Structural study on TypeIII AFP intramolecular dimer RD3

Kazunori Miura, Satoru Ohgiya, Tamotsu Hoshino, Nobuaki Nemoto[1], Katsutoshi Nitta[2], and ○ Sakae Tsuda

Hokkaido National Industrial Research Institute (HNIRI), Sapporo 062-8517, JAPAN [1]Varian Japan, Tokyo 108-0023, JAPAN

[2]Division of Biological Sciences, Graduate School of Science, Hokkaido University, Sapporo 060-0808, JAPAN

Two domain AFP named RD3

A new member of antifreeze protein (AFP) named RD3 extracted from antarctic eel pout is a single polypeptide comprising homologous N-terminal half (res. Asn[1]-Glu[64]) and C-terminal half (res. Ser[74]-Glu[134]) domains, each of which has a high sequence identity with Type III AFP. A 9-residues linker (-D[65]GTTSPGLK[73]-) connects these two domains in tandem. We are currently examines the solution structures of a recombinant N-domain plus the linker of RD3 (RD3-NI: residues 1-73) and a recombinant intact RD3 protein by employing 2D- and 3D-NMR spectroscopy. The N-domain of RD3-NI appears to construct a globular form comprising six β-strands, three type III turns, and several loops, which stabilize a flat ice-binding site constructed on one side of this domain. It appears that the linker portion of RD3-NI constructs a bending structure independently of the globular N-domain. By a combination of the studies of RD3-NI and RD3, we are hopeful to clarify the structure-function relationship of the globular type of AFP.

Primary structure of RD3

- Type III AFP intramolecular dimer (N- and C-domains connected by a 9-residues linker)
- 134 amino acid polypeptide (no disulfide bond, no cation, no suger)
- dissolved in water (p*I* = 4.65)

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1          11         21         31         41         51         61
NKASVVANQL IPINTALTLI MMKAEVVTPM GIPAEEIPNL VGMQVNRAVP LGTTLMPDMV KNYEDGTTSP
71         81         91         101        111        121        131
GLKSVVANQL IPINTALTLV MMKAEEVSPK GIPSEEISKL VGMQVNRAVY LDQTLMPDMV KNYE
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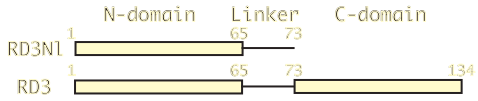
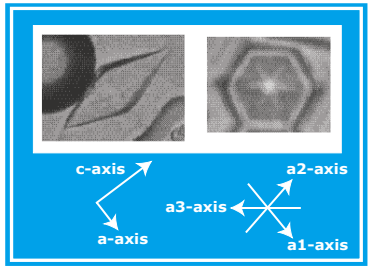
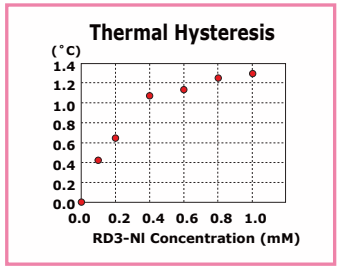


Materials and methods

RD3-NI and RD3 proteins were obtained by using E.coli expression systems constructed by our group. About 50~100 mg of the proteins were isolated from 2.4~3 liter culture of E. coli transformant. In addition, about 15 mg of ^{15}N-labeled RD3-NI and of ^{13}C/^{15}N-labeled RD3 were obtained from 4.0~6.0 liter culture. The antifreeze activity of the recombinant proteins were examined by the observation of the bipyramidal ice crystal and the thermal hysteresis of the solutions.



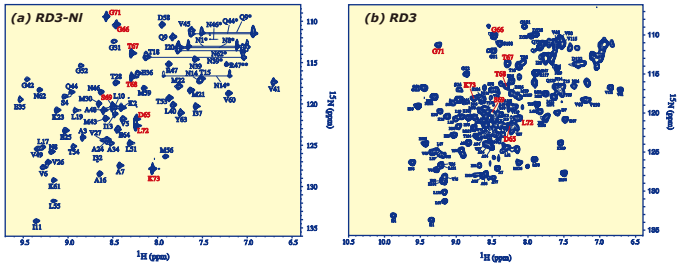


For the expressed recombinant proteins, ^{1}H-, ^{15}N-, and ^{13}C-NMR spectral assignments were performed by employing homo- and heteronuclear multidimensional NMR experiments. Structural restraints were obtained by ^{15}N-edited NOESY (mix=50ms) and HNHA. On the basis of the 958 restraints, well-converged 40 solution structures of RD3-NI were calculated. For RD3 ^{1}H-, ^{15}N-, and ^{13}C-resonance assignments were completed so far. The NMR experiments were performed by JEOL Alpha 500 and Varian UNITY-Inova 500 NMR spectrometers. The structural calculations were carried out using X-PLOR 3.851 on SGI Power Indigo2 (IRIX 6.5.2).

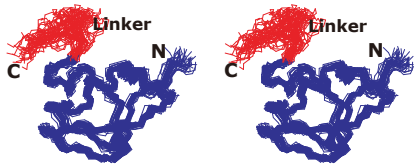
NMR spectral assignments of RD3–Nland RD3

Figures (a) and (b) compare {^{1}H-^{15}N} HSQC spectrum of RD3-NI and that of RD3. Assignments are indicated for each cross-peak. Labels of the peaks originating from the linker residues (DGTTSP GLK) are colored in red. It appears that the cross-peak positions of the linker residues are slightly changed between these two spectra.

^{1}H -^{15}N HSQC Spectra of (a)RD3-NI and (b)RD3 (4˚C)



NMR solution structure of RD3–NI

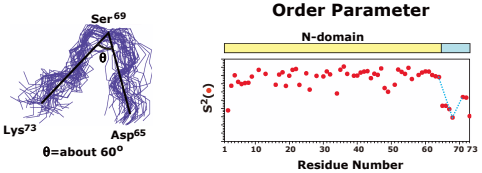


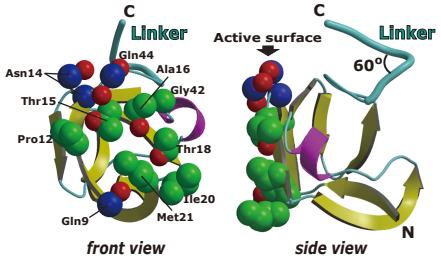
Stereo view of the 40 converged structures of RD3-NI. It appears that the 9-residues linker constructs a definitive structure having a specific orientation.

Structural statistics of the 40 SA structures of RD3-NI

X-PLOR potential energies (kcal/mol)	
E_{total}	105.76 +/- 5.29
E_{bond}	2.70 +/- 0.37
E_{angle}	85.54 +/- 3.17
$E_{improper}$	11.47 +/- 0.28
E_{vdw}	4.70 +/- 1.45
E_{NOE}	1.28 +/- 1.00
E_{cdih}	0.06 +/- 0.06
RMSD from idealized geometry	
bonds (Å)	0.002 +/- 0.000
angles (°)	0.323 +/- 0.010
impropers (°)	0.380 +/- 0.005
RMSD from distance restraints (Å)	
All [915]	0.005 +/- 0.002
intraresidue [460]	0.002 +/- 0.000
sequential [223]	0.004 +/- 0.003
medium-range [49]	0.001 +/- 0.002
long-range [136]	0.004 +/- 0.002
hydrogen bond [40]	0.004 +/- 0.003
RMSD from dihedral restraints (°)	
48 phi	0.134 +/- 0.057
Total number of restraint violations	
NOE > 0.2 Å	0
Dihedral > 2 °	0

A bending structural motif of the short linker

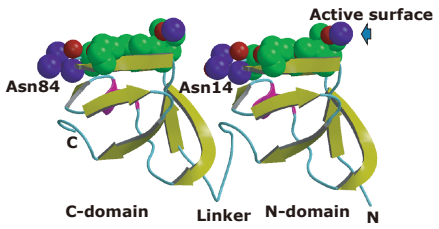




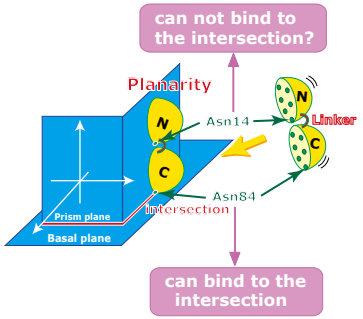
Ribbon representation of RD3-NI, in which the side chain atoms involved in the putative ice-binding site are represented by CPK. They construct a flat surface in the similar manner to the rQAE isoform Type III AFP. The independency of the linker from the globular domain is suggested by only one NOE contact (Tyr^{63}-Pro^{70}) between the linker and the N-domain. The v-shape profile of S^2 for the linker is thought to support its bending formation.

A model structure of RD3

The present identified independency of the linker in RD3-NI molecule may allow us to imagine that the linker keeps its independent nature even in the intact RD3 molecule and restricts the orientation of the two domains in some extent. It should be noted that RD3 possesses 1.9 times higher antifreeze activity compared with the ordinary Type III AFP monomer, implying that any direct or cooperative interaction exists between the two domains. A possible 3D-structure of intact RD3 will be modeled when the linker portion of RD3 is assumed to possess virtually the same bending motif as formed in RD3-NI. In our most updated model structure of RD3, the homologous N- and C-terminal globular domains of RD3 come close in tandem through the 60 degree bending linker so as not to associate directly. The property of the globular domain which does not favor associations is revealed by the present observation of only monomer peaks of RD3-NI even under high concentration (8mg/ml).
In the model structure, the bending linker is able to bring the two flat ice-binding surfaces of both N- and C-domains to the same direction. In such shape of RD3 molecule, the two ice-biding surfaces can interact simultaneously to the same prism plane of the hexagonal ice crystal. Further information will hopefully be provided from the structural analysis on the intact RD3 protein.



It has been pointed out that Asn^{14}, a non-planer residue located on the ice-binding site, is involved in the initial protein-ice interaction at the intersection of the prism and basal planes. In the present model of RD3 Asn^{14} is sandwiched between two globular domains and seems not to be able to bind to the intersection; it is not clear whether the initial ice-binding of RD3 occurs samely with the case of ordinary Type III AFP monomer.



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