The Energy Density of Water and Ice Nucleation

M. Gavish *et al.* hypothesize (*1*) that nucleation is induced by large electric fields found in the narrow cracks of polar crystals. Their calculations show that an ice bilayer structure has a higher energy in a D-alanine crystal crevice than in a D,L-alanine crystal crevice, so they conclude that nucleation is facilitated by the latter crystals. What is pertinent to the nucleation process, however, is the difference between the energy density of an ice nucleus and that of *water* in the crevice of each kind of crystal. Furthermore, a bilayer is not a likely shape for such an ice nucleus because of the heavy cost of surface energy relative to the gain in free energy that results from the chemical potential difference between ice and supercooled water. The bilayer nucleus described in the report would certainly be unstable relative to water unless ice preferentially wet the surface of the crystals. This would constitute surface freezing, which is not consistent with the usual surface melting of ice observed in nature (*2*).

An estimate can be made of the difference in the electrostatic energy density (W) between a small spherical ice nucleus (*3*) and the same volume of water in an electric field. Following Jackson (*4*), one can write

$$W = -\frac{1}{8\pi}(4\pi\sigma)^2 \frac{\epsilon_i - \epsilon_w}{\epsilon_w^2} \frac{3\epsilon_w}{\epsilon_w + 2\epsilon_i}$$

where σ is the surface charge density of the crevice wall and ϵ_i and ϵ_w are the dielectric constants of ice and water, respectively. Because the dielectric constant of ice is greater than that of water, an electric field lowers the free energy of an ice nucleus. For an approximation of the value of σ, one can take the surface density of molecules in the crevice and multiply this value by 0.6e (*5*). Although ϵ_i is anisotropic, if one uses typical values for a nucleus oriented with the *c*-axis along the field, the result is

$$W \approx -2 \text{ to } -5 \text{ J cm}^{-3}$$

The value of W can be related to a shift in the nucleation temperature (*6*) by noting that the nucleation rate J is approximately

$$e^{(-\Delta G^*/kT)}$$

where $\Delta G^* = 16\pi\alpha^3/3(\Delta G)^2$ and $\Delta G = -\Delta S(T_0 - T) + W$.

In these equations, ΔS is the entropy of fusion (about 1.13 J cm^{-3} °C^{-1} for ice), T_0 is the melting temperature, T is the temperature of the supercooled water, k is Boltzmann's constant, ΔG is the free energy difference per unit volume between ice and water, and α is the surface free energy between ice and water.

The effect of the electric field is to raise the temperature at which nucleation occurs (relative to a similar crystal with no electric field present) by the amount $W/\Delta S$, or 2° to 5°C. This is consistent with the results in (*1*). A plot of the shift in the nucleation temperature against σ^2 for all of the crystals that were tested by Gavish *et al.* would reveal any correlation between these two properties.

Finally, several studies (*7*) have attempted to measure the effect of an electric field on nucleation with methods more direct than those used by Gavish *et al.*

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Response: We welcome the macroscopic approach proposed by Wilen. We cannot discount the qualitative results of his analysis. In our report, we explained the phenomenon of induced nucleation of ice on a molecular basis, proposing an intermediate nucleating phase consisting of ice (partially) proton-ordered along the hexagonal axis and thus polar, the idea being that the polar ice nucleus is stabilized in a polar environment. We did not intend to suggest that such an ice nucleus consists of only one or two proton-ordered ice-like bilayers sandwiched into the crevice of an L- or D,L-alanine crystal, but rather we used the small number of bilayers for ease of calculation.

With regard to the "stability" of such a single ice (0001) polar bilayer, we note that it has high density (the perpendicular separation between oxygen atoms across the bilayer is only 0.9 Å) and that each oxygen participates in three hydrogen bonds in a bilayer as opposed to four in crystalline ice. Such a proton-ordered water polar bilayer is part of the complex compound $3AgI \cdot NH_4 \cdot 6H_2O$, as reported by B. L. Davis *et al.* (*1*), who studied it in order to understand the exceptional ice-nucleating ability of aerosols obtained from AgI-NH_4-acetone solution. Each ice-like water bilayer is sandwiched between a layer of Ag and one of I ions, and so must be proton-ordered and polar in a direction normal to the layer, although the H-positions have not been determined. The threshold of ice nucleation for this phase was found to be −1°C, as contrasted with a threshold of −4°C for silver iodide.

Further evidence favoring a polar ice nucleus may be deduced from recent studies on ice nucleation induced from a two-dimensional surface of long-chain alcohols that are arranged as monolayers on drops of water (*2*). These systems involve a partial lattice and structural match (*3*) between the alcohol OH groups and the (0001) layer of ice. Because each oxygen atom in the alcohol molecule must have only one hydrogen bound to it, proton-ordering along the hexagonal axis at the hydroxyl-ice interface would occur and would be propagated for several consecutive bilayers.

We examined the orientation of an isolated water molecule in the crevices of L- and D,L-alanine and found, as expected, a preferred orientation of water in the polar crevice of D,L-alanine, with the symmetry axis of water parallel to the polar crystal axis. The preferred orientation of water in the polar crevice is closer to that of water in ice, which is proton-ordered along the hexagonal axis, than it is to completely proton-disordered ice. These are two distinct orientations of the water molecules in proton-ordered ice with respect to the hexagonal axis, one being fairly close to the preferred orientation of the isolated water molecule. An analysis of the molecular reorientation from water to ice would be required for such an argument to be convincing. Therefore, we did not elaborate on these points in our report, but only referred to the calculations of Zhu and Robinson (*4*) on the structure of

Table 1. Calculated interaction energy E between the D,L and L forms of alanine with $n = 2$ or 3 bilayers of ice intercalated within a crevice of alanine. Polar and nonpolar ice models were used. Energies are in kcal/mol.

n	$E_{D,L}$	E_L	ΔE
	Polar ice		
2	−4.0	−2.7	−1.3
3	−3.3	−1.9	−1.4
	Nonpolar ice		
2	−1.2	−1.3	0.1
3	−1.1	−1.0	−0.1

liquid water between electrically charged plates, and we made a general statement that an electric field mechanism helps align the water molecules into ice-like clusters.

Thus, our analysis does not involve the difference in energy between "ice" and "water" but is rather a comparison of the interaction energy of the polar substrate crystal with an ice nucleus (partially) proton-ordered along the hexagonal axis as against a nonpolar substrate with (completely) disordered ice nucleus.

Model calculations (Table 1) imply that unless polar ice is introduced at the nucleation stage, there is no advantage of D,L-alanine over the L form. With polar ice we have in effect increased the dielectric constant of the intercalated ice medium.

With regard to the last point raised by Wilen, our overall aim was to demonstrate the ice nucleating ability of polar crystals by unraveling the riddle of the difference in ice nucleating behavior of the racemic and chiral forms of α-amino acid crystals. In this way, we also hope to provide other approaches for understanding phenomena such as the ice nucleating ability of frost bacteria.

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HTLV-1 Provirus and Mycosis Fungoides

Human T cell lymphotropic virus type–1 (HTLV-1) is associated with a mature postthymic T cell malignancy, adult T cell leukemia/lymphoma (ATL), which often affects cutaneous tissues. ATL is clinically distinct from mycosis fungoides (MF), although in both conditions the neoplastic $CD4^+$ T cells are epidermotropic (*1*).

W. W. Hall *et al.* found a partially deleted HTLV-1 provirus in a cell line established from one patient with MF (*2*). Defective HTLV-1 provirus has also been found in some cases of ATL (*3*). The findings of Hall *et al.* and others (*4*) implicate HTLV-1 or a related retrovirus in the pathogenesis of cutaneous T cell lymphoma in some patients.

To assess the frequency of this association, we analyzed genomic DNA from freshly isolated peripheral blood mononuclear cells and from lesional skin biopsies of 40 patients with MF (*5*). Under low stringency conditions, Southern (DNA) blot hybridization with a full-length HTLV-1 probe (λ 23-3) revealed identical multiple bands in the 40 patients and in normal controls, which presumably indicates the presence of endogenous HTLV-1–like sequences within human genomic DNA (*6*). In contrast, at high stringency we found discrete bands in tissue DNA from only three (seronegative) Caucasian patients. The sizes of these bands were different from those characteristic of HTLV-1–associated ATL samples. Specifically, in one patient a truncated 8-kb Eco RI fragment was detected (Fig. 1), while the other two patients had an extra 3-kb Hind III fragment. We did not succeed in amplifying HTLV-1 sequences from these latter two patients, but in the former patient amplification by polymerase chain reaction with primers flanking a conserved HTLV-1 *pol* sequence yielded a 119–base pair fragment. Subcloning into bacteriophage M13 and single-strand DNA sequence analysis revealed a 119–base pair *pol* sequence which, with the exception of a single base substitution, was identical to that of HTLV-1 (*7*).

These data indicate monoclonal integration of exogenous defective HTLV-1 sequences, which agrees with the findings of Hall *et al.* However, our results indicate that defective HTLV-1 sequences can be detected only in about 10% of patients with MF. Therefore, in our view, the role of these defective retroviruses in the pathogenesis of mycosis fungoides remains in question.

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Cutaneous T cell leukemias/lymphomas (CTCLs) are rare neoplasms that include mycosis fungoides (MF), its variant Sézary syndrome (SS) (considered to be the leukemic phase of MF), and adult T cell leukemia/lymphoma (ATL) (*1*). ATL, in its acute or chronic forms, is closely associated with HTLV-I infection (*2*) and consequently has a marked geographic predominance, with a high incidence in Japan, the Caribbean, and parts of eastern Europe. The role played by HTLV-I in MF and SS has been the subject of contradictory reports.

Hall *et al.* (*3*) report finding deleted HTLV-I provirus in cutaneous lesions of patients with MF. Polymerase chain reaction (PCR) analysis with HTLV-I *gag*, *pol*, *env*, pX, and long terminal repeat oligonucleotide primers, showed amplification of HTLV-I sequences in tissue samples from lesions of the five patients that they tested and in the blood of one of them. On this basis, they suggest that HTLV-I infection may be involved in at least certain cases of

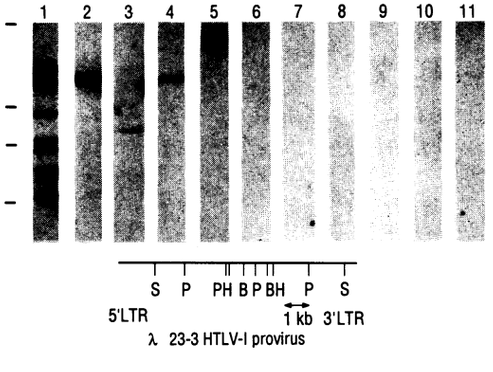

Fig. 1. Southern DNA blot autoradiograph of Eco RI–digested DNA hybridized with a full-length HTLV-1 probe (λ 23-3) under high stringency conditions. A restriction enzyme map of the HTLV-1 provirus and the sizes of λ DNA fragments digested by Hind III (23.3, 9.4, 6.6, 4.4 kb) are shown. Positive controls are indicated by multiple bands in DNA from cell line MT2 (*1*) and a single 13 kb band in DNA from a patient with ATL (*2*). Single discrete Eco RI bands (3/8, 4/13.4, and 5/19.8 kb) are present in tissue DNA samples from three patients with MF, while negative results are seen in six other patients with MF (lanes 6 through 11). S, Sst I; P, Pst I; H, Hind III; B, Bam HI.