A Debate on the Parity-Violating Energy Difference of D- and L-Alanine [*]

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Abstract   The chiral purity of biochemical monomers——only L-amino acids are present in proteins and D-sugars in the DNA and RNA of living organisms——is today accepted as an absolute necessity for the existence of life. All previous studies on amino acids reported the naturally occurring L-form to be stabilized by weak neutral currents. Two recent papers dealing with electroweak quantum calculations of the parity-violating energy shifts of alanine in gaseous phase and in solution have defied this long held belief. The parity-violating energy difference (PVED) of D- and L-alanine was undertaken as a function of the dihedral angle θ of the carboxylate plane with respect to the C_{α}-H_{α} plane. Dihedral angles was calculated from the atomic coordinates of O(1) O(2) C(1) C(2) H(1) of D- and L-alanine under the temperature dependence of X-ray diffraction data. According to Quack′s theoretical results by means of highest level *ab initio* studies (MC-LR), the ΔE_{PV} value is 1.2×10^{-19} Hartree (3.3×10^{-18} eV/ molecule), namely L-alanine is more stable than D-alanine. The present paper proved definitely that in the single crystal state L-alanine is lower in energy than D-alanine both by X-ray diffraction data and the differential scanning calorimetry.

Keywords: Parity-violating energy difference,   D- and L-alanine,   Dihedral angle,   Second order phase transition,   X-ray diffraction

1   Introduction

Life is based on L-amino acids and D-sugars rather than the enantiomeric D-amino acids and L-sugars. This broken symmetry is now believed to be a feature of fundamental physics——a result of symmetry broken by the weak force, which is chiral——it can give the difference between left and right, and makes one enantiomer very slightly more stable than the other. The consequence of parity violation of the electroweak interaction in chiral molecules is the existence of a parity-violating shift of the electronic binding energy E_{PV} with a positive value for one enantiomer and negative for its mirror image, but of equal magnitude ($-E_{PV}$) [1] . All previous studies on the parity-violating potentials of L-alanine are considered that the naturally occurring L-form is favored in aqueous solutions by weak neutral currents[2-5] . It has been argued that this subtle energetic preference and the corresponding excess of the more stable L-enantiomer may have been enhanced by an amplification mechanism to give a chiral environment in which life evolved[6-8].

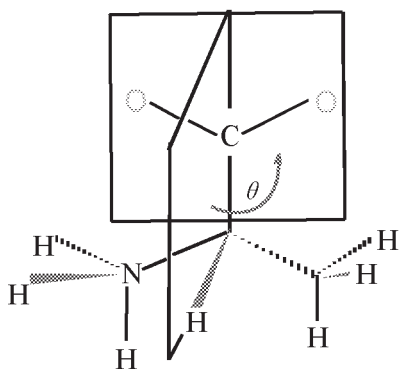**The question of "how much difference are the energies of D- and L-alanine"**

Fig. 1   Comformation of the zwitterionic form L- alanine with explicit definition of the angle θ between O_2C & C_α-H_α planes

Recently, Berger and Quack[9] introduced the multi-configuration linear response (MC-LR) approach to a study of parity-violating effects in alanine, and found that the L-configuration is not always more stable than the D-form. In order to evaluate theoretically the contribution of the PVED, a parity-violating component (H_{PV}) was added to the Hamiltonian describing D- and L-chiral states. For multi-particle systems, H_{PV} should include the contributions of other particle-particle interactions, such as the electron-electron(H_{ee}), the proton-electron (H_{pe}) and the neutron-electron (H_{ne}) interactions. E_{PV} was generally utilized as a criterion to judge the preferable configuration. Quack's study in a detailed analysis of dynamic chirality[10] proved that the dihedral angle (θ) between the O_2C and C_α-H_α planes played an important role in determining the intrinsic energies of the molecules. The ordinary, parity-conserving electronic potential energy $E_{PC}(\theta)$ and the parity-violating potential $E_{PV}(\theta)$ were displayed as functions of the conformational angle(θ) of the carboxylate plane with respect to the C_α-H_α plane (Fig. 1). They found that the sign of ΔE_{PV} changed from negative (namely, L-alanine is more stable than the D-alanine) to positive values (that is, D-alanine is stabilized) through the complete range between 0 and 360°. According to Quack's calculation[10], near

the minimum of the parity-conserving potential at $\theta <$ 60°, E_{PV} is negative. However, near 60° (at thermal energies), E_{PV} changes to positive values (namely, D-alanine is more stable than L-alanine).

Laerdahl and Schwerdtfeger[11] investigated the gas phase parity-violating energy shifts of the L- alanine. Due to the lack of accurate experimental gas-phase structures available for alanine conformers, they explored the molecular potential energy surface using gradient-corrected density functional theory and searched for the global and all local minima. Thirteen minima were found and displayed together with the calculated parity-violating energy shifts for each conformation. Theoretical calculation data show that seven conformers of the L-form are stable. Notably, for the remaining six conformers, the D-form is more stable. Cintas[12] concluded that the weak neutral currents should not be invoked as a source of chiral discrimination in living systems unless a feasible amplification mechanism can be demonstrated.

Definition of PVED——Magnitude and sign

A parity-violating energy difference (PVED) between two enantiomeric structures at a given angle (θ) is defined and denoted by

$$PVED = \Delta E_{PV}(\theta) \equiv E_{PV,L}(\theta) - E_{PV,D}(\theta)$$
$$= 2E_{PV,L}(\theta)$$

We add in quotation the angle θ to stress the importance of dihedral angle in our experiments. θ is calculated from the torsion angle ω, $\omega = 180° - \theta$, which has a properly defined sign, determined by X-ray diffraction with high accuracy.

Dihedral angle of alanine —— in gaseous phase, single crystal and aqueous solution

The dihedral angles of alanine in solution or in gas phase are hard to be measured, therefore Quack's study is generally of theoretical significance only. In order to avoid the complicated conformational changes and solvent effects, we select single crystals to search for the PVED. In the case of crystal, the rotational angle and the crystal conformation have

definite and not random behavior. In this study, single crystals of D- and L-alanine were prepared and the X-ray diffraction was performed under 300 K, 270 K and 250 K. The collected data are accurate to the fourth decimal place according to the specifications of the instrument. For alanine, the unit cell dimensions and the atomic coordinates were obtained, and the dihedral angles could be easily calculated from the torsion angle.

2   Experimental

2.1   Sample characterization

D- and L- alanine single crystals were characterized by elemental analysis (C, H and N) and a good agreement was shown between theoretical and experimental data. By using X-ray diffraction crystallography on a Rigaku RAXIS-RAPID imaging plate diffractometer at 300 K, the cell dimensions of D- and L-alanine crystals were determined as the same space group $P2_12_12_1$, orthorhombic, $a = 0.60344$ nm, $b = 1.23668$ nm, $c = 0.57879$ nm, $V = 0.43193$ nm³, which agree with Simpson[13], $a = 0.6032$ nm, $b = 1.2343$ nm, $c = 0.5784$ nm, $V = 0.4306$ nm³. It indicates that D- and L-alanine are pure single crystals containing no crystalline H_2O molecule. The rotation angle ζ of the D- and L-alanine solution was measured on Polarimeter PE-241 MC at 293 K at the wavelength of 589.6 nm. By using the formula $[\alpha] = \zeta / (L \times C)$, the corresponding α values of D- and L-alanine were shown to be the enantiomer[14].

2.2   Temperature dependent X-ray diffraction of D-and L-alanine

The structure of L-alanine has been determined with X-ray crystallography by Simpson (1966)[13] at room temperature and by Destro *et al*. (1988)[15] at 23 K. A neutron diffraction structure of L-alanine has been carried out by Lehmann *et al*. (1972)[16]. In this study, the unit cell dimensions and cell parameter of X-ray crystallographic data were collected on a Rigaku RAXIS-RAPID imaging plate diffractometer with

graphite monochromated Mo-K_α radiation at 300 K, 270 K and 250 K. For simplicity, only data of 270 K and 250 K were listed in Table 1.

For example, the data were collected at a temperature of 250 ± 1 K to a maximum 2θ value of 54.9°. A total of 44 images, corresponding to 220.0° oscillation angles, were collected with 2 different goniometer settings. Exposure time was 1.00 minute per degree. Indexing was performed from 1 oscillation which was exposed for 2.5 minutes. The camera radius was 127.40 mm. Readout was performed in the 0.100 mm pixel mode. Data were processed by the PROCESS-AUTO program package. Of the 3935 reflections collected, 600 were unique ($R_{int} = 0.026$); equivalent reflections were merged. The linear absorption coefficient, μ, for Mo-K_α radiation is 1.2 cm^{-1}. A symmetry-related absorption correction using the program ABSCOR was applied which resulted in transmission factors ranging from 0.77 to 0.92. The data were corrected for Lorentz and polarization effects. A correction for secondary extinction was applied (coefficient = 1.64408×10^{-4}).

The structure was solved by direct methods (SAPI91) and expanded using Fourier techniques. The non-hydrogen atoms were refined anisotropically. Hydrogen atoms were refined isotropically. The final cycle of full-matrix-least-squares refinement was based on 600 observed reflections ($I > -10.00 \sigma(I)$) and 85 variable parameters and converged with unweighted and weighted agreement factors of:

$$R = \Sigma (F_o^2 - F_c^2) / \Sigma F_o^2 = 0.070$$
$$R_w = \sqrt{\Sigma w (F_o^2 - F_c^2) / \Sigma w (F_o^2)^2} = 0.078$$
$$R_1 = \Sigma ||F_o| - |F_c|| / \Sigma |F_o| = 0.030$$
$$\text{for } I > 2.0 \ \sigma(I) \text{ data}$$

The standard deviation of an observation of unit weight was 1.34. The weighting scheme was based on counting statistics and included a factor ($p = 0.050$) to downweight the intense reflections. Plots of $\Sigma w (F_o^2 - F_c^2)^2$ versus F_o^2, reflection order in data collection, $\sin \theta / \lambda$ and various classes of indices showed

Table 1　　Unit-cell dimensions of D- and L- alanine

Temperature				270 K			
Samples		D-Alanine				L-Alanine	
Empirical formula				$C_3H_7NO_2$			
Formula weight				89. 10			
Crystal system				Orthorhombic			
Space group				$P2_12_12_1$			
Unit cell dimensions（nm）	$a = 0. 60073(5)$					0. 60095(5)	
	$b = 1. 23030(7)$					1. 23388(7)	
	$c = 0. 57732(4)$					0. 57904(3)	
Volume（nm³）	0. 42669(5)					0. 42936(5)	
Z				4			
Atomic coordinates	X	Y	Z		X	Y	Z
O(1)	0. 7280(1)	0. 08419(6)	0. 3723(1)		0. 7279(1)	0. 08414(6)	0. 3725(1)
O(2)	0. 4488(1)	0. 18497(6)	0. 2388(1)		0. 4489(1)	0. 18494(6)	0. 2388(1)
C(1)	0. 4744(2)	0. 16118(8)	0. 6442(2)		0. 4746(2)	0. 16115(8)	0. 6445(2)
C(2)	0. 5591(2)	0. 14146(7)	0. 3978(1)		0. 5593(2)	0. 14146(7)	0. 3980(2)
H(4)	0. 441(2)	0. 237(1)	0. 656(2)		0. 440(2)	0. 238(1)	0. 656(2)
Dihedral angle θ		45. 52°				45. 20°	
Temperature				250 K			
Samples		D-Alanine				L-Alanine	
Empirical formula				$C_3H_7NO_2$			
Formula weight				89. 1			
Crystal system				Orthorhonbic			
Space group				$P2_12_12_1$			
Unit cell dimensions（nm）	$a = 0. 60041(3)$					0. 60002(4)	
	$b = 1. 23179(5)$					1. 23293(6)	
	$c = 0. 57892(3)$					0. 42775(4)	
Volume（nm³）	0. 42815(3)					0. 42775(4)	
Z				4			
Atomic coordinates	X	Y	Z		X	Y	Z
O(1)	0. 7282(1)	0. 08412(6)	0. 3727(1)		0. 7279(1)	0. 08404(6)	0. 3730(1)
O(2)	0. 4481(1)	0. 18482(6)	0. 2388(1)		0. 4480(1)	0. 18484(6)	0. 2387(1)
C(1)	0. 4734(2)	0. 16125(8)	0. 6443(2)		0. 4736(2)	0. 16115(8)	0. 6447(2)
C(2)	0. 5582(2)	0. 14134(7)	0. 3980(1)		0. 5587(2)	0. 14139(7)	0. 3984(1)
H(4)	0. 437(2)	0. 237(1)	0. 653(2)		0. 440(2)	0. 237(1)	0. 658(2)
Dihedral angle θ		43. 97°				45. 72°	

no usual trends. All calculation was performed using the teXsan crystallographic software package of Molecular Structure Corporation.

From the X-ray data, the dihedral angles of D-alanine is 45. 52°, 43. 97° and L-alanine is 45. 20° and 45. 72° at 270 K and 250 K respectively. A computational method used a multi-configuration self-consistent reaction field linear response approach to simulate effects from surrounding solvent molecules （H_2O）. For example, the energy of the L-alanine was shown in（SCRF）$\sim \theta(°)$ plot[9], $E_{PV, L}$ is $-6. 2 \times 10^{-20}$ E_h（1 Hartree energy $E_h = 4. 360 \times 10^{-18}$ J $= 27. 2$ eV）

and that of D-alanine, $E_{PV,D}$ is 6.2×10^{-20} E_h. The PVED is 1.2×10^{-19} $E_h = 3.3 \times 10^{-18}$ eV. The atomic coordinates of D- and L-alanine have shown obvious difference at 250 K. It was found that all the dihedral angles were $\theta < 60°$ in the crystal state from 300 K→ 293 K→270 K→250 K→223 K, where E_{PV} is negative. The energy of L-alanine contributed by electroweak force is lower than D-alanine by 1.2×10^{-19} Hartree （3.3×10^{-18}eV／molecule）.

3   Results and discussion

The classical chemist has hitherto used the electromagnetic force as the only fundamental force, which can produce chemical effects. He has not considered the electroweak force, and in particular its Z^0 component, since the effects due to Z^0 are supposed very small at low temperatures. The electroweak interactions（for which there is nothing comparable in classical chemistry）can give rise to a phase transition[8, 17-18]. In our previous studies, temperature dependence of the specific heat of D-／L-alanine（D-／ L-valine）have been measured by differential scanning calorimeter （DSC）with adiabatic continuous heating in the temperature region from 77 K to 300 K[20-21]. The differential peak of（D−L）valine was shown to be in the order of 6.2×10^{-5} eV·molecule^{-1}·K^{-1}, which is argued as the impetus of the second order phase transition. The differential specific heat measurement definitely proved the energy of D-alanine is higher than the L-alanine[14]. In addition, knowledge of the conformational flexibility of amino acids in aqueous solution is required for understanding the molecular structure and the interactions of proteins in biological systems. It is important to estimate the most stable conformation of L-alanine zwitterion in aqueous solution. Osama Kikuchi *et al.* [22] used *ab initio* molecular orbital （MO）and Monte Carlo （MC） simulation study on the conformation of L-alanine zwitterion in aqueous solution. The energy variation along the θ rotation indicated that the value of $\theta =$

$5 \sim 10°$ is favorable for L-alanine zwitterion in aqueous solution. The barrier to the rotation of the O_2C group of L-alanine is high, whereas that of the NH_3 group is very low. The effect of the CH_3 group on the dissymmetry of the molecular structure is evident, but its effect is small.

It is noticeable that there is a significant difference between the dihedral angles of L- and D-alanine originating from the atomic coordinate variations in the course of temperature lowering, apart from their signs, which should be another indication of the asymmetry of their configuration——they are not true mirror images. A true mirror must be parity charge conjugation （CP）but not parity （P）. Strictly speaking, L- and D-alanine molecules are really diastereoisomers, not enantiomers, owing to the handedness of their constituent elementary particles. The true enantiomer of an L-amino acid is the D-amino acid made of anti-matter. L- and D-alanine molecules should therefore differ in many properties, e. g. NMR chemical shifts[23], and, most importantly, in energy[24].

Combining the theoretical calculation and the experimental results, PVED exactly exist between D- and L-alanine crystals. We conclude that the role of weak neutral currents for an explanation of biomolecular homochirality has still to be established. These forces are the source of the asymmetry of all living organisms, provided there is a reliable and reproducible amplification mechanism. Our findings indicate that the phase transition existed in both D-and
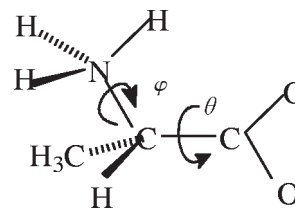

Fig. 2   Definition of two dihedral angles of the L- alanine zwitterions

θ is the rotation of the CO_2 group and φ is the rotation of the NH_3 group in the direction indicated[22]

L-alanine and the mechanism is reversible and repro-ducible[18,20]. We speculate on an alternative mechanism and the enhancement is due to phase transitions[20-21]. In general, when global cooperative and condensation phenomena do take place, low temperatures（or high densities, as for example for neutron stars）are nec-essary. The maximum surface temperatures have been estimated as 135 K for Jupiter, 120 K for Saturn, 85 K for Uranus, 55 K for Neptune, and 20 K for Pluto. The prebiotic temperatures may be less than 300 K[25]. Ellis-Evans[26] reported that a great lake-Lake Vostok lies below a flowing ice sheet, and a freezing point of the lake water is -3.15 ℃（270 K）. Microbiological studies of the Vostok ice core have revealed a great diversity of microbes including yeasts and actino-mycetes（with antibiotic synthesizing potential）which remain viable in ice after 3000 years, and viable mycelial fungi present up to 38 600 years old. We have shown that chirality may provide a boundary condition for theories of chemical evolution and the origin of life and that Z^0 ought to play a central role in this process. Life as manifested to us is a function of the asymmetry of the Universe and of the conse-quences of the fact. The Universe is asymmetrical. Life is dominated by asymmetrical actions[27].

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"D 和 L- 丙氨酸宇称破缺能差正负"的争论 *

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摘要   在宇宙开始大爆炸的时候,电荷变号与镜象反射共轭 (CP)是对称的. 但现在我们的宇宙绝大部分是正物质核子和电子等组成的,所以我们的宇宙是不对称的. D 和 L- 丙氨酸通常称为对映体 (enantiomer),实际上它们并不是由正、反粒子组成的真正的对映体,而是空间反演的,即 $x \rightarrow -x$, $y \rightarrow -y$, $z \rightarrow -z$ 的非对映异构体 (diastereoisomer),所以 D- 和 L- 丙氨酸是不对称的,两者间有能量的差别. 自然界的力只有弱力是宇称不守恒的. 在分子物理中,电弱力宇称不守恒是导致 D- 和 L- 丙氨酸能差的根源. 所有以前的研究都认为 L 型丙氨酸比 D 型稳定. 但是,最近以 Quack[9]和 Schwerdtfeger[11]为首的理论物理学家计算了 L-丙氨酸在气相和溶液两种状态下,宇称破缺能差与分子构象的关系,提出 "D- 和 L- 丙氨酸究竟哪一个稳定"的质疑. 由于气相和液相中两面角较难测定,我们用 X 射线四圆单晶衍射法,测定了 270 K 和 250 K D 和 L- 丙氨酸的 O (1)O (2)C (1)C (2)H (4)的原子坐标,算出了二面角,按照 Quack[9]的 MC-LR 方法,D- 和 L- 丙氨酸宇称破缺能差为 1.2×10^{-19} Hartree, 相当于 3.3×10^{-18} eV/分子或 3.2×10^{-16} kJ·mol^{-1},从而得出 D-丙氨酸能态高于 L- 丙氨酸的结论.

关键词: 宇称破缺能差,   D- 和 L- 丙氨酸,   二面角,   二级相变,   X 射线四圆单晶衍射

中图分类号: O641

勘误 (Correction)

本刊 2001 年 17 卷第 12 期 1080 页 Table 2 题目中的 D-valine 应更改为 L-valine. 特此更正.

D-valine in the title of Table 2, in 2001, 17(12): 1080, should be L-valine.