Search for Electroweak Interactions in Amino Acid Crystals. II. The Salam Hypothesis

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Received: December 9, 2002; In Final Form: May 28, 2003

In 1991, A. Salam proposed that the electroweak interaction might promote tunneling through the potential barrier existing between chiral molecules (i.e., a phase transition at a critical temperature, T_c), which could change the structure of D-amino acids into the reputedly more stable L-form of the enantiomer. A recent report by Wang et al. has presented experimental evidence for such a transition at $T_c \approx 270 \pm 1$ K in enantiomorphs of L- and D-alanine and -valine crystals using differential scanning calorimetry (DSC), magnetic susceptibility, and Raman spectroscopy. Experimental verification of the Salam prediction has great implications for the origins of specific homochirality in biomolecules, that is, the exclusive use of L-amino acids in proteins. In this contribution, we reexamine these measurements, as well as present measurements of the temperature dependence of X-ray diffraction and C-13 solid-state NMR for alanine. While the DSC measurements show interesting features similar to those reported by Wang et al. at ∼270 K, there are significant differences, including the observation that the small feature observed in the DSC experiments becomes even smaller upon recrystallization (i.e., purification). Also, the small change in specific heat at ∼270 K is found to be absent in (natural) L-valine. We find no unusual behavior in our additional X-ray diffraction or NMR experiments in this temperature range. We also present arguments against the Salam hypothesis for the molecules under study.

Introduction

The parity-violating (P-odd) weak interaction is the only known chiral force in nondecaying atoms and molecules. The standard model of low-energy nuclear physics describes this weak interaction as the virtual exchange of Z^0-bosons between elementary particles. Following the prediction and observation of parity nonconservation in the late 1950s, P-odd effects were observed in the optical activity for atoms[1] and in the occurrence of weak transitions between atomic hyperfine states of the same parity.[2] Although expected, similar effects have not been detected in molecules. Interest in P-odd effects in molecules has focused upon the measurement of the so-called parity-violating energy difference (PVED) predicted to exist between *R* and *S* enantiomers of a chiral molecule. The PVED calculated for small molecules such as amino acids is exceedingly small [see accompanying paper and recent studies][3] and has not yet been detected. A small ($\sim 10^{-10}$ eV) energy difference between enantiomorphic crystals of a chiral iron complex was recently reported using Mössbauer spectroscopy; however, the possibility of strain within the crystal precludes an unqualified claim of the measurement of PVED.[4] Early PVED calculations for the amino acids[5−8] predicted that the L-enantiomer lies lower in energy than the D-enantiomer; however, this energy difference is generally believed to be too small to account for the exclusive occurrence of L-amino acids in biomolecules. Furthermore, results presented in the accompanying paper provide no clear evidence that L-amino acids are stabilized as a result of the weak interaction. Clearly, there is no solid evidence that PVED can account for biomolecular homochirality through the Yamagata hypothesis[3].

In 1991, Salam introduced a startling new hypothesis, which was proffered to account for biomolecular chirality, that does not rely upon the long times involved in the Yamagata hypothesis.[9,10] Salam proposed that the subtle energy difference, PVED, together with a type of Bose condensation phenomenon, may allow for a second-order phase transition below a critical temperature T_c allowing the "less" stable D-enantiomer to tunnel into the more stable L-enantiomer. The critical temperature T_c for this transition was identified with that for the Bardeen, Cooper, and Schrieffer (BCS) theory of superconductivity. Wang et al. have recently reported extensive experimental studies of L- and D-alanine and -valine crystals, which were designed to test the Salam hypothesis.[11] These authors report three experimental results that were presented as evidence for the Salam hypothesis: (1) specific heat measurements for both crystals show a second-order phase transition at $\sim 270 \pm 1$ K that are different in magnitude for the two enantiomorphs of each amino acid, (2) differences in the mass susceptibilities for the two enantiomorphs were detected using a superconducting quantum interference device (SQUID) magnetometer in the same temperature region, and (3) Raman spectra of the $C^{\alpha}-H$ deformation modes disappear for the D-alanine at ∼270 K but reappear again at ∼100 K; the same vibrational mode for L-alanine does not vanish over this temperature range. In this contribution, we reexamine these measurements and, in addition, study the temperature dependence of X-ray diffraction and C-13 solid-state NMR for alanine. We conclude with some critical comments on the Salam hypothesis.

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10.1021/jp0225673 CCC: $25.00
Published on Web 07/31/2003

Experimental Section

Both enantiomers of alanine and valine were purchased from Sigma Aldrich Chemical Co. The optical purities of the samples were quoted to be 99% and at least 98% for the L- and D-forms, respectively. The D- and L-alanine and -valine crystals were grown in aqueous solutions using HPLC water in Petri dishes at room temperature, 298 K. The crystallization process took about a week because plastic Saran wrap lids with 80 0.5-mm holes were placed in the Petri dishes to control the evaporation of the water. These lids also prevented larger biological debris and other particles from falling into the solution during crystal growth. The crystals were recrystallized twice to eliminate further impurities in the crystals. As discussed later, measurements were performed after each stage of recrystallization. The alanine crystals were shaped like rectangular parallelepipeds. The average alanine crystal had the dimensions of 5 mm × 4 mm × 2 mm. Crystals of both D- and L-alanine were grown using these methods. The production of both D- and L-valine crystals proved difficult. Also, the L-valine crystals were more difficult to grow than the D-valine crystals because their aqueous solution was more prone to bacteria growth over long periods of time. The growing of valine crystals from an aqueous solution was unsuccessful. Thus, the data for D- and L-valine crystals were obtained directly from the material purchased from the Sigma Aldrich Chemical Co.

X-ray Diffraction

X-ray diffraction patterns for single crystals of D- and L-alanine were obtained at 296 K using a Bruker AXS single-crystal X-ray diffractometer with the Smart 1000 system. The space group was found to be $P2_12_12_1$ and orthorhombic with cell dimensions of $a = 5.7811$ Å, $b = 6.0282$ Å, and $c = 12.3401$ Å. The temperatures of the crystals were varied from 296 to 258 K, and the unit cell dimensions stayed the same as those shown in Table 1. The X-ray diffraction measurements do not take into account the motion of hydrogen atoms to form zwitterions.

TABLE 1: X-ray Diffraction Data for the D- and L-Alanine Crystals at 296 K

sample	D-alanine	L-alanine
empirical formula	$C_3H_7NO_2$	$C_3H_7NO_2$
formula weight	89.10	89.10
T, K	296	296
wavelength, Å	0.710 73	0.710 73
cryst syst	orthorombic	orthorombic
space group	$P2_12_12_1$	$P2_12_12_1$
unit cell dimensions		
a, Å; α, deg	5.7811(3); 90°	5.7905(3); 90°
b, Å; β, deg	6.0282(3); 90°	6.0220(3); 90°
c, Å; γ, deg	12.3401(6); 90°	12.3435(7); 90°
vol, Å^3	430.05(4)	430.05(4)
Z	4	4
density (calcd), Mg/m^3	1.367	1.375
abs coeff, mm^{-1}	0.115	0.115
F(000)	192	192
cryst size, mm^3	0.3 × 0.4 × 0.3	0.3 × 0.4 × 0.3
θ (deg) range for data collection	3.30−28.32	3.30−28.32
index ranges	$-7 \le h < 7$; $-8 \le k < 8$; $-16 \le l < 16$	$-7 \le h < 7$; $-8 \le k < 8$; $-16 \le l < 16$
reflns collected	5563	5562
independent reflns	1044 [R(int) = 0.0249]	1043 [R(int) = 0.0252]
completeness to $\theta = 28.32°$	98.9%	99.1%
refinement method	full-matrix least-squares on F^2	full-matrix least-squares on F^2
data/restraints/parameters	1044/0/83	1043/0/83
GOF on F^2	1.124	1.114
final R indices [$I > 2\sigma(I)$]	R1 = 0.0293; wR2 = 0.0752	R1 = 0.0296; wR2 = 0.0746
R indices (all data)	R1 = 0.0302; wR2 = 0.0758	R1 = 0.0308; wR2 = 0.0760
absolute structure parameter	0.4(11)	0.3(10)
largest diff. peak and hole	0.220 and −0.244 e Å^{-3}	0.151 and −0.258 e Å^{-3}

Raman Spectroscopy

The vibrational modes of the C−H bond in D- and L-alanine were recorded using a Dilor XY modular laser Raman spectrometer. Raman spectra were taken with an argon-ion laser with a wavelength of 514.53 nm and power of 100 mW. A variable temperature sample holder device was constructed using a cold plate, a thermoelectrical cooler, and a heat sink. An ILX temperature controller was used to vary precisely the temperature settings. Raman spectra of alanine crystals were recorded over a temperature range from 298 to 270 K. The Raman data reported in Figures 1 and 2, parts a, b, c, or d, show no changes over this temperature range when special attention was focused upon the C−H peaks at 2600 and 2750 cm^{-1}. An alanine crystal in the shape of a rectangular parallelepiped was placed on the platform with its broad side facing up. When the long crystal axis was rotated 90° with respect to the plane of polarization of the laser beam, an additional peak appeared on the spectra around 3100 cm^{-1} at all of the temperatures observed. This additional peak was not observed in the Raman spectra when the long crystal axis was parallel to the plane of polarization of the laser. The Raman data are seen in Figures 1 and 2, parts a, b, c, or d. The Raman emissions were collected at 180° (back reflected) with respect to the incident beam. Once again, no changes in the Raman spectra occurred around ∼270 K. Wang et al.[11] recorded their Raman spectra using an Ar-ion laser with a power of 200 mW at 488 nm, while the emissions were collected at 90° to the incident beam. Their Raman lines decreased in intensity to the point that some disappeared around 270 K. This effect was not observed in our studies as seen in Figures 1 and 2.

NMR Spectroscopy

C-13 magic angle spinning NMR spectroscopy of alanine and valine samples was performed on a 400 MHz solid-state NMR Varian spectrometer. Alanine crystals were grown from both aqueous solutions and deuterium oxide. The D- and L-alanine crystals displayed three peaks at 178, 51, and 21 ppm, representing the COOH, CH, and CH_3 groups, respectively. As the temperature was decreased from 298 to 248 K in both D- and L-alanine, the peaks became less intense without broadening. The peaks almost completely disappeared at 248 K. Also, no shifts were observed during the temperature-dependent experiment as seen in Figure 3. These temperature effects were observed for D- and L-alanine grown in either water or deuterium oxide.

C-13 NMR spectra for D- and L-valine obtained from Sigma Aldrich revealed five resonances at 178, 61, 32, 23, and 19 ppm. These peaks are due to the C^{13} in the COOH, CH, CH_3, and two CH_2 groups, respectively. We could detect no change in the NMR spectra over the temperature range from 298 to 248 K. The peak widths were also unchanged as presented in Figure

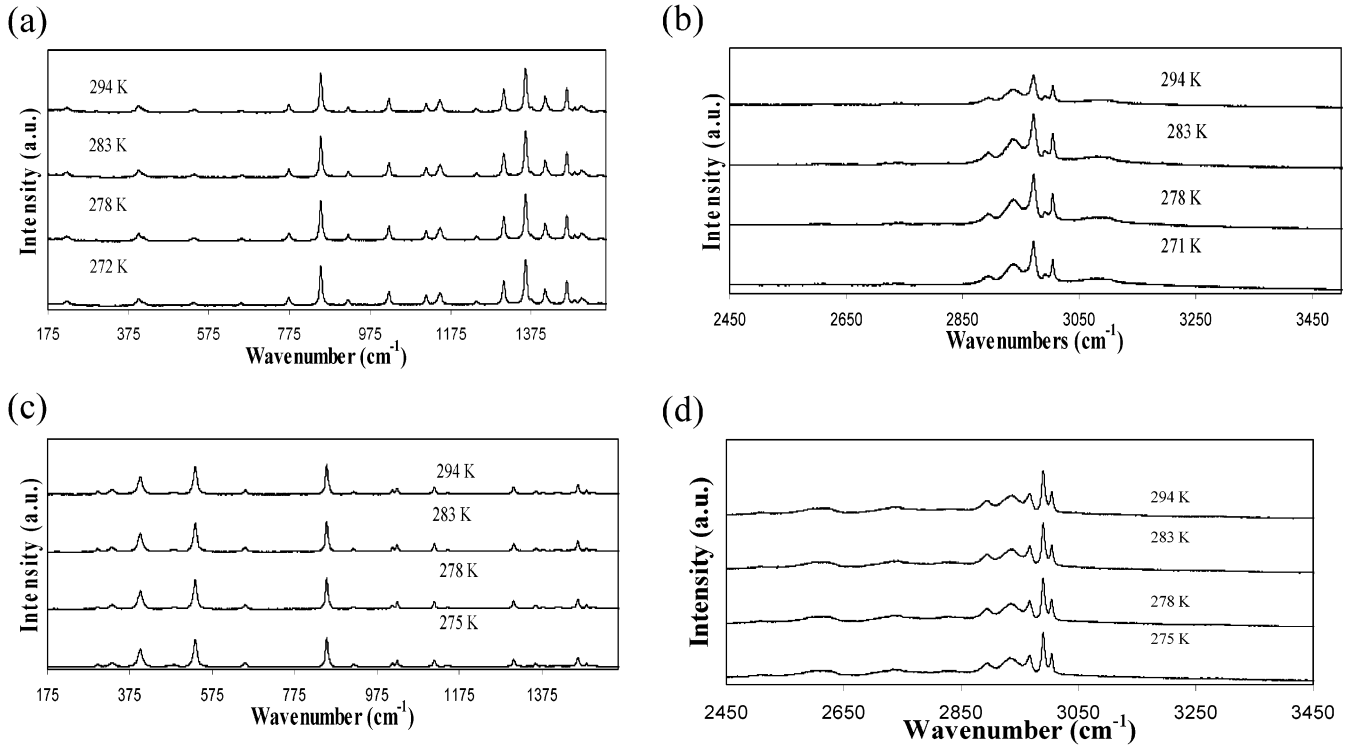


Figure 1. The temperature-dependent Raman spectra for the D-alanine crystal with its long crystal axis (a,b) perpendicular and (c,d) parallel to the laser beam's polarization.

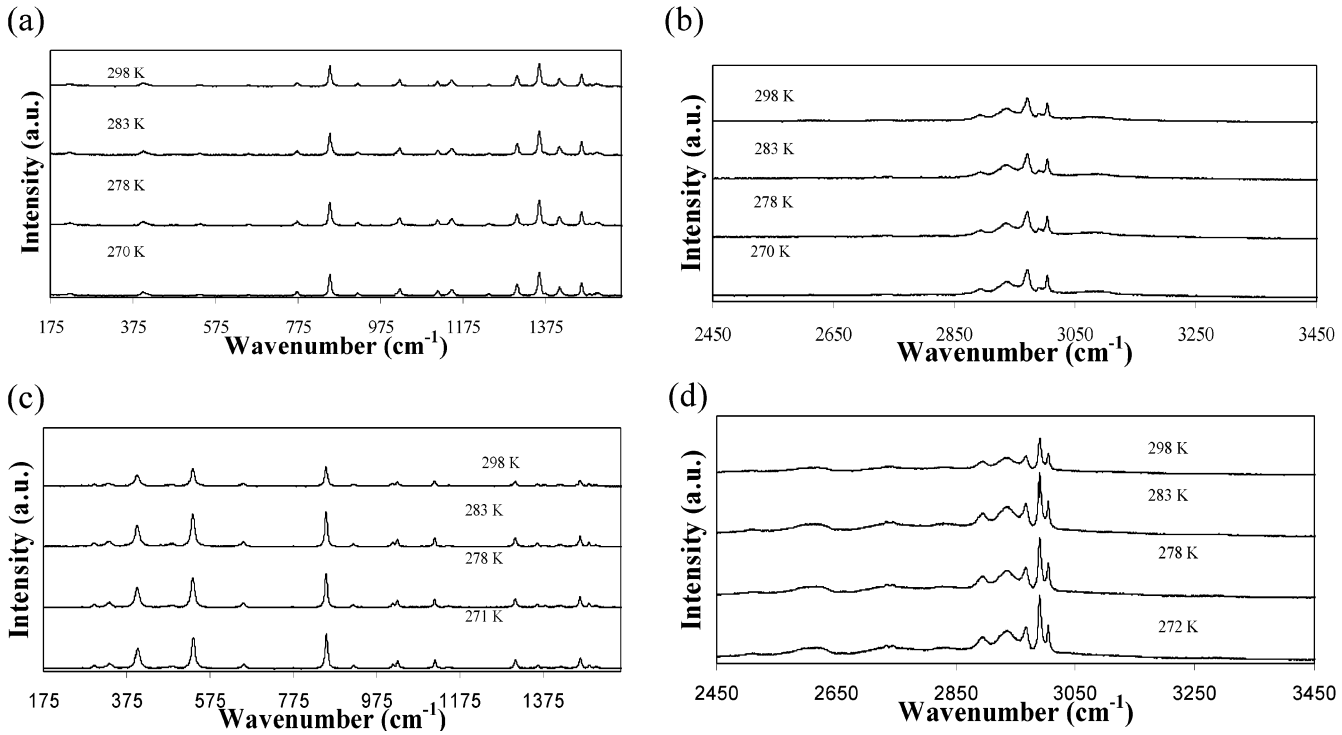


Figure 2. The temperature-dependent Raman spectra for the L-alanine crystal with its long crystal axis (a,b) perpendicular and (c,d) parallel to the laser beam's polarization.

4. The spectra were identical for D- and L-valine grown in water and deuterium oxide. No phase transition is evident in the NMR spectra over this temperature range.

Magnetic Susceptibilities

The magnetic susceptibility of single crystals of D- and L-alanine was investigated in the temperature range 5−330 K using a SQUID-based magnetometer (Quantum Design model MPMS-7). The crystals were prepared by repeated recrystallizations. They were stored for an extended period (~6 months) in a small vial with desiccant to ensure dryness. Two crystals were selected (mass of 169.0 mg for D-alanine and 129.3 mg for L-alanine) and mounted sequentially in the same long plastic tube for measurement. The two crystals were roughly rectangular and the magnetic field was applied parallel to the longest axis. For the magnetic study, a field $H = 10$ kOe was applied, and

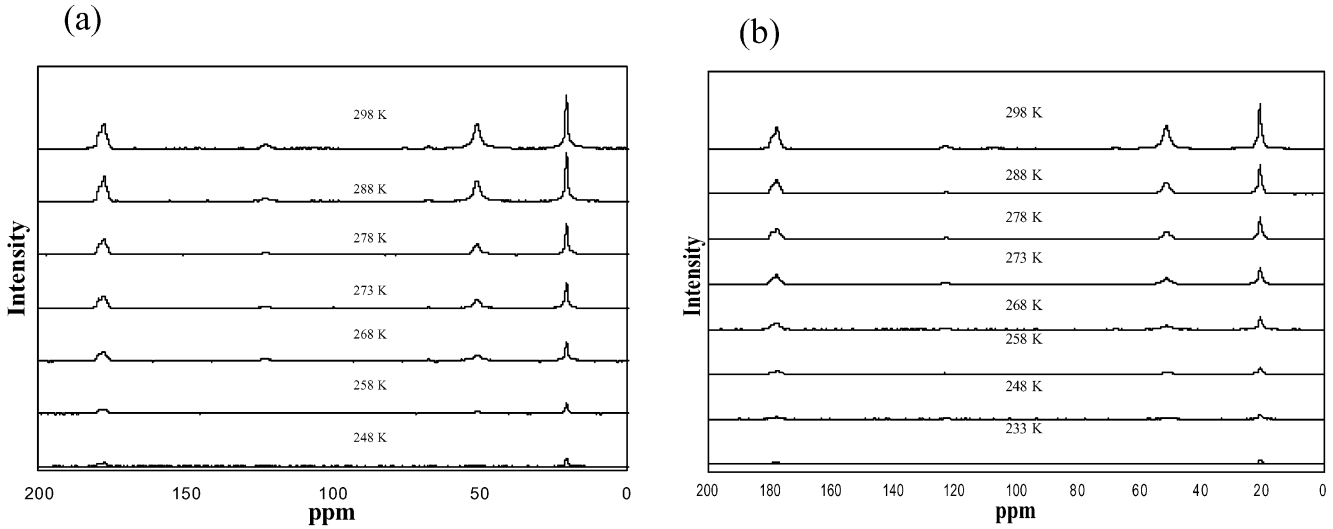


Figure 3. The temperature-dependent solid-state NMR spectra of (a) D-alanine and (b) L-alanine crystals. The small feature at ~120 ppm is due to the rotor.

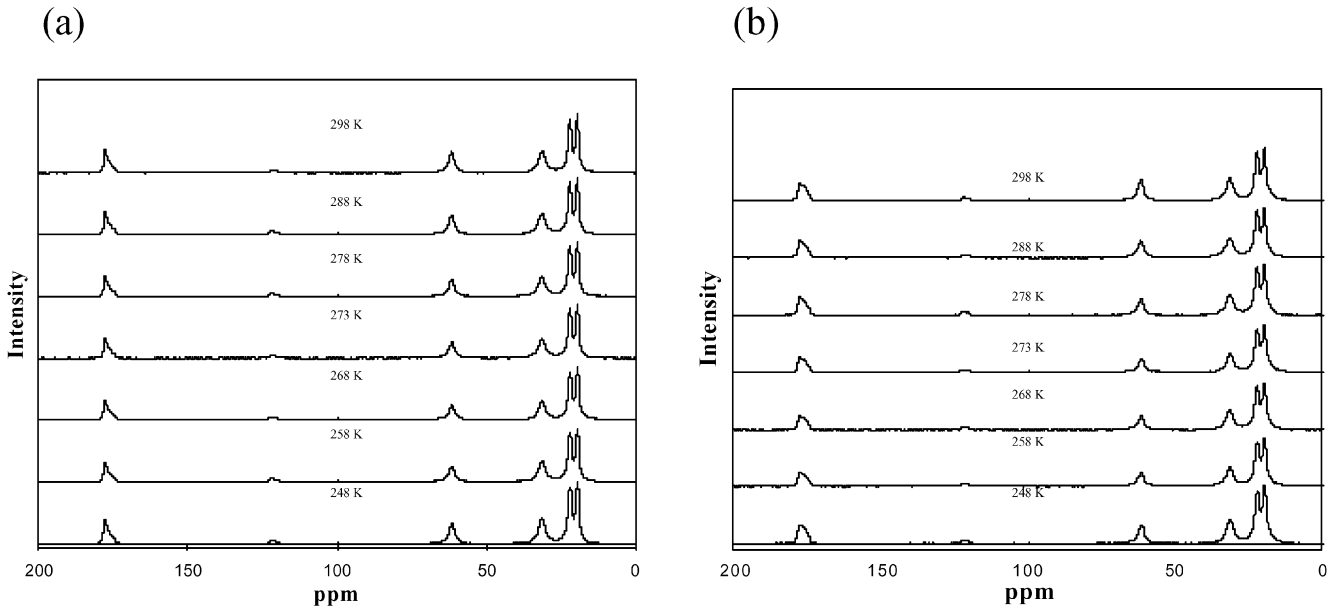


Figure 4. The temperature-dependent solid-state NMR spectra of (a) D-valine and (b) L-valine. The small feature at ~120 ppm is due to the rotor.

the system was allowed to stabilize for 1 h before beginning measurements with 4-cm scan lengths. Measurements on the two crystals were conducted consecutively with no change in the magnetic field by sweeping the temperature T from 300 to 5 K, then back to 300 K, and continuing to 330 K. There was very little hysteresis, as shown in Figure 5.

The cgs mass magnetic susceptibility, $\chi = m/(H \times \text{mass})$, versus temperature is presented in Figure 5. To first order, χ is diamagnetic and nearly temperature-independent. Curiously, the absolute value of χ is ~3% greater for the D-alanine crystal than for the L-alanine. This may arise in part from (1) small differences in sample size and geometry that slightly affect the magnetometer sensitivity or (2) crystalline anisotropy [Jean Bernard Robert, private communication], or both. Most importantly, the behavior of $\chi(T)$ is nearly identical for the two cases of D- and L-alanine: χ hardly changes near room temperature and then increases by ~0.6% upon cooling to 5 K. The similarity in temperature dependencies is evident in Figure 5, in which the left and right vertical scales are shifted by 2.5%. These findings on magnetic susceptibility do not concur with those reported by Wang et al.[11]

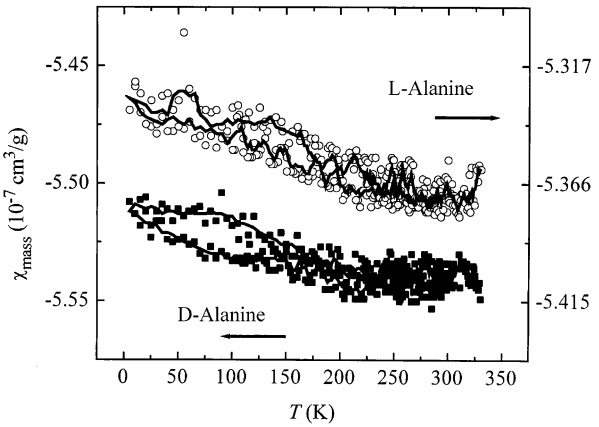


Figure 5. The magnetic susceptibility versus temperature is shown for D- and L-alanine. Note the separate vertical scales.

Differential Scanning Calorimetry (DSC)

The heat capacity and transition phases of crystalline L- and D-alanine and -valine were measured from 220 to 340 K by

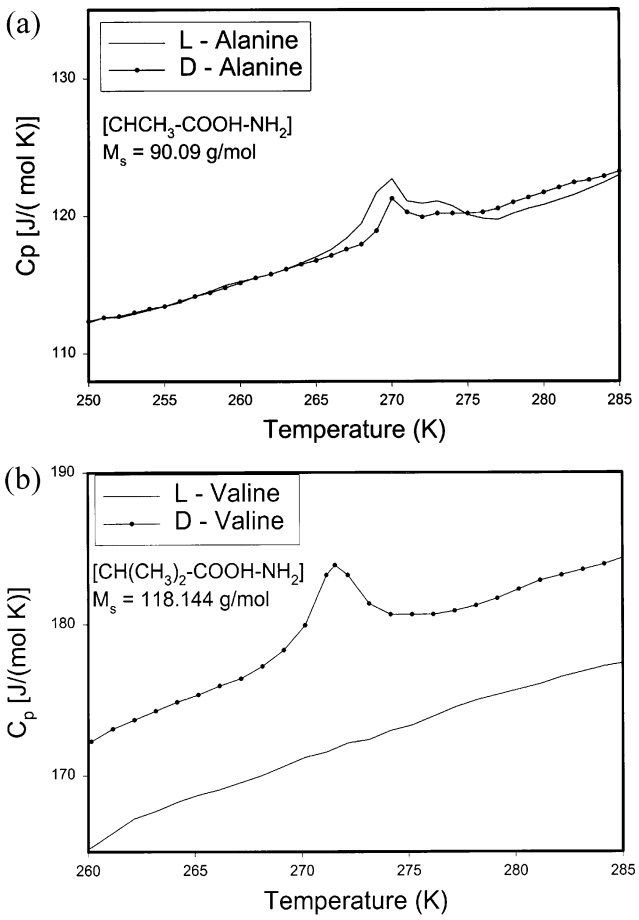


Figure 6. A typical heat capacity measurement of (a) D- and L-alanine and (b) D- and L-valine by standard DSC.

standard DSC. Two differential scanning calorimeters were used to measure the transition behavior and heat capacities: a modulated differential scanning calorimeter (MDSC) 2920 of TA Instruments, Inc. and a DSC 820 from Mettler Toledo Inc. Both the MDSC and DSC are of the heat-flux type. Figure 6a shows a typical example heat capacity measurement from 250 to 285 K on the crystalline samples of L-alanine and D-alanine grown from aqueous solutions by standard DSC. Figure 6b shows the heat capacity measurement of L- and D-valine by standard DSC. In both L- and D-alanine, a small endothermic transition occurs around 270 K. However, for valine, only the D-form exhibits an endothermic peak. Figure 7 shows a typical example of heat flow for L- and D-valine by standard DSC. A comparison of the experimental data reveals an endothermic peak at −1.54 °C (271.61 K) for D-valine but none for L-valine in the same temperature region. The heat of transition for D-valine was estimated to be $\Delta H_t = 0.15$ J/g (17.8 J/mol), which corresponds to a change of entropy of $\Delta S_t = 0.0655$ J/(K mol). All transition parameters are given in Table 2. The absence of a peak in L-valine suggests that an impurity is present in the synthetic D-valine but not in the naturally occurring L-valine, which one can reasonably assume to be of higher purity. Identical DSC curves were observed for crystals grown in deuterated water.

It is interesting to note that all entropy values are hundreds of times smaller than any entropy of transition in liquid crystals and much smaller than the entropy of fusion [$\Delta S_t \gg 53$ J/(K mol) for alanine]. The heat of transition in all samples was checked after recrystallization from water. The heat transition

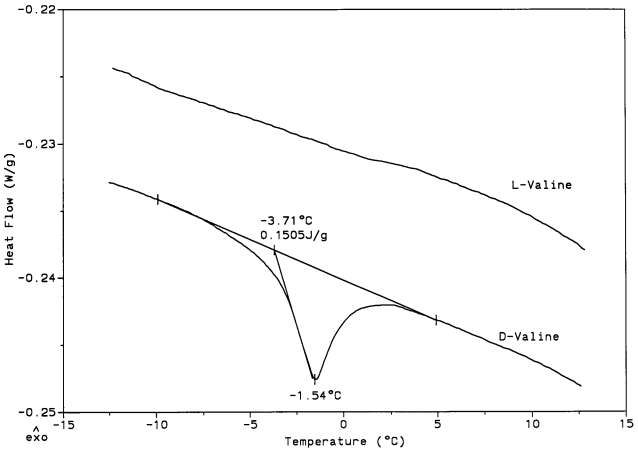


Figure 7. Heat flow of D- and L-valine by standard DSC.

TABLE 2: Transitional Results of L- and D-Alanine and -Valine Crystals

sample	*T* of peak (K)	*T* of onset (K)	heat of transition (J/mol)	entropy of transition (J/(K mol))
L-alanine	269.59 ± 0.01	267.78 ± 0.01	20.3 ± 0.1	0.076 ± 0.001
D-alanine	270.02 ± 0.01	268.81 ± 0.01	5.8 ± 0.1	0.021 ± 0.001
L-valine				
D-valine	271.61 ± 0.01	269.44 ± 0.01	17.8 ± 0.1	0.065 ± 0.001

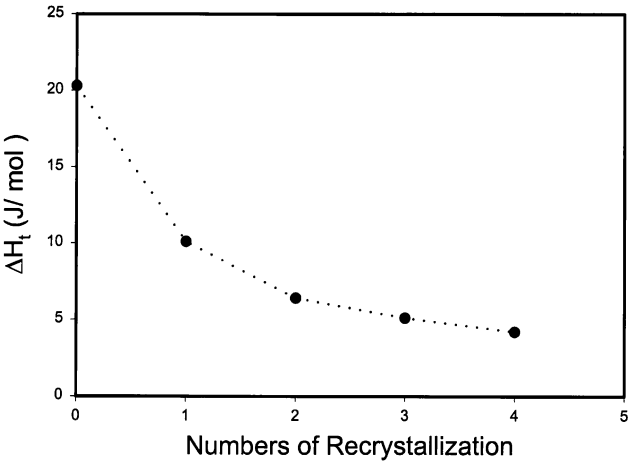


Figure 8. Heat of transition for L-alanine at 270 K as a function of crystallization.

at 270 K decreased with the number of recrystallizations (see Figure 8), again suggesting a reduction in impurity level.

The experimental heat capacities over the entire temperature range are linked to vibrational motion in the solid state.

Conclusion

This study reports the results for a number of experiments designed to search for phase transitions in two amino acids crystals, alanine and valine. Specifically, we reexamined all of the experiments reported by the group of Wang et al.,[11] which were presented as evidence for a phase transition at a critical temperature of $T_c \approx 270 \pm 1$ K. These data were taken as support of the Salam hypothesis. Our differential scanning calorimetric heat capacity measurements do show an anomaly at ∼270 ± 1 K for both crystals; however, the effect becomes smaller upon recrystallization. Also, no feature is seen in the naturally occurring L-valine. Careful magnetic susceptibility and Raman spectroscopy measurements show no anomalies over this temperature range. Temperature-dependent C-13 solid-state

NMR and X-ray diffraction also show no anomalies. In light of these measurements, we will examine the Salam hypothesis with regards to the energy barrier existing between enantiomers.

Consider the chemical reaction that can interconvert the two enantiomeric forms,

$$\text{L-amino acid} \rightleftharpoons \text{D-amino acid} \qquad (1)$$

Such racemization reactions are well-known in solution[12] and are catalyzed by a base or an acid.[13] It is well-known that aspartic acid racemizes quite rapidly in solution compared to other amino acids, and the mechanism is now well understood.[14] The formation of D-amino acids as required in certain bacteria or in mammalian brain is produced by enzymatic conversion from the L- to the D-form.[15,16] Racemization reactions of amino acids in the solid state or in the gas phase have not been observed to date. We note, however, that aspartic acid racemization in dentine is used as a measure of aging.[17] Although the exact process is not understood in our opinion; this reaction is catalytic in nature and would not occur in a pure sample of aspartic acid.

Salam proposed that at lower temperatures a Bose−Einstein-type phase transition would favor the more stable enantiomeric form.[9,10] He regarded reaction 1 as an equilibrium problem and estimated the critical temperature T_c to be lower than 250 K, that is, for temperatures below the melting point of amino acids. For a single molecule in the solid state (or in the gas phase), the mechanism for hydrogen migration may occur classically by overcoming a rather low barrier for process 1 or by tunneling of the hydrogen atom at the chiral carbon center through the inversion barrier. Bimolecular reactions in the solid state are energetically not preferred.

The stereomutation of CH_4 has been previously investigated, and the transition state is estimated to be 439 kJ mol^{-1} above the T_d minimum structure at the MP2 level of theory.[18] This transition state can be best described as a pyramidal complex between methylene in the $^1A_1$ electronic state and H_2.[18] According to the Wentzel−Kramers−Brillouin (WKB) approximation such a high barrier (with a thickness on the order of angstroms) would prevent any hydrogen tunneling at low temperatures in a reasonable time scale. This barrier can be decreased significantly to about 180 kJ mol^{-1} for amino acids adsorbed on transition metals.[19] Because the mechanism for the unimolecular racemization of amino acids is not known, the hydrogen migration process for the alanine inversion was investigated by means of quantum chemical procedures (second-order Møller−Plesset, MP2, and density functional B3LYP level of theory using 6-311+G* basis sets).[20]

After an intensive search of possible transition states for the unimolecular gas-phase racemization of alanine, the lowest possible energy path as illustrated in Figure 9 was found. Here, the hydrogen atom migrates from the chiral carbon center (denoted as C*) over a high-energy path bridging the C* and N atoms to the final intermediate state in which the hydrogen is bound to the amino group. Racemization then occurs when another hydrogen atom at the amino group migrates back to the chiral carbon center. Other possible paths of lower energy cannot be excluded because the potential energy hypersurface (PES) is quite complicated. However, we could not find an intermediate state of lower energy than that found in Figure 9. This intermediate configuration, $CH_3C(NH_3)COOH$, is a minimum at the PES and can be best described by the following resonance structures:

This calculation explains the rather high stability of this metastable compound, which is only 156 kJ mol^{-1} above the

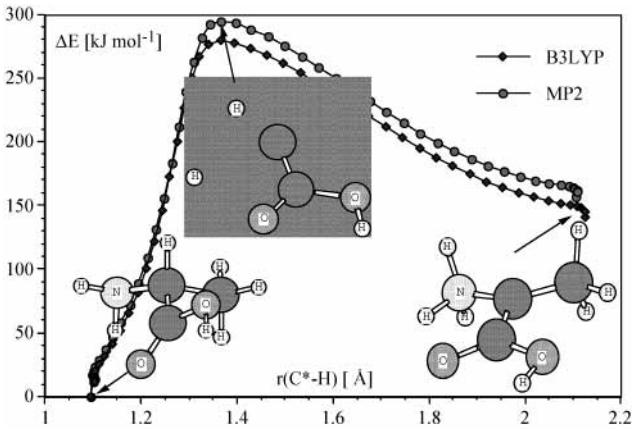
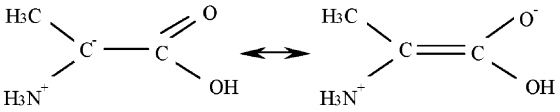


Figure 9. Minimum energy path for the hydrogen migration from the chiral carbon center (C*) to the amino group. For the reaction coordinate, the C*−H bond distance is chosen.



(local) minimum structure shown in Figure 9 at the MP2 level of theory (140 kJ mol^{-1} at the B3LYP level). Here, the hydrogen atom is clearly bound to the nitrogen center at a distance of 1.02 Å at the MP2 level. The transition state is 294 kJ mol^{-1} above the minimum (279 kJ mol^{-1} at the B3LYP level) and below the dissociation limit for the hydrogen abstraction, $CH_3C(NH_2)COOH + H$ (330 kJ mol^{-1} at the MP2 and 328 kJ mol^{-1} at the B3LYP level of theory). Furthermore, the transition state is energetically well below that calculated for CH_4;[18] the barrier happens to be too high and thick to be overcome either classically by hydrogen migration at temperatures below the melting point of alanine (570 K)[21] or quantum mechanically by hydrogen tunneling. One may assume that the solid-state environment would severely restrict any molecular rearrangements, thus increasing the barrier even more for the racemization process. Hence, the Salam phase transition is kinetically hindered and a Bose−Einstein condensation from a racemic mixture to the energetically more stable form due to electroweak interactions is not expected to take place in the solid state at lower temperatures. Bose−Einstein condensations observed in nature usually have no or extremely small barriers to overcome, and this is apparently not the case for amino acids.

In conclusion, we have presented a wide range of experimental measurements on enantiomorphs of L- and D-alanine and -valine in an effort to detect a weak interaction phase transition as described by Salam[10] and to reexamine the results reported by Wang et al.[11] supporting such a transition. Although a small difference in the DSC for both L- and D-alanine and -valine is seen at ∼270 K as reported by Wang et al.,[11] we present evidence that this effect becomes smaller upon successive recrystallization and is absent in L-valine. Other features reported by Wang et al.[11] in support of the Salam hypothesis are not corroborated. On a positive note, we report a number of new physical measurements on the important amino acid crystals and provide further theoretical insight into the unimolecular racemization of amino acids (alanine).

Acknowledgment. This work is supported through a National Science Foundation Grant and the James Cook Fellowship (P.S.) of the Royal Society of New Zealand.

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