsequently refuted.⁴ Unfortunately, studies based on this fortuitous correlation are still being published.⁵ A more thorough approach toward understanding the spectra of eukaryotic cells, including the first indication of cell-cycle-dependent spectral changes, was reported in 1998.⁶ This work was

based on separation of cells according to stages within the cell cycle by elutriation and identification of the cell cycle stages via fluorescence activated cell sorting (FACS). Since these procedures are quite involved, a simpler way to differentiate growth phase, based on cell size, was proposed by Holman et al.⁷ The understanding of cell-growth-related effects is required for an eventual cytological application of infrared (IR) microspectroscopy. This article is a preliminary report on the spectral variance of the individual cells.

EXPERIMENTAL

Cell Culture/Sample Preparation

HeLa cells (cell line CCL-2, ATCC, Manassas, VI) were grown in 75-cm² cell culture bottles (Sigma–Aldrich, Milwaukee, WI) at 37°C in a 5% CO₂ atmosphere in minimum essential Eagle's medium supplemented with 10% (v/v) fetal calf serum (both from ATCC). Cells were removed from the bottles by trypsin–EDTA (ATCC), washed repeatedly, and resuspended in BSS (ATCC⁸). In order to prepare samples of dried cells, they were deposited onto low-e slides (see below) using a CytoSpin4 centrifuge (Thermo Sheldon, Inc., Pittsburgh, PA) from 0.5 mL of cell suspension in BSS, containing an estimated 2×10^5 cells/mL.

Low-e slides (Kevley Technologies, Chesterfield, OH) are glass microscope slides that are coated with a thin Ag/SnO₂ layer. They are chemically inert and nearly transparent to visible light. However, they reflect midinfrared radiation nearly completely. Thus, they are an ideal (and inexpensive) substrate for reflection/absorption infrared microspectroscopy, since visual images and IR spectra can be collected from the same sample.

Spectral Measurements

Single cell data presented here were recorded in reflection/absorption or transmission mode. In the former, the IR beam passes the sample, is reflected by the thin silver layer on the slide, and passes the sample again. In transmission mode, samples are on IR-transparent windows (CaF₂). Reflection/absorption spectra reported here were recorded using either an IlluminatIR infrared microspectrometer, manufactured by SensIR, Inc. (Danbury, CT), or a Spectrum One/Spotlight 300 (Perkin–Elmer, LLC, Shelton, CT). Both instruments have been described, and their performance has been compared.⁹

Spectra recorded using the IlluminatIR employed fixed circular apertures of 12, 25, 50, and 100 μm diameter. The apertures were selected to match the cell's size as closely as possible. No variation of the spectral pattern with the aperture size was detected (see below). Depending on the aperture size, between 100 and 1200 interferograms, collected at 4 cm⁻¹ spectral resolution, were coadded and Fourier transformed. At 4 cm⁻¹ resolution, 600 interferometer scans can be collected in less than 5 min.

Spectra collected using the Spotlight system were recorded either in imaging mode at 6 μm spatial resolution or using the single element detector with the aperture set to straddle the individual cells. Sixty-four interferograms, collected at 4 cm⁻¹ resolution, were coadded in each mode of data acquisition. The total number of individual spectra collected to date, using both instruments, is about 500.

RESULTS AND DISCUSSION

Figure 1A shows a typical section (ca. 250 $\mu\text{m} \times 200 \mu\text{m}$) of a microscope slide preparation of dried HeLa cells. During the drying procedure, the cells preserve their integrity quite well and range in diameter from about 15 to over 45 μm . However, their thickness decreases to below 10 μm , based on the amide I absorption strength (vide infra). Selected baseline corrected,

unnormalized spectra are shown in Figure 1B. These spectra reproduce those reported by Holman et al. very well.⁷

First, the dependence of spectra on the selected aperture size will be discussed. If the aperture is significantly smaller than the size of the cells, different compartments of the cell will produce different spectra (such as the ones reported previously in cell mapping experiments^{10,11}). On the other hand, an aperture much larger than the cell's dimension will reduce the amplitude of the spectrum, since the cell's spectrum is averaged into a larger area devoid of any signal. In this case, a normalization of the spectrum produces spectral traces that are identical within the noise limit.

Next, we discuss the overall intensity and intensity variations of the observed spectra in the mid-IR range. This intensity will depend on the thickness of the dried cells and other factors. When in suspension, these cells are approximately spherical in shape. Upon drying, they become quite thin; however, one may expect that large cells form a thicker layer than small cells. Thus, large cells would exhibit a more intense absorption spectrum than small cells. This is not the case: when cells are grouped by size, each group shows a variation of about an order of magnitude in integrated absorption intensity. This is summarized in Table I. These variations must be caused by differences in the concentration of biochemical components. Indeed, Holman et al.⁷ postulated that the cells with the highest absorption values (such as the unusual spectrum depicted by blue trace in Figure 1B) were due to cells in mitosis and that a simple correlation existed between cell size and spectral patterns observed for them. While we agree with the overall spectra observed for cultured cells, we do not find the same correlation with the cell size (*vide infra*). However, Holman et al.⁷ used cells grown onto a substrate, rather than spin-deposited cells, which may influence the way they dry.

We have collected in excess of 500 individual spectra of HeLa cells in reflection/absorption mode, using both instruments in the laboratory. In order to find objective means to distinguish the spectral patterns for cells found in exponentially growing cell cultures, we subjected these spectra to unsupervised multivariate methods of analysis.¹ We found that the spectra readily cluster into five distinct groups, whose mean spectra (normalized) are shown in Figure 2. These spectra differ mostly in the amide I/amide II intensity ratios and amide I to phosphate stretching intensities (ca. 1240 and 1080 cm^{-1}) and slight shifts in frequencies. Detailed results of the cluster analysis of these and other cell types will be reported at a later date.

In previous studies,^{6,7} similar variations in the amide I/amide II intensities and an increase in the phosphate intensity have been reported. In both studies, the spectral changes were correlated to the progression of a cell through the division cycle by FACS analysis. Here, we present results from a much larger and still increasing data set. Within this set of hundreds of spectra, we observed that the simple size/spectral pattern does not hold rigorously. For example, we find that all cells that exhibit the unusual spectral pattern depicted by the blue trace in Figure 1B are, indeed, very large. However, we find that other cells of similar size may present spectral patterns devoid of the broad background seen in the blue trace. Furthermore, most small cells (presumably those in G0 or G1 phases) exhibit spectra which are quite similar, with low symmetric and antisymmetric phosphate stretching intensities (cf. green trace in Figure 1B). However, even in this subset of spectra, the overall spectral intensity varies significantly. Obviously, further identifiers are needed to determine the exact cell cycle stage at which a cell is trapped in the drying process.

CONCLUSIONS

We demonstrate that it is feasible to collect high-quality mid-IR data of individual human cells without the use of synchrotron light sources. Large spectral heterogeneity is observed for HeLa

cells spin-deposited onto low-e microscope slides. This heterogeneity appears to be related to the cells' progression through the cell cycle; however, we do not find a clear correlation of the dried cells and their spectral patterns.

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FIGURE 1.

A: Photomicrograph of HeLa cells spin-deposited onto low-e slides from BSS buffer. The area shown measures about $250 \times 200 \mu\text{m}^2$ and contains about 50 cells. B: Typical mid-IR spectra of selected cells. See text for data acquisition conditions.

FIGURE 2.

Mean cluster spectra obtained from unsupervised hierarchical cluster analysis of about 350 individual spectra from dried HeLa cells. Spectra demonstrate the increase in amide I/amide II intensity ratio and the increase in phosphate intensities observed previously.

Table I

Variation of Amide I Intensity (in Absorbance Units) with Cell Size

	<20 μm	20–30 μm	30–40 μm	>40 μm
Number of cells	29	165	101	22
Average amide I intensity	0.010	0.0091	0.011	0.0132
Std Dev amide I intensity	0.0035	0.0045	0.0063	0.0083
Minimum and maximum amide I intensity	0.0020, 0.016	0.0023, 0.0208	0.0015, 0.0337	0.0030, 0.0355