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Study on the absolute configuration of levetiracetam via density functional theory calculations of electronic circular dichroism and optical rotatory dispersion

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ABSTRACT

Theoretical calculation of chiroptical properties has been a powerful tool for the absolute configuration assignment of chiral compounds including synthetic drugs and natural products. In the present work, time-dependent density functional theory (TDDFT) calculations of electronic circular dichroism (ECD) and optical rotatory dispersion (ORD) were employed to investigate the absolute configuration of levetiracetam, which is a widely used anticonvulsant drug. Nine conformers were generated by conformation search using the MMFF94 molecular mechanics force field, and the geometries were then optimized using the Becke 3–Lee–Yang–Parr (B3LYP) exchange-correlation functional. The population-averaged ECD spectrum was obtained by adding ECD spectrum of each conformer using Boltzmann statistics. The predicted ECD spectrum is in excellent agreement with the measured ECD spectrum of levetiracetam. Theoretical ORD spectra show the same tendency as the experimental data of levetiracetam, further confirming the absolute configuration derived from the ECD spectra. Our results demonstrated that the only chiral carbon atom of levetiracetam is unambiguously to be S configuration.

1. Introduction

Different enantiomers of chiral compounds may exert different or even totally opposite functions. Enantiopure drugs have been the focus of pharmaceutical industry since they often display higher efficiency and less toxicity than racemates [1]. Therefore, determination of the absolute configuration of bioactive compounds is crucial for drug discovery and development. Many countries have introduced requirements on the identification and characterization of the stereochemistry of novel drugs. Single-crystal X-ray diffraction is conventionally regarded as the only direct way to assign the absolute configuration. However, this method requires a sizable single crystal of a pure enantiomer and the lack of heavy atoms would prevent use of Flack's methodology. Electronic circular dichroism (ECD) is a convenient and sensitive technique to detect the stereochemistry of organic and inorganic molecules [2]. A good ECD spectrum can be obtained using 0.1–1.0 mg of the sample, which can be solid or liquid. ECD is associated to the electronic transitions of molecules and often gives complex curve, which contains important stereochemical information. Thus, the interpretation of ECD spectra is pivotal to assign the absolute configuration. Several empirical rules and semi-empirical models have been established including octant rule and exciton chirality method. However, specific requirements of molecular structures limit the application of these approaches. In the past 10 years, a novel alternative methodology based on the chiroptical properties has been developed and implemented via comparison of *ab initio* quantum chemical calculations with experimental results [3–6]. Amongst all the theoretical prediction methodologies, time-dependent density functional theory (TDDFT) has been proved to be a reliable and feasible approach. Many studies on the application of TDDFT to the ECD calculation have been reported over the past few years [7]. Combined with other chiroptical properties, ECD prediction has been demonstrated to be a most valuable tool for the absolute configuration assignment of chiral compounds [8].

Levetiracetam ((S)-2-(2-oxopyrrolidin-1-yl)butanamide) obtained marketing authorization from the Food and Drug Administration (FDA) as an anticonvulsant medication used to treat epilepsy and neuropathic pain (Scheme 1) [9]. Levetiracetam has also potential benefits for other psychiatric and neurologic conditions [10]. It is the S-enantiomer of etiracetam, structurally similar to the prototypical nootropic drug piracetam. Levetiracetam can be prepared from chiral source (S)-2-aminobutanoic acid or (S)-2-aminobutanamide, which is synthesized and optically resolved with tartaric acid. Neither single-crystal X-ray nor circular dichroism data of levetiracetam has been reported to identify its absolute configuration. In this paper, the predicted ECD and ORD

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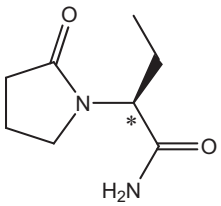

Scheme 1. Structure of levetiracetam ((S)-**1**).

spectra by quantum chemical calculation were compared with the experimental data of levetiracetam to determine its absolute configuration.

2. Materials and methods

Levetiracetam was synthesized and characterized according to literature methods. ECD spectra of levetiracetam, at a concentration of 0.2–1.0 mg/mL in distilled water, were recorded in a quartz cuvette of 1 mm optical path length using a Jasco J-815 CD spectrometer (Jasco Inc., Japan). The conditions of measurement were as follows: scanning speed, 50 nm/min; bandwidth, 1 nm; and 3 accumulations. ECD spectrum of the solvent was used as the baseline and subtracted from the experimental spectra. Optical rotations of levetiracetam were measured on a Perkin-Elmer Model 341 LC polarimeter (Perkin-Elmer Inc., Norwalk, CT) in H_2O or CH_2Cl_2 at different wavelengths (589, 546, 436 and 365 nm) and room temperature.

3. Computational details

All calculations have been carried out on S configuration of compound 2-(2-oxopyrrolidin-1-yl)butanamide ((S)-**1**, Scheme 1). Initial conformational analysis was performed using the MMFF94 molecular mechanics force field via the MOE software package [11]. Full geometry optimization of the MMFF94 conformations obtained was then completed in the framework of density functional theory (DFT) using the Becke 3–Lee–Yang–Parr (B3LYP) exchange-correlation functional at the 6-31+G(d,p) basis set level. It has been checked that stationary points were true minima of the potential energy surface by verifying they do not exhibit vibrational imaginary frequencies. The polarizable continuum model (PCM) was adopted to consider solvent effects using the dielectric constant of water (78.36) and dichloromethane (8.93). The 20 lowest electronic transitions were calculated for each conformer. Rotational strengths of electronic excitations were showed using both dipole length (R_{len}) and dipole velocity (R_{vel}) representations. ECD spectra were simulated by using a Gaussian function

$$\Delta\in(E) = \frac{1}{2.297\times10^{-39}}\frac{1}{\sqrt{2\pi\sigma}}\sum_{i}^{A}\Delta E_i R_i e^{-((E-\Delta E_i)/2\sigma)^2}$$

where σ is the width of the band at $1/e$ peak height, and ΔE_i and R_i are excitation energies and rotatory strength for transition i, respectively. Equilibrium populations of conformers at 298.15 K were calculated from their relative free energies (ΔG) using Boltzmann statistics. The overall ECD spectra were then generated according to the Boltzmann weighting of each conformer. All quantum computations were performed by using Gaussian03 program package [12], on an IBM cluster machine located at the High Performance Computing Center of Peking Union Medical College.

4. Results and discussion

4.1. Conformation analysis

Since compound (S)-**1** is a relatively flexible molecule, a complete conformational analysis was carried out to obtain the multiple possible conformations. A systematic conformation search using the MMFF94 molecular mechanics force field identified nine conformations of (S)-**1** within a 10 kJ/mol window. Conformers **Ia**–**Id** and **IIa**–**IIe** differ principally with regard to the position of β-carbon atom C4, below and above the C1C22N10C7 plane of cyclic amide, respectively (Fig. 1).

The geometries of the MMFF94 conformers were then re-optimized using DFT at the B3LYP/6-31+G(d,p) level and nine conformers were obtained (Fig. 2). Harmonic vibrational frequencies were then calculated to check the stability and also to give the free energies of each conformer, with the results shown in Table 1. Solvents may affect the population of conformers and the calculation of chiroptical properties through the solute-solvent interaction. Regarding (S)-**1** in aqueous solution, it may form hydrogen bond with the solvent. Therefore, the influence of H_2O has been taken into account and compared with CH_2Cl_2 using PCM, which is a commonly used method to model solvation effects. It is interesting to find that (S)-**1** possesses a similar conformation distribution in H_2O and CH_2Cl_2 regardless the possible formation of hydrogen bond with the solvent. Conformers **Ia**, **Ib**, **IIb**, **IIc** and **IIe** are dominant, constituting >98% in the solution phase and >80% in the gas phase, respectively.

The difference between **Ia** and **Ib** lies in that the torsional angle of C11–C13 bond and C14–C17 bond changed from anti-periplanar to 65°. Conformers **Ic** and **Id** differ with **Ia** and **Ib** in the N10C11C13N24 dihedral angle, respectively. The relative free

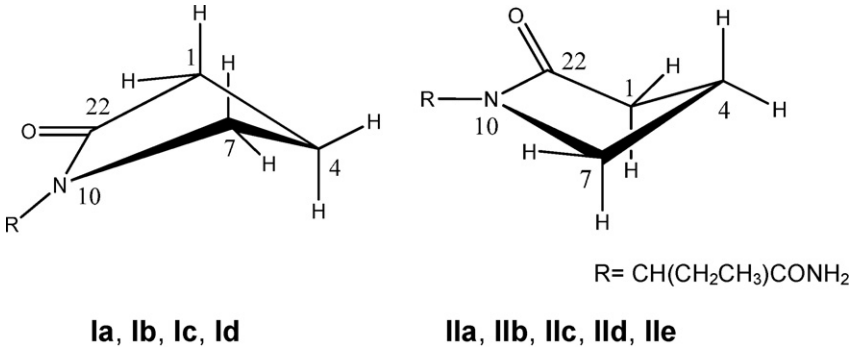

Fig. 1. Schematic representation of the envelope conformations of (S)-**1**, with the β-carbon atom below (conformers **Ia**, **Ib**, **Ic** and **Id**) and above the C1C22N10C7 plane (conformers **IIa**, **IIb**, **IIc**, **IId** and **IIe**).