Collective Vibrational Modes in Biological Molecules Investigated by Terahertz Time-Domain Spectroscopy

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ABSTRACT: We present well-resolved absorption spectra of biological molecules in the far-IR (FIR) spectral region recorded by terahertz time-domain spectroscopy (THz-TDS). As an illustrative example we discuss the absorption spectra of benzoic acid, its monosubstitutes salicylic acid (2-hydroxy-benzoic acid), 3- and 4-hydroxybenzoic acid, and aspirin (acetylsalicylic acid) in the spectral region between 18 and 150 cm^{-1}. The spectra exhibit distinct features originating from low-frequency vibrational modes caused by intra- or intermolecular collective motion and lattice modes. Due to the collective origin of the observed modes the absorption spectra are highly sensitive to the overall structure and configuration of the molecules, as well as their environment. The THz-TDS procedure can provide a direct fingerprint of the molecular structure or conformational state of a compound. © 2002 Wiley Periodicals, Inc. Biopolymers (Biospectroscopy) 67: 310–313, 2002

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INTRODUCTION

Vibrational spectroscopy is widely used as a powerful technique to investigate the thermodynamic properties and structure of molecules. The absorption spectrum arising from vibrational motions of a molecule in its electronic ground state shows bands in the IR region. The resonance frequency of an individual vibrational mode is determined by the reduced mass and the curvature of the potential energy surface along the respective vibrational coordinate of the system. The weaker the potential forces and the larger the moving masses are, the lower are the vibrational frequencies. This implies that, for example, strongly localized stretching vibrations have resonance frequencies in the mid-IR whereas out of plane vibrations or torsions involving larger numbers of atoms are resonant in the far-IR (FIR) range.

Because of the collective nature of modes in the FIR, their position and strength is highly sensitive to the conformation and structure of the molecule and its environment. In addition to internal motion, the intermolecular modes (e.g., vibrations and torsions around hydrogen bonds in the presence of dimers) or lattice vibrations in the case of crystalline samples may contribute to the FIR absorption spectrum. This illustrates the importance of this spectral range, providing a unique

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fingerprint of the conformational state of molecules.

Due to considerable experimental difficulties and the complexity of theoretical models, only a small amount of work on recording and interpreting FIR spectra of large molecules with biological relevance has been done compared to the extensive work in the higher frequency region.

Terahertz time-domain spectroscopy (THz-TDS)[1] has proven to be a versatile tool in physics and chemistry for spectroscopy on a wide variety of different samples in the FIR. It was recently applied to investigate biomolecules like retinal chromophores[2] or DNA.[3] The THz-TDS procedure relies on the coherent generation and electric field sensitive detection of an ultrashort probe pulse with a correspondingly large bandwidth covering the spectral region from 0.1 to 5 THz (3–167 cm^{-1}). A terahertz pulse represents a broadband light source in the FIR range with a pulse duration of less than 1 ps, which is also applicable in transient FIR spectroscopy with a time resolution well into the femtosecond regime.[4–6]

Recently we demonstrated the capability of this technique to distinguish between different isomeric configurations of retinal chromophores.[2] In this article we present the absorption spectra of benzoic acid and its monosubstituted derivatives 2-, 3-, and 4-hydroxybenzoic acid. In spite of their similarity these molecules show individual absorption bands as a fingerprint of their structure. We also recorded the absorption spectrum of the closely related aspirin (acetylsalicylic acid). Our results may be useful for medical diagnostics because the distinct FIR fingerprints allow a clear discrimination between aspirin and its breakdown product 2-hydroxybenzoic acid.

METHODS AND MATERIALS

Benzoic acid, 2-, 3-, and 4-hydroxybenzoic acid, and acetylsalicylic acid were purchased from Sigma–Aldrich Co. and used without further purification. Samples were prepared by mixing the dry powder with polyethylene powder at a mass ratio of approximately 1:2.5 and by pressing the mixture to disks of thicknesses between 400 and 510 μm by applying a pressure of 400 bar. Polyethylene is nearly transparent in the FIR and is therefore a suitable material for spectroscopic applications in this spectral region.

We use a standard terahertz spectrometer setup[1,7] as illustrated in Figure 1. Laser pulses (40 fs) from a titanium:sapphire oscillator are used to drive two photoconductive antennas. One emits short pulses of terahertz radiation, and the other acts as a gated detector monitoring the temporal shape of the radiated terahertz field. Silicon lenses and paraboloidal mirrors are used to collimate, guide, and focus the terahertz beam through free space and the sample onto the detector. The sample is mounted in a cryostat equipped with Teflon windows, which are transparent for terahertz radiation. The temperature is measured near the sample with a calibrated silicon diode. The cryostat can be moved so that the terahertz beam passes through either the sample or an empty aperture identical in size to the clear aperture of the sample. The terahertz beam path is purged with dry nitrogen to minimize absorption by water vapor. The setup has a useful bandwidth of 3–150 cm^{-1} (100 GHz to 4.5 THz) and a spectral resolution of better than 1 cm^{-1}. The inset in Figure 1 shows a typical terahertz pulse and its corresponding frequency spectrum recorded with and without a benzoic acid sample in the beam path.

RESULTS AND DISCUSSION

The electric field of the terahertz pulse transmitted through a sample is modified by dispersion and absorption of the sample material. The ratio of the electrical field strengths before E_r and after transmission E_s is given by $E_s/E_r = T(n) \cdot \exp\{-\varepsilon K d + in\omega d/c\}$, where d is the thickness and K is the concentration of the sample, ω is the frequency of the radiation, c is the speed of light, and $T(n)$ is a factor that accounts for reflection losses at the sample surfaces. Both the refractive index n and the molar absorptivity ϵ (i.e., the complete dielectric function of the sample) are evaluated from the ratio of the measured terahertz fields.

In Figure 2 we show the absorption spectra of benzoic acid and its 2-, 3-, and 4-hydroxy substitutes recorded at 10 K. Below 20 cm^{-1} oscillatory features due to multiple reflexions occur. Above 125 cm^{-1} the noise increases significantly and dominates the spectra. Narrow features at 101 and 127 cm^{-1} that are attributable to residual water vapor absorption are superimposed on the broader absorption bands. All spectra show a small background increase with frequency due to scattering.

There is a pronounced difference between the

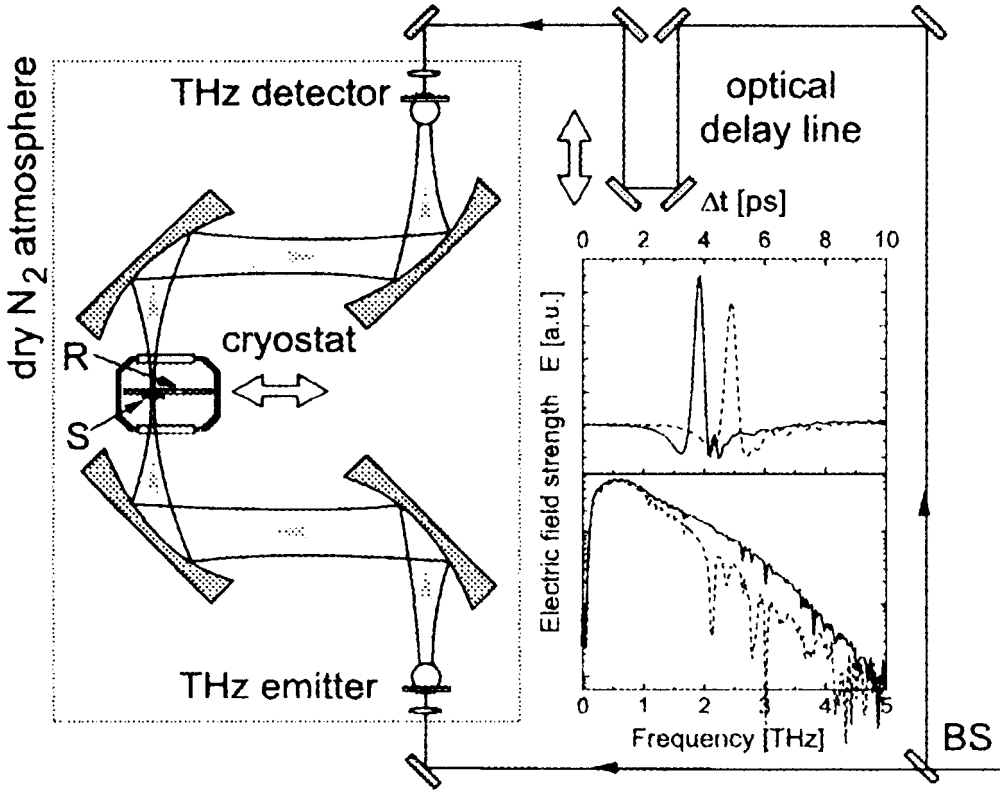

Figure 1. The terahertz spectrometer setup with the cryostat containing reference (R) and sample (S) apertures in the terahertz beam path. The inset shows a typical terahertz pulse and its corresponding frequency spectrum after transmission through (—) the empty aperture and (- - -) the benzoic acid sample, respectively, at 10 K.

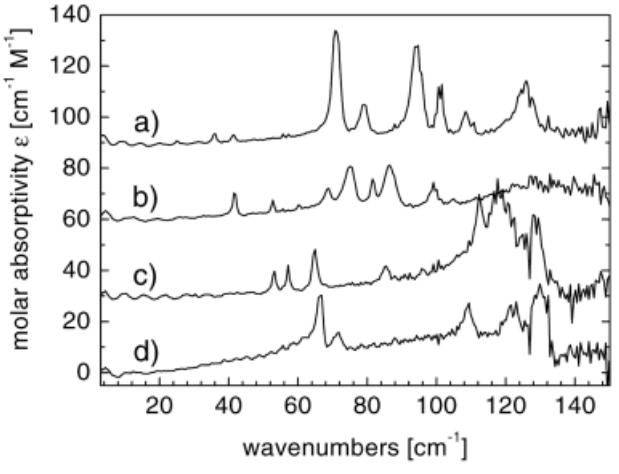

Figure 2. The absorption spectra of benzoic acid (spectrum a), 2-hydroxybenzoic acid (salicylic acid, spectrum b), 3-hydroxybenzoic acid (spectrum c), and 4-hydroxybenzoic acid (spectrum d) at 10 K. The traces are vertically offset from each other by 30 cm^{-1} for better representation.

four spectra in spite of the very similar molecular structures. This indicates the importance of this spectral range as a conformational fingerprint region where even minor changes in the molecular configuration lead to major differences in its FIR absorption. The current sensitivity of the experiment allows detection of amounts as small as 5 $\times 10^{-8}$ mol of the molecule in the beam path.

An assignment of the different modes is dramatically complicated by the fact that benzoic acid and its derivatives form dimers that are arranged in a crystal structure in the solid phase.[8] A thorough analysis and assignment of the individual FIR absorption bands of benzoic acid was carried out by Zelsmann and Mielke.[9] Their assignment attributes the modes below 72 cm^{-1} to translations between the dimers (i.e., lattice vibrations) and the modes at higher frequencies to internal dimer modes, namely, torsions, out of plane bends, and asymmetric stretches of the hydrogen bonds. They find the lowest intramolecular mode at around 188 cm^{-1}, which is outside of our range.

The temperature dependence of the absorption bands can be used for assignment purposes. Specifically, it can provide information on the shape of the potential energy surfaces governing the individual molecular dynamics. The absorption spectrum of benzoic acid at different temperatures is shown in Figure 3. The explicit behavior will be discussed elsewhere.[10] Generally, line profiles shift to lower frequencies and broaden as the temperature is increased due to the anharmonicity of the potential.

Figure 4 shows the FIR absorption spectrum of aspirin. We find striking similarities to the absorption of benzoic acid. The reason for this observation is yet unknown and will be the subject of further studies. The main difference in the accessible spectral range is a strong feature at low frequencies between 50 and 62 cm^{-1}. This band is likely to have its origin in dynamics involving the acetyl group because it is absent in the spectra of benzoic and salicylic acid.

CONCLUSION

In this article we report measurements of the FIR absorption spectra of benzoic acid and its derivatives 2-, 3-, and 4-hydroxybenzoic acid and aspirin by THz-TDS. We observe well-resolved absorption features due to collective intra- and intermolecular motion and show their strong sensitivity to the structure of the molecules. This illustrates how FIR spectroscopy provides a fingerprint of the molecular conformation. The distinct band structure is already visible at liquid nitrogen temperature, allowing a rather inexpensive experimental effort. It must be pointed out that THz-TDS is an optical technique that can pinpoint the molecular structure without using visible light, thereby avoiding effects like photobleaching in light-sensitive materials.

The observed distinct differences between these molecules may be useful in drug tracing applications where the detection of small amounts of a substance is needed.

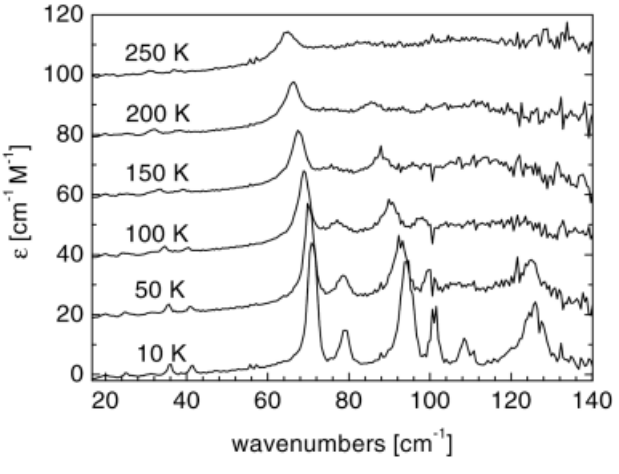

Figure 3. The temperature-dependent absorption of benzoic acid (drawn with an offset for better representation).

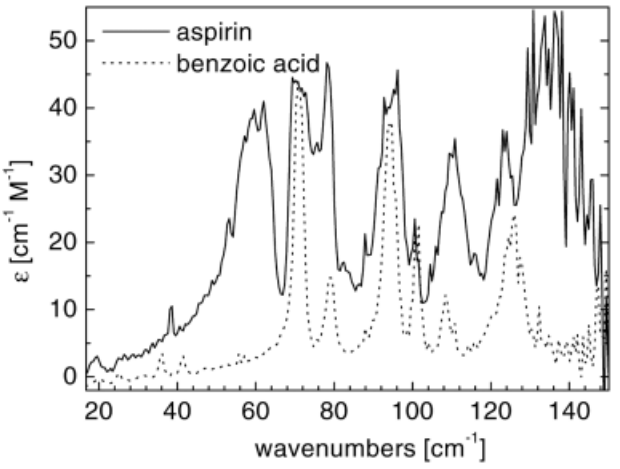

Figure 4. The absorption spectrum of aspirin and of benzoic acid at 10 K.

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