Enantioselective Adsorption of the D- and L-Forms of an α-Helical Antifreeze Polypeptide to the {20$\bar{2}$1} Planes of Ice

Richard A. Laursen,*,† Dingyi Wen,† and Charles A. Knight‡

Department of Chemistry, Boston University
Boston, Massachusetts 02215
National Center for Atmospheric Research
Boulder, Colorado 80307

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Many cold water fishes survive in subzero seawater by secreting antifreeze polypeptides and glycoproteins that bind to and inhibit the growth of ice crystals in their body fluids.[1−3] Of these, the most intensively studied are the α-helical type I antifreeze polypeptides (AFPs), such as those found in the winter flounder.[4−9] Using an ice crystal etching technique, Knight et al.[6] provided the first evidence that type I AFPs bind to the {20$\bar{2}$1}[10] hexagonal bipyramidal planes of ice and that they are oriented along directions ⟨$\bar{1}$102⟩, whose repeat spacings of 16.7 Å match the repeat spacings of polar groups on one face of the helix. Thereafter, Wen and Laursen[8] described a detailed binding model in which both threonine and aspartic acid oxygen atoms and asparagine nitrogen atoms of the AFP oriented along [$\bar{1}$102] occupy specific water oxygen sites on the "ridges" and "valleys" of the (20$\bar{2}$1) surface. Similar models were also deduced by Lal *et al.*,[11] based on molecular modeling studies, and by Knight *et al.*[12] The model predicts that the AFP can bind only along [$\bar{1}$102], with the amino terminus oriented toward the apex of the hexagonal bipyramid but not along the reflected, but chemically equivalent, direction [0$\bar{1}$12]. It was further predicted by molecular modeling and energy minimization that an AFP composed of all D-amino acids should bind along [0$\bar{1}$12], but not [$\bar{1}$102].[8] Subsequently, a D-AFP was synthesized and shown, as expected, to have antifreeze activity identical to that of the natural L-AFP.[13] In addition, molecular modeling and energy minimization calculations by Madura and coworkers[14] showed that the L- and D-AFPs should bind in the mirror image directions [$\bar{1}$102] and [0$\bar{1}$12], respectively. Despite these predictions, however, there has been no direct experimental evidence for chiral binding sites on ice surfaces. In this communication, we provide that evidence.

The AFPs used in this investigation were the same synthetic products used for antifreeze activity studies,[13] namely, the 37-residue winter flounder AFP (S00), DTASDAAAAAALTAANAKAAAELTAANAAAAAAATAR. Single crystals of ice were grown as hemispheres of about 4.5 cm diameter on a cold finger from AFP solutions as described previously.[6] The total AFP concentration in each solution (D-AFP, L-AFP, and D+L-AFP) was 0.015 mg/mL. The hemispheres were then etched by evaporation at −10 to −15 °C.[6]

Figure 1a shows an etching pattern for the synthetic L-AFP that is identical to that reported[6] earlier for the native AFP isolated from winter flounder, while Figure 1b shows the predicted[8,14] mirror image etching pattern for the D-AFP. In contrast, a mixture of D- and L-AFPs gives an X-shaped pattern resulting from superimposition of the D and L patterns.

In the study that first established the direction of orientation of the winter flounder AFP on ice, Knight et al.[6] argued that if a native AFP were to bind equally well along the chemically equivalent reflected orientations on the adsorption plane of ice, an etching pattern in the form of a "fat X" would be observed. The fact that only a single orientation was seen, as in Figure 1a, provided the evidence that binding was stereospecific, in this case, along [$\bar{1}$102]. In the case of the DL mixture, the observation of a "fat X" (Figure 1c) indicates that binding occurs along both orientations, at least in the center of the X. This is consistent with the observation that a mixture of D- and L-AFPs has the same antifreeze activity and generates the same ice crystal morphology (a hexagonal bipyramid) as either the D- or L-AFP alone.[13] The origin of the etching patterns on the ice hemisphere has been discussed,[6] but can be envisioned by imagining the ice hemisphere as a terraced, dome-shaped hill, with the surface of the terraces parallel to the (20$\bar{2}$1) plane. The top terrace is large and circular, but the lower terraces are annular and become successively narrower downhill. If one then places a large number of long cylindrical objects (AFPs) on the terraces and allows them to orient only in two directions ([$\bar{1}$102] and [0$\bar{1}$12]), they will generate a pattern in the form of an X (Figure 2). Both orientations are possible on the entire surface of the wide upper terraces, but only on parts of the surface of the narrow terraces.

These results provide direct experimental confirmation of earlier predictions[8,13,14] that the D enantiomer of the winter flounder AFP should bind to the reflected direction [0$\bar{1}$12] on the (20$\bar{2}$1) surface of ice and demonstrate that this particular surface is *prochiral,* in that the constellation of ice lattice water molecules that make up the binding site for the D-AFP is the mirror image of the site for the L-AFP. Some other surfaces, such as the [0001] basal face of hexagonal ice, where all oxygen atoms are in the same plane, are not prochiral. The ability of

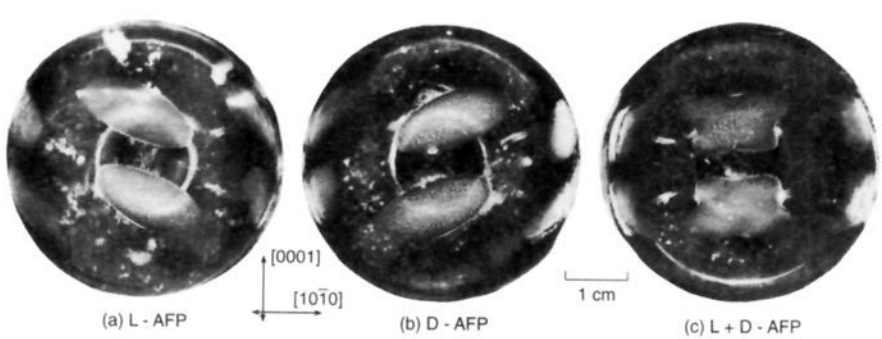

Figure 1. Etched single crystals of ice grown as hemispheres from solutions containing 0.015 mg/mL of (a) L-AFP, (b) D-AFP, and (c) D+L-AFP. The hemisphere is viewed from the top, normal to the *c*-axis [0001]. Each etched area is centered at a (20$\bar{2}$1) hexagonal bipyramidal surface, six out of 12 of which can be seen on the surface of each hemisphere. The apexes of the bipyramid lie along [0001], the *c*-axis.

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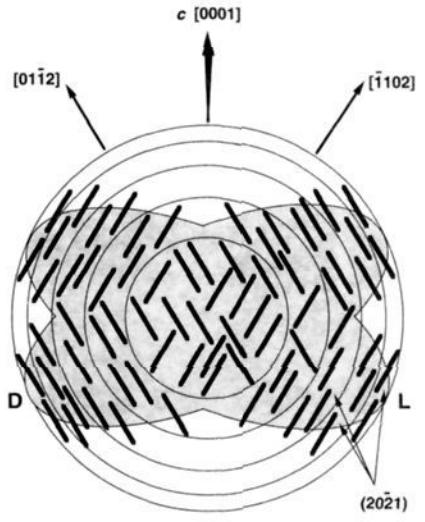

Figure 2. Proposed orientation of D- and L-AFP molecules on the etched surface of an ice hemisphere giving rise to a "fat-X" pattern. The arrangement of the AFP molecules (solid rods) is governed by the width of the steps or terraces and their permitted orientations with respect to the ice lattice. The projection shown is normal to the (20$\bar{2}$1) planes (the etched area) which are tilted with respect to the [0001] *c*-axis.

apparently achiral surfaces to bind biological molecules stereospecifically may find analogy in other systems where crystal surfaces interact with proteins, for example, in mineralized tissue such as bone and shell.[15,16] A recent report[17] describing the differential interaction of cultured epithelial cells with the {011} faces of the *R,R* and *S,S* calcium tartrate crystals represents another type of stereospecific protein−crystal surface interaction, but also serves to demonstrate that crystalline materials can present chiral sites for enantioselective binding of biological molecules.

* Address correspondence to Dr. Richard A. Laursen, Department of Chemistry, Boston University, 590 Commonwealth Ave., Boston, MA 02215. Tel: (617) 353-2491. FAX: (617) 353-6466. Internet: laursen@chem.bu.edu.
† Boston University.
‡ National Center for Atmospheric Research.

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(10) In the presence of AFPs, water crystallizes as a hexagonal bipyramid with 12 equivalent surfaces {20$\bar{2}$1} and 12 corresponding AFP alignment positions ⟨01$\bar{1}$2⟩, one of which is [$\bar{1}$102]. The specific alignment directions used in this discussion, [$\bar{1}$102] and [01$\bar{1}$2] for the L- and D-AFPs, respectively, are those appropriate for the specific plane (20$\bar{2}$1). The hexagonal four-index notation [*abcd*] can be converted to Miller indicies [*xyz*] as follows: $x = a - c$; $y = b - c$; and $z = d$. Thus [$\bar{1}$102] and [01$\bar{1}$2] become [$\bar{1}$12] and [$\bar{1}$22], respectively.
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