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Laser Spectroscopy

Conformation-Specific Circular Dichroism Spectroscopy of Cold, Isolated Chiral Molecules**

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Abstract: *The CD spectroscopy of a chiral compound in solution yields an average CD value derived from all of the conformations of a chiral molecule. By contrast, CD spectroscopy of cold chiral molecules in the gas phase distinguishes specific conformers of a chiral molecule, but the weak CD effect has limited the practical application of this technique. Reported herein is the first resonant two-photon ionization CD spectra of ephedrines in a supersonic jet using circularly polarized laser pulses, which were generated by synchronizing the oscillation of the photoelastic modulator with the laser firing. The spectra exhibited well-resolved CD bands which were specific for the conformations and vibrational modes of each enantiomer. The CD signs and magnitudes of the jet-cooled chiral molecules were very sensitive to their conformations and thus offered crucial information for determining the three-dimensional structures of chiral species, as conducted in combination with quantum chemical calculations.*

The building blocks of living organisms are molecules of identical chirality. For instance, all amino acids except glycine have levorotatory-type chirality, which gives rise to the enantiomeric selectivity of proteins. This enantioselectivity facilitates the incorporation of molecules of a specific chirality throughout biological reactions, thus maintaining the homochiral nature of life.[1] This enantioselectivity occurs through noncovalent interactions in weakly bound chiral molecular complexes. To understand those weak, specific interactions at the molecular level, many high-resolution spectroscopy studies have been performed to identify the structures of these complexes under solvent-free conditions.[2,3] However, conventional laser spectroscopy cannot be used to unambiguously determine the structures of highly complex, large, flexible chiral complexes which are conformationally diverse.

The absorption coefficients of a chiral molecule differ for left- and right-handed circularly polarized (LCP and RCP) light. This phenomenon is known as circular dichroism (CD). Since the first observation of CD in 1895,[4] CD spectroscopy has been used extensively to investigate the structures and structural changes of chiral compounds in various reactions.[5,6] However, most CD spectra have been measured in solution, where molecules exist in many conformations; thus, the values obtained represent averaged CD values. CD spectroscopy of jet-cooled chiral molecules is a promising tool for elucidating conformation-specific configurations but has not been sufficiently developed for practical use.

A few researchers have reported CD spectra of gaseous molecules using a gas cell in a CD spectrometer.[7–10] The CD values of molecules in a supersonic jet have also been measured at selected wavelengths using circularly polarized (CP) laser pulses generated through a Fresnel rhomb (FR) or quarter-wave plate (QWP).[11–14] However, CD spectra of jet-cooled chiral molecules over a broad wavelength range have not been reported, primarily because existing CD techniques are inadequate for measuring the weak CD effect for low-density jet-cooled molecules while scanning the laser wavelength. For instance, to generate LCP or RCP pulses with FR or QWP, FR or QWP must be rotated by −45° or +45° from the axis of laser polarization, and this rotation is not possible on a pulse-to-pulse basis. This pulse-to-pulse alternation between LCP and RCP pulses is necessary to reduce signal fluctuations from short- and long-term variations of laser-pulse energy and gas density. To circumvent this difficulty, we used a photoelastic modulator (PEM).

The PEM is used to modulate the polarization of continuous light in a conventional CD spectrometer with wide wavelength coverage. The birefringence of the optical element in the PEM oscillates at its natural frequency as a result of the photoelastic effect,[15,16] thus altering the retardation value either from $+\lambda/4$ to $-\lambda/4$ or from $+\lambda/2$ to $-\lambda/2$, depending on the choice of the oscillation amplitude.

Zare and co-workers[17] first combined a PEM with a nanosecond (ns) laser to generate LCP and RCP pulses at a fixed wavelength. Later, Bonmarin and Helbing[18] developed a technique synchronizing the PEM oscillation with femtosecond laser pulses at 1 kHz for transient vibrational CD measurements. However, we determined that this technique is not appropriate for use with a ns laser at a low repetition rate because of the time jitter problem. Here, we modified this technique to generate ns CP laser pulses alternating between left and right on a pulse-to-pulse basis over a wide wavelength range. By using those pulses, we obtained the first well-resolved, resonant two-photon ioniza-

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tion (R2PI) CD spectra of (1*S*,2*S*)-(+)- and (1*R*,2*R*)-(−)-pseudoephedrine (*S*- and *R*-pED) and (1*R*,2*S*)-(−)-ephedrine (*R*-ED) produced in a supersonic jet.

pED and ED are well-known neurotransmitters and differ from each other at only one of two chiral centers. However, these molecules have very different physical and physiological properties, thus emphasizing the role of chirality.[19] In addition, pED and ED contain a UV chromophore, and their CD values in the UV region are similar to those of aromatic organic molecules and are well-suited to our purpose of verifying the feasibility of R2PI CD spectroscopy for jet-cooled molecules.[5,20,21]

The R2PI CD spectra of pED and ED exhibit well-resolved CD bands which are specific for conformations as well as vibrational modes. Theoretical calculations based on time-dependent density functional theory (TDDFT) were performed to reproduce the conformation-specific CD bands.

Figures 1 a and 1b show the experimental set-up and the scheme of synchronization between the PEM oscillation and the laser pulse, respectively (see the Experimental Methods in the Supporting Information). Figure 2 a shows the R2PI spectrum of *S*-pED obtained by monitoring the fragment ion at m/z 58. The parent ion at m/z 165 was not detected owing to efficient fragmentation following R2PI, as reported previously.[19,22] The spectrum exhibits three strong bands, which were assigned previously as the origin bands of conformers AG(a), AG(b), and GG(a).[22] Figure 2 b presents the CD spectra of *S*- and *R*-pED obtained by recording the difference between the ion signals produced by the LCP and RCP pulses. Both spectra exhibit well-resolved CD bands which are mirror images of each other. The CD bands for unlabeled weak vibronic bands in Figure 2 a were not observed because of their small CD values. The asymmetry factors $g = 2(I^{LCP} - I^{RCP})/(I^{LCP} + I^{RCP})$ at the origin bands of AG(a), AG(b), and GG(a) of *S*-pED were measured as -0.026 ± 0.005, $+0.025 \pm 0.006$, and -0.024 ± 0.005, respectively. Here, I^{LCP} and I^{RCP} represent the ion signals produced by LCP and RCP pulses, respectively. Interestingly, not all CD bands of *S*- or *R*-pED have the same signs. For instance, the CD signs for AG(a) and GG(a) of *S*-pED are negative, whereas that of AG(b) is positive. These results are in contrast to the CD spectrum in solution, which displayed only a broad negative band in the same wavelength region.[21]

The different CD signs of the different conformers of *S*- or *R*-pED might not be unexpected, considering that CD arises from the interaction between the electric (μ) and magnetic transition dipole moments (M), which vary with the molecular structure.[23] However, differences in CD signs have never been observed explicitly because most previous CD spectra have been measured in solution, in which the separation of different conformers either spatially or spectroscopically is not possible.

Figure 2 c shows the theoretical CD spectra in which the rotatory strengths (R) of the S_0–S_1 transition were calculated using TDDFT at the M06-2X/6-311++G(d,p) level of theory (see Table S1 in the Supporting Information).[23,24] The oscillator strengths as well as the relative abundances of each conformer, which were estimated from the intensities of the origin bands in Figure 2 a, were also considered in the simulation. The simulated CD spectrum is in good agreement with that obtained experimentally.

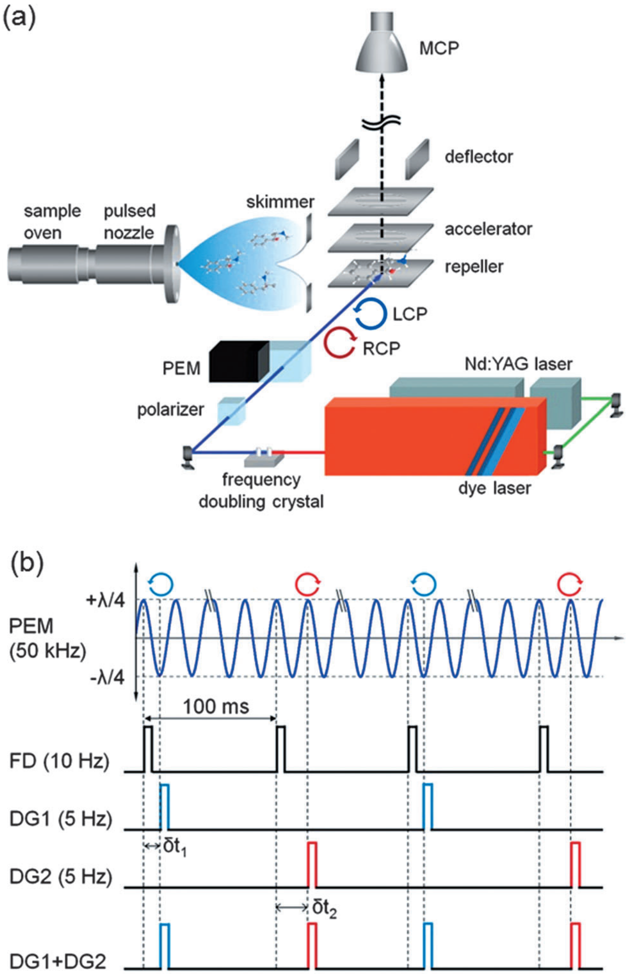

Figure 1. a) Experimental scheme. The jet-cooled molecules in the ionization region of the time-of-flight (TOF) mass spectrometer were irradiated with the frequency-doubled output from the dye laser, which had passed through the polarizer and PEM. The ions were accelerated to the field-free region and were detected by a multichannel plate (MCP). b) Synchronization scheme between the PEM oscillation and the firing of laser pulses. The 50 kHz reference pulses from the PEM were down-modulated to 10 Hz by a frequency divider (FD), then fed into two digital delay generators (DG1 and DG2) as trigger pulses. DG1 and DG2 further divided the 10 Hz pulses into 5 Hz pulses alternating with each other, and were combined into 10 Hz pulses for triggering the laser. δt_1 and δt_2 were adjusted such that the laser pulses fired by the 5 Hz pulses from DG1 and DG2 passed through the PEM when its retardation became $-\lambda/4$ and $+\lambda/4$, respectively (see Figures S1 and S2).

R, which is given as the imaginary part of the scalar product between μ and M, represents the CD value.[23] Hence, the CD sign is determined by the angle between μ and M as shown in Figure 3. This angle is slightly larger than 90° only in AG(b) of *S*-pED, thus leading to positive CD, whereas AG(a) and GG(a) have angles smaller than 90°, and give rise to negative CD. This result illustrates how subtle differences in molecular structures or electronic configurations in the S_0 or S_1 states are reflected in the CD sign.

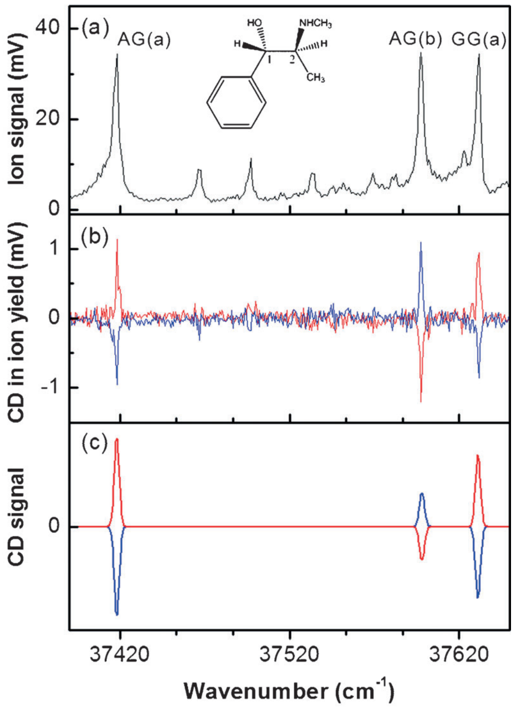

Figure 2. a) R2PI spectrum of *S*-pED near the origin band of the S_0–S_1 transition. The inset shows the structure of *S*-pED. The number of ions produced by a single-laser pulse at the origin bands was roughly estimated as about 900. b) R2PI CD spectra of *S*- (blue line) and *R*-pED (red line). c) Theoretical CD spectra of *S*- (blue line) and *R*-pED (red line) obtained with the rotatory strength, *R*, of the S_0–S_1 transition. The widths and excitation energies of the bands were adapted from those of the corresponding bands in (b).

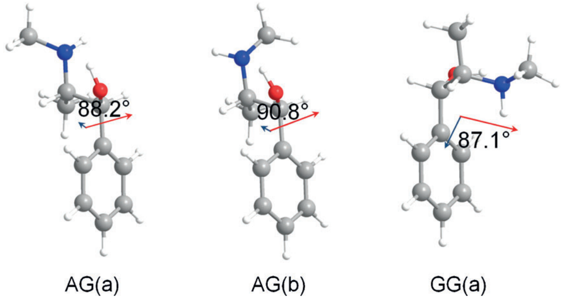

Figure 3. Optimized structures of *S*-pED conformers. The μ and M estimated using TDDFT at the M06-2X/6-311++G(d,p) level are represented as blue and red arrows, respectively, with the angle between them.

The R2PI CD spectra represent not the CD of the S_0–S_1 transition but the cumulative CD of the two-step R2PI process. In the plot of $\log I_{\text{ion}}$ versus $\log P$ (see Figure S3), in which I_{ion} and P are the fragment ion intensity at m/z 58 and the laser intensity, respectively, the slope is 2.0, indicating that two photons are involved in producing the ion. One photon excites pED to the origin band, while the other photon ionizes it. Then, the pED ion dissociates to the fragment ion with the excess energy deposited from R2PI.[22] Therefore, the agreement between the R2PI CD and theoretical CD values may imply little CD effect of the ionization process in the R2PI of pED. This small CD effect for the ionization has also been reported for the R2PI CD of a few chiral molecules and was attributed to the overlap of many different states in the transition to the continuum, thus leading to leveling or canceling of the CD effects.[12,13,25] However, further investigation on the CD effect for the ionization process is necessary to relate the R2PI CD value mainly to the CD of the S_0–S_1 transition.

To determine whether mode-specific CD values can also be measured using the same technique, we performed the experiment with *R*-ED. Figure 4a presents the R2PI spectrum obtained under basically the same experimental conditions as in Figure 2a. In contrast to pED, most of the vibronic peaks in the spectrum are from the most stable conformer of ED.[26] Peaks 1–3 were assigned as the origin band of the S_0–S_1 transition and the vibronic bands corresponding to the 6^1_0 band of benzene and the 1^1_0 band of benzene, respectively. The CD spectrum of Figure 4b exhibits clear mode specificities of the CD bands. The *g* value of peak 2 is more than twofold larger than those of peaks 1 and 3. In addition, peaks 1 and 3 have negative CD bands, whereas peak 2 has a positive CD band.

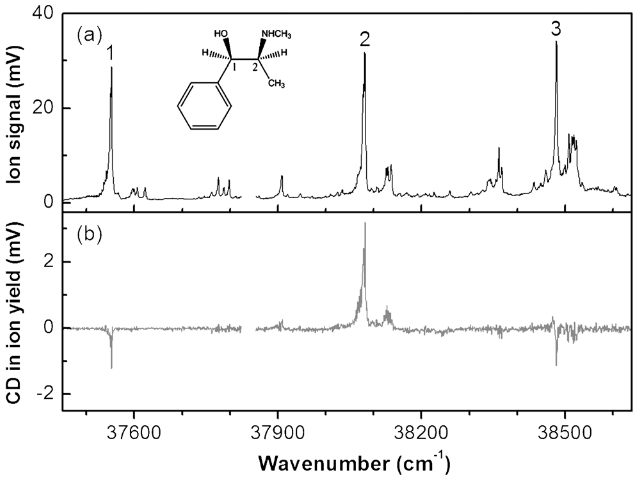

Figure 4. a) R2PI spectrum of *R*-ED near the origin band of the S_0–S_1 transition. The inset shows the structure of *R*-ED. The discontinuous region between 37825 and 37855 cm^{-1} is where the grating order of the dye laser changes. b) R2PI CD spectrum of *R*-ED. The *g* values of 1–3 bands were measured as -0.030 ± 0.011, $+0.091\pm0.007$, and -0.041 ± 0.013, respectively.

The vibronic CD is determined as the sum of contributions from the electronic transition and the vibronic coupling between the S_0 and S_1 states.[27] However, the CD of an asymmetric ring deformation mode such as ν_6 is thought to be dominated by the contribution of vibronic coupling and is responsible for the inversion of the sign in the electronic CD spectra of several chiral benzene derivatives, whereas the CD of a symmetric ring breathing mode such as ν_1 is determined by the contribution of the electronic transition.[28] Accordingly, the CD sign of peak 3, assigned as ν_1, is as negative as that of the origin band, peak 1, whereas the CD sign of peak 2, assigned as ν_6, is positive. A more detailed analysis using high-level theoretical calculations is in progress.

The experimental realization of CD spectroscopy for jet-cooled chiral molecules not only broadens the scope of CD spectroscopy but also provides a powerful tool for measuring conformation-specific and vibrational-mode-specific CD values. Furthermore, the use of this technique will facilitate the development of theory with which to compute accurate CD values. These CD values, combined with quantum chemical calculations, offer a greater possibility of determining the conformation-specific absolute configurations of chiral compounds, which is crucial to elucidate the roles of chirality in chiral recognition and thus the origin of homochirality in nature.

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