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Analysis of the DNA Fourier transform-infrared microspectroscopic signature using an all-reflecting objective

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ABSTRACT

The Fourier transform-infrared (FT-IR) signature of dry samples of DNA and DNA-polypeptide complexes, as studied by IR microspectroscopy using a diamond attenuated total reflection (ATR) objective, has revealed important discriminatory characteristics relative to the PO_2^- vibrational stretchings. However, DNA IR marks that provide information on the sample's richness in hydrogen bonds have not been resolved in the spectral profiles obtained with this objective. Here we investigated the performance of an "all reflecting objective" (ARO) for analysis of the FT-IR signal of hydrogen bonds in DNA samples differing in base richness types (salmon testis vs calf thymus). The results obtained using the ARO indicate prominent band peaks at the spectral region representative of the vibration of nitrogenous base hydrogen bonds and of —NH and —NH_2 groups. The band areas at this spectral region differ in agreement with the DNA base richness type when using the ARO. A peak assigned to adenine was more evident in the AT-rich salmon DNA using either the ARO or the ATR objective. It is concluded that, for the discrimination of DNA IR hydrogen bond vibrations associated with varying base type proportions, the use of an ARO is recommended.

1. Introduction

Infrared (IR) spectroscopy of solid samples, currently performed with modern modular spectrometers associated with light microscopy devices, has proved useful for several purposes pertinent to biological investigations. IR spectral signatures obtained with a Fourier transform algorithm (FT-IR), which turns the raw data into a spectrum, provide information on the vibrational characteristics of a sample's chemical functional groups. IR absorption peaks at specific wavenumber ranges may reveal bonds or groups of bonds that tend to vibrate at characteristic frequencies. Mathematical analysis of FT-IR profiles using appropriate softwares may allow identification of complex molecular structures. Details on the theory and applications of FT-IR have been discussed extensively elsewhere (Griffiths and Haseth, 2007).

The infrared absorption profile and marker bands of dry samples of DNA and DNA–protein complexes obtained using an IR microspectrometer with a diamond attenuated total reflection (ATR) objective have recently been reported to support forthcoming interpretation of FT-IR signatures of chromatin (Mello and Vidal, 2012). The FT-IR spectral profiles obtained have proved to be important tools for establishing the vibrational properties of these chromatin components. However, IR spectral differences between DNA types containing different nitrogenous base proportions (for instance, AT-biased DNA vs plurimodal base DNA) in some FT-IR profile regions, including the region associated with hydrogen bonds have not yet been resolved using the ATR objective for dry samples. The examination of samples on gold-covered slides using an "all reflecting objective" (ARO) has been recommended for certain FT-IR analyses to avoid direct contact with the preparations (Whitley et al., 2009). However, no data on the IR spectral regions associated with hydrogen bonds for dry DNA samples has been reported using the ARO.

In the present study, we investigated the performance of an ARO objective for analysis of the FT-IR spectral profiles of dry samples of DNA bearing different base richness, with emphasis on the spectral region representative of the vibration of hydrogen bonds.

2. Materials and methods

2.1. Materials

Samples of double-stranded DNA derived from calf thymus and salmon testis (Sigma–Aldrich, St. Louis, USA) differing in base

Abbreviations: ARO, all reflecting objective; AT, adenine thymine; ATR, attenuated total reflection; FT-IR, Fourier transform-infrared; IR, infrared; ν_{as}, antisymmetric vibration; ν_s, symmetric vibration.

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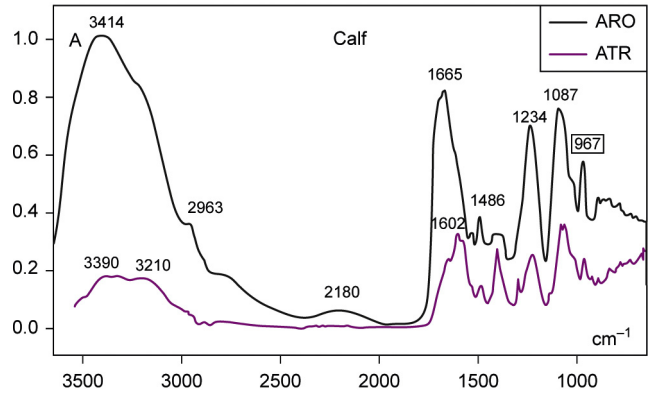

Fig. 1. FT-IR spectral profile of calf thymus DNA using the ARO (black line) and the ATR (purple line) objectives.

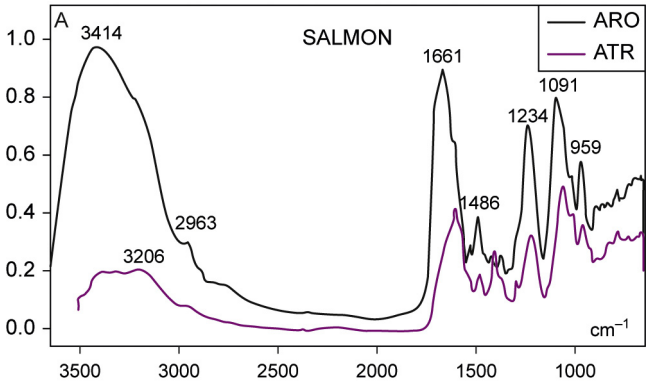

Fig. 2. FT-IR spectral profile of salmon testis DNA using the ARO (black line) and the ATR (purple line) objectives.

richness (calf DNA, plurimodal; salmon DNA, AT-biased) (Chargaff et al., 1951; Pivec et al., 1972) were prepared as previously described (Mello and Vidal, 2012) and spread on glass slides for analysis with the ATR objective or on gold-covered glass slides for analysis with the ARO.

2.2. Equipment/software

The DNA FT-IR spectral profiles were obtained using the Illuminat IR II^{TM} microspectrometer (Smiths Detection, Danbury, USA) equipped with a liquid nitrogen-cooled mercury-cadmium-telluride (MCT) detector; Olympus microscope; ARO (16×), ATR diamond objective (36×); and Grams/AI 8.0 spectroscopy software (Thermo Electron Co., Waltham, USA). The performance validation of the equipment used a low signal-to-noise ratio (7929:1) (Vidal and Mello, 2011). The measurement site area was 50 μm per side; the absorbances of the samples and background were measured using 64 scans for each preparation.

2.3. Procedures

The spectral absorption signatures were obtained at wavenumbers ranging from 4000 cm^{-1} to 650 cm^{-1}, with a spectral resolution of 4 cm^{-1}. Ten spectral profiles were obtained for each sample. Baseline correction and normalization with respect to the highest peak were performed for each spectral profile; an average profile was then calculated for each sample using the Grams software. When pertinent for comparisons, peak fitting and peak fitting "estimate" procedures using a Gaussian function and low sensitivity level were subsequently applied to spectral regions of absorption band peaks, using the Grams software. This software allowed the area of the selected bands to be calculated using the trapezoidal rule of integration and the center of mass, defined as the *X* coordinate of the point where the peak areas are equal on either side, to be estimated.

3. Results and discussion

The absorbance intensities for both the calf and salmon DNA FT-IR profiles obtained with the ARO were higher than those obtained with the ATR objective (Figs. 1 and 2). The FT-IR average profile of the calf DNA using the ATR objective as shown in Fig. 1 had been previously reported (Mello and Vidal, 2012). In contrast to results obtained with the ATR objective, the most prominent band peak obtained using the ARO (~3420–3410 cm^{-1}) was found at the spectral range associated with hydrogen bonds for both DNA types (Figs. 1–3). When the DNA FT-IR profile at this spectral region was analyzed using the peak fitting procedure of the Grams software, 17 and 19 peaks were revealed for the calf and the salmon DNA, respectively (Fig. 4a and c). The peak fitting "estimate" resolved all these peaks into one peak positioned at ~3340 cm^{-1} for the calf thymus DNA and at ~3337 cm^{-1} for the salmon testis DNA, revealing practically no difference in the electromagnetic spectral frequency at such spectral region between these DNA types (Fig. 4b and d). Even the band center of mass did not much differ when comparing the two DNA types to each other (Table 1). However, the total area of the absorption band peak which includes the vibrational region of the hydrogen bonds and the region associated with the stretching vibration of —NH and —NH_2 groups (3600–3000 cm^{-1}), was found to be higher for the calf thymus DNA using the ARO contrasting with results obtained using the ATR objective (Table 1).

When using the ATR objective the spectral profile for the calf DNA at 3539–2991 cm^{-1} showed absorbances smaller than those obtained for the salmon DNA (Figs. 1 and 2). Using the peak fitting procedure of the Grams software, 9 and 29 peaks were revealed in this case for the calf and the salmon DNA, respectively (data not shown). The peak fitting "estimate" resolved these peaks into one peak positioned at 3280 cm^{-1} for the calf thymus DNA and at 3261 cm^{-1} for the salmon testis DNA (Table 1).

According to Eglinton (1970), most of the IR light absorption bands observed in the 4000–650 cm^{-1} spectral region correspond to the "quantized uptake of energy into the fundamental vibration modes of the molecules under study". In magnetic resonance spectroscopy, the area of the signal strength is directly related to the number of absorbed photons (Eglinton, 1970). For gas IR absorption, the quantity used for comparison with theory is the integrated absorption coefficient for unit pressure, where the integration is carried out over the frequency range covered by a given band (Wilson and Wells, 1946). Yugami et al. (1996) have determined

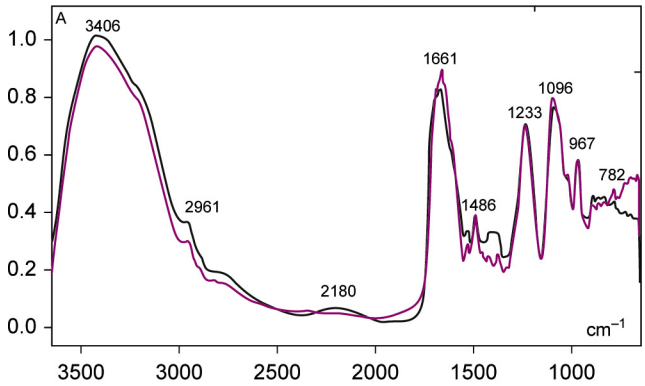

Fig. 3. FT-IR spectral profile of the salmon DNA (purple line) compared to that of the calf DNA (black line) using the ARO.