Interactions of the D- and L-Forms of Winter Flounder Antifreeze Peptide with the {201} Planes of Ice

Jeffry D. Madura,*,† Andrzej Wierzbicki,†
John P. Harrington,† Richard H. Maughon,†
James A. Raymond,‡ and C. Steven Sikes‡

Department of Chemistry and Department of Biological Sciences, University of South Alabama Mobile, Alabama 36688

Received August 16, 1993

Antifreeze peptides and glycopeptides found in cold water fishes have been studied experimentally for two decades[1-6] and computationally for the last eight years.[7-10] In their pioneering work, Knight et al. used etch marks on single ice crystals to identify the planes on which, and the probable directions along which, several antifreezes bind to ice.[11,12] They proposed that the winter flounder antifreeze peptide (WF), a right-handed α-helix, binds to the (201)[13] bipyramidal planes of ice I_h along the [$\bar{1}$12] direction, a direction whose repeat distance, 16.7 Å, nearly matches that of the WF polar groups. Recently, Wen and Laursen synthesized an all-D-isomer of WF that had the same antifreeze activity as its natural L-enantiomer.[14] They suggested that the D-isomer, being a mirror image of the L-isomer, should bind in a mirror symmetry-related direction along the [$\bar{1}\bar{2}$2] vector. In this communication, using molecular modeling and energy minimization calculations, we demonstrate that indeed the D-isomer preferentially binds along the mirror-related [$\bar{1}\bar{2}$2] direction and analyze the nature of the binding preference for the L- and D-isomers.

We employed molecular modeling and energy minimization methods to study the binding of WF to the (201) bipyramidal planes of ice I_h along the two mirror symmetry-equivalent vectors [112] and [122]. The ice surfaces were constructed from the asymmetric fractional coordinates of ice[15] using CERIUS 3.2.[16] Once the surface was constructed, the hydrogen positions of ice were randomized by running a 5-ps, 2000K dynamics calculation, in which the oxygen positions were held fixed, followed by 500 steps of steepest descent minimization. Initial structures for WF along with the positioning of WF on the ice surface were prepared using QUANTA 3.4.[17] Energy minimizations,[18] which permitted translational, rotational, and conformational changes of the peptide with respect to the ice surface, were performed with CHARMM 22.[19] During the energy minimizations, only the oxygen positions of ice were held fixed, allowing the water molecules to rotate freely.

Table 1. Binding Energies of Winter Flounder Antifreeze Peptide on the (201) Plane of Ice I_h

isomer	direction	energy/(kcal/mol)[a] N^+	N^-
L	[$\bar{1}$12]	−282	−199
L	[$\bar{1}\bar{2}$2]	−234	−195
D	[$\bar{1}$12]	−242	−195
D	[$\bar{1}\bar{2}$2]	−288	−203

[a] N^+ represents the N-terminus pointing in the direction of the bipyramidal apex, while N^- represents the opposite direction.

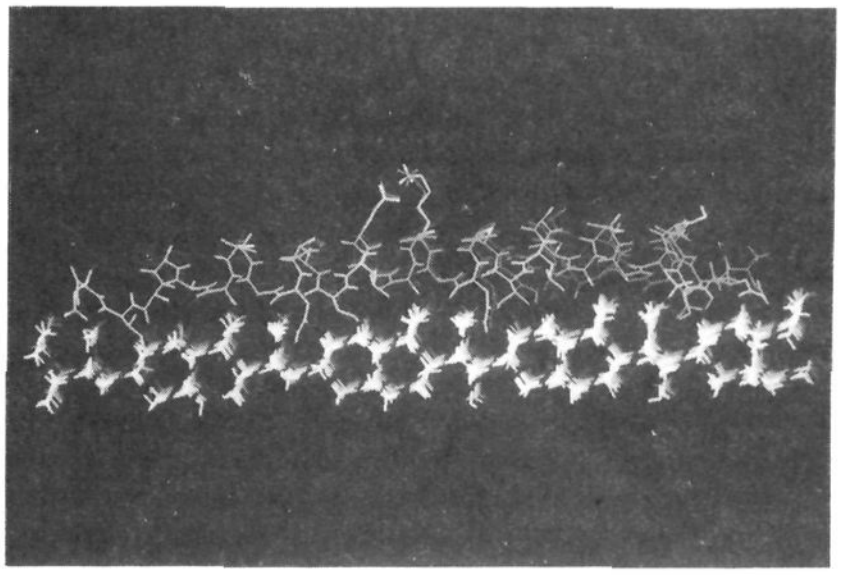

Figure 1. Side view of winter flounder D-isomer (foreground) and L-isomer (background) on the (201) ice surface. Both N-termini are on the left and point toward the bipyramidal apex. Two asparagine and two threonine side chains are visible near the center of the figure, with the asparagine side chains embedded in the ice lattice.

The energy minimizations showed that WF L-isomer binds significantly more strongly along the [$\bar{1}$12] direction than along the [$\bar{1}\bar{2}$2] direction. The difference, 48 kcal/mol (Table 1), is similar to the 40 kcal/mol difference observed by Wen and Laursen. The calculations also show a preference in orientation in which the N-terminus points toward the bipyramidal apex, (N^+). Binding in the opposite orientation (N^-) was found to be 83 kcal/mol weaker (Table 1). This orientational difference is due to the ice surface topography, i.e., the binding sites along the [$\bar{1}$12] vector do not have 2-fold rotational symmetry on the (201) plane.

The D-isomer preferentially binds in the [$\bar{1}\bar{2}$2] direction with a 46 kcal/mol preference over the D-isomer in the [$\bar{1}$12] direction. Preferential binding energy for the D-isomer in the [$\bar{1}\bar{2}$2] direction is the same as that for the L-isomer in the [$\bar{1}$12] direction (Table 1). The slight differences in binding energies are due not to intrinsic differences in binding but to the following: first, in the process of minimization, which was done for each isomer independently, the final positions of the isomers resulted in slight structural differences (Figure 1); second, although the D- and L-isomers of the peptide and the [$\bar{1}$12] and [$\bar{1}\bar{2}$2] ice oxygen lattice positions are mirror images, the positions of the ice

* To whom correspondence should be sent.
† Department of Chemistry.
‡ Department of Biological Sciences.

(1) DeVries, A. L. *Phil. Trans. R. Soc. London B, Biol. Sci.* **1984**, *304*, 575–588.
(2) Yang, D. S. C.; Sax, M.; Chakrabartty, A.; Hew, C. L. *Nature* **1988**, *333*, 232–237.
(3) Davies, P. L.; Hew, C. L. *FASEB J.* **1990**, *4*, 509–517.
(4) Cheng, C. C.; DeVries, A. L. *Life Under Extreme Conditions*; Springer-Verlag: Berlin, 1991; pp 1–14.
(5) Hew, C. L.; Yang, D. S. C. *Eur. J. Biochem.* **1992**, *203*, 33–42.
(6) Wilson, P. W.; Beaglehole, D.; DeVries, A. L. *Biophys. J.* **1993**, *64*, 1878–1884.
(7) Sansom, C. E.; Atkins, E. D. T.; Upstill, C. *Gums and Stabilizers for the Food Industry*; Elsevier: New York, 1986; pp 565–575.
(8) Chou, K. C. *J. Mol. Biol.* **1992**, *223*, 509–517.
(9) Wen, D.; Laursen, R. A. *Biophys. J.* **1992**, *63*, 1659–1662.
(10) Jorgensen, H.; Mori, M.; Matsui, H.; Kanaoka, M.; Yanagi, H.; Yabusaki, Y.; Kikuzono, Y. *Protein Eng.* **1993**, *6*, 19–27.
(11) Knight, C. A.; Cheng, C. C.; DeVries, A. L. *Biophys. J.* **1991**, *59*, 409–418.
(12) Knight, C. A.; Driggers, E.; DeVries, A. L. *Biophys. J.* **1993**, *64*, 252–259.
(13) Planes are denoted by parentheses, while vectors are identified by brackets. The planes and vectors are defined by three Miller indicies. Four-index notation for planes may be converted to the three-index notation by dropping the third index, e.g., (20$\bar{2}$1) becomes (201). Conversion of four-index notation for a vector [*abcd*] to a three-index notation [*xyz*] is done as follows: $x = a - c$; $y = b - c$; and $z = d$.
(14) Wen, D.; Laursen, R. A. *FEBS* **1993**, *317*, 31–34.
(15) The space group used for ice, I_h, was No. 194 $P6_{3/mmc}$, unit cell constants were $a = b = 4.516$ Å, $c = 7.354$ Å, $\alpha = \beta = 90°$, $\gamma = 120.0°$, and fractional coordinates O(0.3333,0.6667,0.0629), H_a(0.3333,0.6667,0.1989), and H_b-(0.4551,0.9102,0.0182).
(16) CERIUS molecular modeling software materials research from Molecular Simulations Inc. of Burlington, MA, and Cambridge, U.K.
(17) QUANTA 3.4 molecular modeling software from Molecular Simulations Inc. of Burlington, MA.
(18) All energy minimizations were run with a constant dielectric, vgroup, group, switch, and vswitch cutoffs with a cutoff of 8.0 Å. TIP3 water model was used in all calculations.
(19) Brooks, B.; Bruccoleri, R. E.; Olafson, B. D.; States, D. J.; Swaminathan, S.; Karplus, M. *J. Comput. Chem.* **1983**, *2*, 187–217.

0002-7863/94/1516-0417$04.50/0

hydrogens are not, thereby giving rise to the slight differences in the binding energies. The possibility of being trapped in a local minimum was eliminated by generating a set of (ca. 20) different rotational and translational perturbations of the best binding arrangement and recomputing the binding energy.

The binding energy differences in the N^+ orientation for the L-isomer when changing the binding direction from the preferred [$\bar{1}12$] to the mirror image-related [$\bar{1}\bar{2}2$], 48 kcal/mol, and for the D-isomer when changing from preferred [$\bar{1}\bar{2}2$] to [$\bar{1}12$], 46 kcal/mol, reflect the stereospecificity of binding. Analysis of the L-isomer configuration, when bound along [$\bar{1}\bar{2}2$] N^+ direction when compared to binding along the optimal [$\bar{1}12$] N^+ direction shows that the α-carbons occupy the same positions with respect to the ice oxygen atoms but the side chains (e.g., Asn, Thr, Leu) are improperly oriented to promote strong binding. Exactly the same holds true for the D-isomer when the binding configurations along [$\bar{1}12$] N^+ and optimal [$\bar{1}\bar{2}2$] N^+ are compared.

Figure 1 shows the D- and L-isomers bound to (201) with the N-termini pointing toward the bipyramidal apex. It can be seen that the threonines are in positions to continue the coordination polyhedra of the ice surface, while the asparagines fit within the polyhedra cages.

Figure 2 shows the L- and D-isomers of WF bound in their lowest energy configurations to the (201) surface, viewed with the *c*-axis normal to the page. It can be seen that the two helicies are mirror images of each other with the asparagine, leucine, and threonine side chains positioned on the outside of each helix. We have found this to be a second requirement for binding (in addition to lattice matching), which accounts for the preference for a particular direction.

In summary, the preferential binding is due to (i) a lattice match between the ice oxygen atoms and the polar groups of the peptide (which narrows the number of possible binding directions to [$\bar{1}12$] and [$\bar{1}\bar{2}2$]) and (ii) the fitting of the peptide shape with that of the ice surface topography. This stereospecific binding accounts for the N^+/N^- and L/D preferences.[20] Although the [$\bar{1}12$] and [$\bar{1}\bar{2}2$] binding directions appear to be rotationally equivalent (i.e., the right-handed helix can be rotated from [$\bar{1}12$] to [$\bar{1}\bar{2}2$] within the (201) plane), the helix will encounter a different binding environment due to the fact that the two directions are mirror reflections of one another, and only the D-isomer, which is a mirror reflection of the L-isomer, satisfies the symmetry requirement.

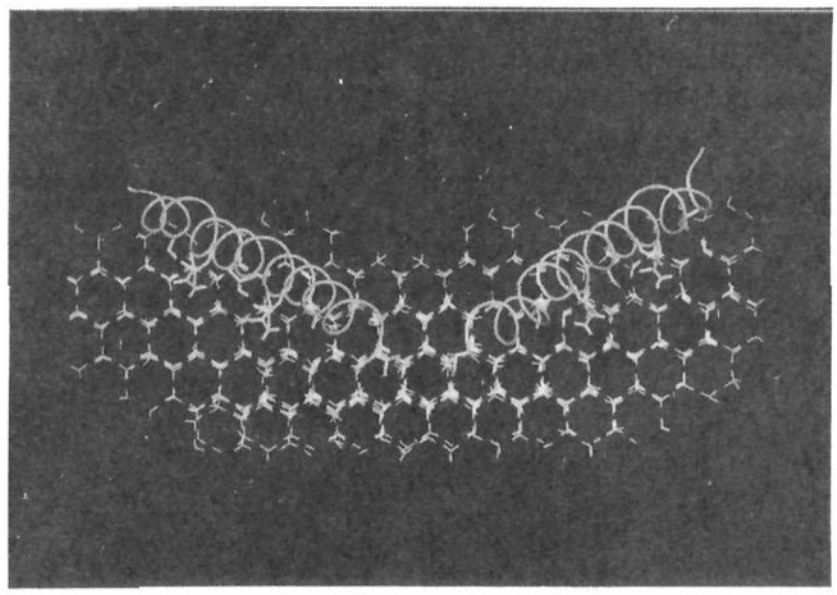

Figure 2. View of the model in Figure 1 from the right looking down the *c*-axis toward the bipyramidal apex. The D-isomer is on the left and the L-isomer is on the right. The peptide is drawn as a helical backbone with the asparagine, threonine, and leucine side chains explicitly drawn as thick bonds.

Acknowledgment. We are grateful for financial support of this work from Research Corporation under (C-3121), NSF/EPSCoR (EHR-9108761), and NOAA Sea Grant Program (NA16RG0155-03). We also thank Dr. C. A. Knight for enlightening discussions and Mark Taylor for computational assistance.

(20) Weissbuch, I.; Addadi, L.; Lahav, M.; Leiserowitz, L. *Science* **1991**, *253*, 637–645.