IAP/Belspo P7/44 project : Integrative Protein Science (iPROS)

Modeling of the cyclic lipodepsipeptide Pseudodesmin A self-assembly through molecular dynamic simulations

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Abstract

The self-assembly of short peptides into supramolecular structures represents an active field of research with potential applications, ranging from material sciences to medicine. Pseudodesmine A is a cyclic lipodepsipeptide of nine residues which presents a moderate antibacterial activity and whose structure has been resolved by X-ray and NMR. In acetonitrile, Pseudodesmine A is monomeric while in chloroform, it has the same structure but assembles in a supramolecular complex. This structure could associate with membranes and be responsible for the biological activity of the peptide. Comparison of NMR data in the two solvents has given indications on the intermolecular contacts that arise in chloroform and a model for the self-association was proposed. To study in more details this assembly, molecular dynamics simulations have been carried on. The results were compared with detailed information given by NMR, regarding the dimensions of the assembly and the orientation of the individual peptide building blocks inside the supramolecular assembly. In acetonitrile, the simulations show that the peptide has transient interactions while in chloroform, interactions between monomers are always observed. In agreement with NMR, these interactions arise mainly between the backbone protons of the LEU1 and the GLN2, the GLN2 sidechain and the loop located on the opposite end of the monomer structure. From 10 simulations of dimerization, hydrogen bonds were followed and specific interaction patterns were identified regarding the hydrogen bonds formed. Peptide interactions are mainly described by 13 interaction patterns characterized by 2 to 4 hydrogen bonds. In dimers, the peptides can have a linear, a perpendicular or a side by side configuration. From the linear dimer, it is possible to reconstruct filaments and, by combining a linear and a lateral dimer, it is possible to build fibrils with multifilaments, as found in the NMR-derived model. Two self-consistent supramolecular models can be built from dimers and they present a very good correlation with NMR data regarding the supramolecular organization. Besides, the perpendicular dimer can gives peptide rings that can also explain the potential ability of this peptide to form ion pores in membranes.

1. Introduction

Pseudodesmin properties

- Antimicrobial cyclic lipodepsipeptide
- Unique structure defined by NMR in acetonitrile and chloroform
- Monomeric in acetonitrile versus multimeric in chloroform
- Informations derived from NMR about the supramolecular assembly
  Intermolecular contacts, orientation of monomers within the assembly, size, ...

Aim of the molecular modeling study

- Study the behaviour of Pseudodesmin A in different solvents
- Study interactions between Pseudodesmin A
- Propose a model for the supramolecular assembly

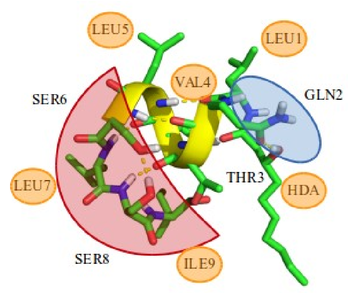
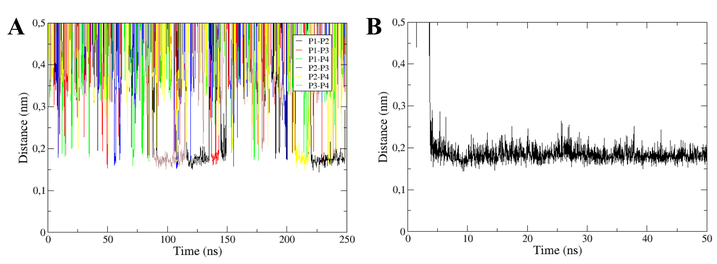


Fig. 1 : NMR structure of Pseudodesmin A (Sinnaeve D. *et al*. 2009). H donor and acceptor sides of the peptide are in red and blue and amino acids of the hydrophobic side are in orange.

2. Results : *PD assembly in acetonitril vs chloroform*

- Pseudodesmin A self assemble in chloroform but not in acetonitrile
- MD have been carried on with Gromacs and the G53a6 force field
- Topologies have been added for D amino acids, HDA and ester bond
- NMR distance restraints were used during simulations

Fig. 2 : A) Minimal distance between 4 Pseudodesmin in acetonitrile during MD of 250 ns and B) between 2 Pseudodesmin in chloroform during MD of 50 ns



3. Results : *PD dimerization in chloroform*

- Interactions were analyzed from 10 simulations of 50 ns
- H bonds patterns are formed during the self-assembly
- Clusters can be defined from these H bonds patterns

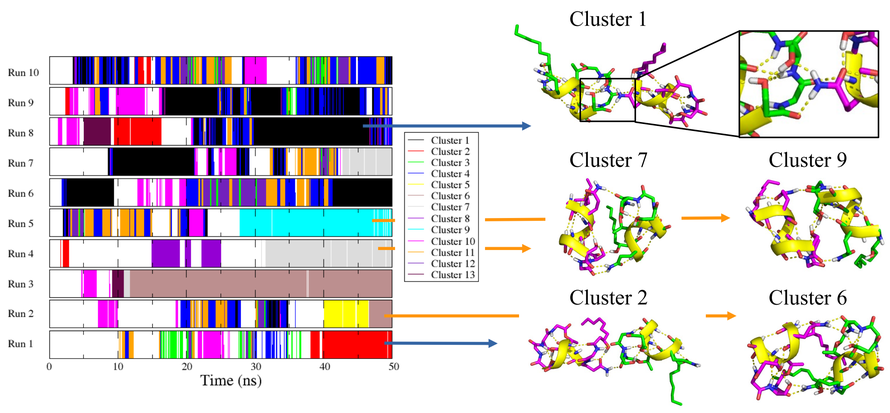


Fig. 3 : Clusters occurrence in each simulation runs with representative structure for long lasting clusters

- Intermolecular contacts arise through H bond formation between N- and C-terminal residues
- The same clusters are observed in different simulations
- Several clusters are more stable along the simulation time

Table I : H bonds defining each clusters with the peptides relative positions

Clusters	Relative position	Hbonds involved	Clusters	Relative position	Hbonds involved
Cluster 1	linear	DGLN2 NH – LEU17 O DGLN2 NH$_E$ – DSER16 O	**Cluster 8**	linear	HDA0 OH – DVAL14 O LEU1 NH – DLEU15 O DGLN2 NH – DSER16 O DGLN2 NH$_E$ – LEU17 O
Cluster 2	linear	LEU1 NH – LEU17 O DGLN2 NH$_Z$ – DSER18 O			
Cluster 3	linear	LEU1 NH – DSER16 O DGLN2 NH$_Z$ – DLEU15 O	**Cluster 9**	anti-parallel	DGLN2 NH$_E$ – DSER16 O DGLN12 NH$_E$ – DSER6 O
Cluster 4	linear	LEU1 NH – LEU17 O DGLN2 NH$_Z$ – DSER16 O	**Cluster 10**	linear	LEU1 NH – DSER18 O DGLN2 NH$_Z$ – LEU17 O
Cluster 5	perpendicular	DGLN2 NH – DSER18 O DGLN2 NH$_E$ – DSER18 OG DGLN2 NH$_Z$ – ATHR13 OG1	**Cluster 11**	linear	HDA0 OH – DSER16 O LEU1 NH – LEU17 O DGLN2 NH$_Z$ – DLEU15 O
Cluster 6	anti-parallel	DGLN2 NH$_E$ – DSER18 OG DGLN12 NH$_E$ – DSER8 OG	**Cluster 12**	linear	HDA0 OH – DLEU15 O LEU1 NH – DSER16 O DGLN2 NH$_Z$ – DVAL14 O
Cluster 7	anti-parallel	DGLN2 NH$_E$ – DSER18 O DGLN12 NH$_E$ – DSER6 O	**Cluster 13**	anti-parallel	DGLN2 NH$_E$ – DLEU15 O DGLN12 NH$_E$ – DSER8 O

- There is a good correlation between intermolecular contacts identified by NMR and their atomic distance computed from several clusters

Table II : Distance for intermolecular contacts identified by NMR in each cluster. Colored values are beneath 5A. Yellow, green and pink are for linear, perpendicular and anti-parallel dimers.

	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13
HDA HA – SER8 HA	4.6	5.0	10.4	3.6	8.4	11.0	6.9	10.3	9.8	4.5	4.2	8.3	9.8
HDA OH – SER8 HA	3.2	3.8	9.0	4.8	6.9	7.9	3.9	7.7	7.7	3.3	5.7	9.0	7.5
LEU1 HN – LEU7 HA	6.4	4.0	5.4	3.3	9.6	13.1	9.6	5.0	10.5	7.9	2.9	3.2	10.8
LEU1 HN – SER8 HA	2.7	3.1	7.8	3.8	5.9	9.4	5.9	8.7	10.4	3.7	4.4	6.6	7.3
LEU1 HN – ILE9 HB	6.9	8.0	12.2	8.7	4.6	9.8	9.9	11.7	16.3	5.9	9.6	10.9	11.3
GLN2 HN – LEU7 HA	4.1	7.0	3.8	3.9	8.8	11.0	7.9	3.3	8.1	7.7	4.0	4.2	9.2
GLN2 HN – SER8 HA	3.1	4.0	7.3	6.3	4.6	7.9	5.7	6.7	8.6	4.5	7.2	8.5	5.5
GLN2 HN – ILE9 HB	8.7	8.5	11.5	11.0	6.6	9.5	8.3	11.1	14.1	7.0	11.8	11.6	8.2
GLN2 NH$_E$ – SER8 HA	3.9	4.9	8.5	8.2	4.5	4.4	4.3	3.6	4.9	4.4	9.4	9.6	4.0
GLN2 NH$_Z$ – SER8 HA	2.8	3.5	8.4	7.1	5.2	4.9	3.6	4.5	4.4	2.8	7.9	8.3	4.8

4. Results : *Modeling of PD filaments*

- Filaments are build from the linear dimers
- Multifilaments are build from linear and lateral dimers
- Filaments present different topologies depending on the cluster
- Only few cluster combination give acceptable double filaments
- **Double filaments C1+9 and C4+9 are the only possibilities**

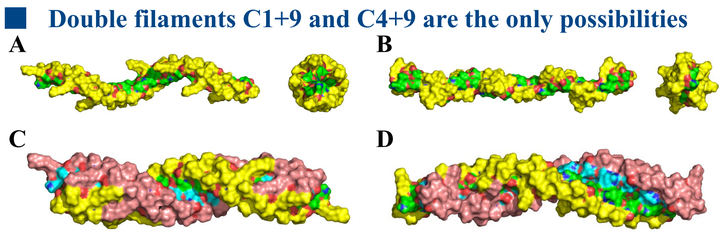


Fig 4 : Model of filaments build from A) cluster 1 and B) cluster 3 and combining C) clusters 1 + 9 and D) clusters 4 + 9.

- The two second filaments are almost identical to the first one
- There is a good correlation with NMR on the filament size
- Width calculated from C1+9 is 21Å and 23Å from C4+9 versus 25Å measured from NMR
- A length of 64 Å means that there is 5 peptides per filament

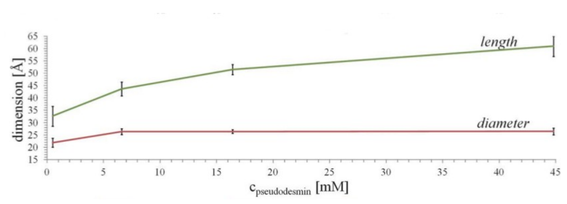


Fig 5 : Dimension the multimolecuar Pseudodesmin A as a function of concentration (Sinnaeve *et al*. 2012).

- There is a good correlation with NMR on the angles between CαH and helix axis

Table III : Average angles between CαH and the filament axis

	Leu1 CHα	Gln2 CHα	Thr3 CHα	Val4 CHα	Leu5 CHα	Ser6 CHα	Leu7 CHα	Ser8 CHα	Ile9 CHα	Thr3 CHβ
NMR	**138,1**	**72,9**	**47,0**	**42,4**	**74,2**	**69,2**	**34,3**	**14,0**	**79,5**	**132,8**
C1	125,3	55,7	50,4	36,6	60,0	56,6	32,7	33,9	97,7	114,7
C2	131,7	76,9	53,1	32,3	54,6	73,8	47,0	14,7	81,6	117,9
C3	115,2	44,6	38,1	48,0	40,4	49,4	18,9	50,7	102,9	130,9
C4	107,1	56,5	23,5	47,9	72,9	55,4	20,7	44,1	82,7	153,1
C8	87,8	152,8	130,5	102,2	121,5	155,9	165,3	108,9	54,0	72,7
C10	66,5	105,4	156,2	130,9	85,2	104,8	143,6	151,3	106,7	21,8
C11	104,9	37,3	41,9	60,8	63,0	36,8	6,1	46,8	122,7	124,2
C12	52,5	128,0	122,8	122,4	143,4	138,7	141,2	134,9	72,2	51,5

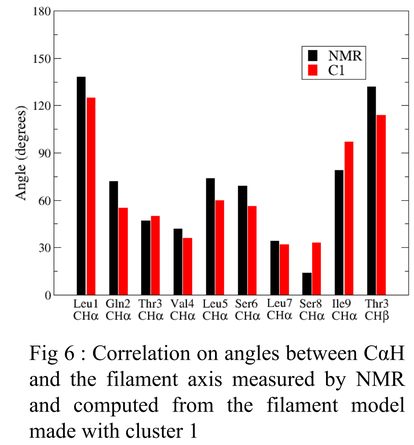


Fig 6 : Correlation on angles between CαH and the filament axis measured by NMR and computed from the filament model made with cluster 1

5. Results : *Modeling of a PD ring*

- Besides filaments, a ring can also be made from cluster 5
- The ring could correspond to a smaller oligomeric species
- Rings forms spontaneously when there is at least 4 Pseudodesmin A in the simulation box
- They could have a biological activity

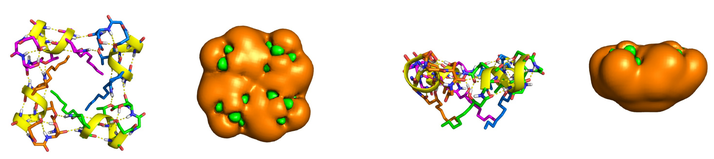


Fig 7 : Model of filaments build from cluster 1 and 3 and combining clusters 1 + 9 and 4 + 9.

6. Conclusions

- Behavior in chloroform and acetonitrile is correctly simulated
- Pseudodesmin self interactions have been sampled and characterized
- Two filaments and a ring model have been proposed
- General agreement between modeling and NMR results
- The filament C1+9 is the best model

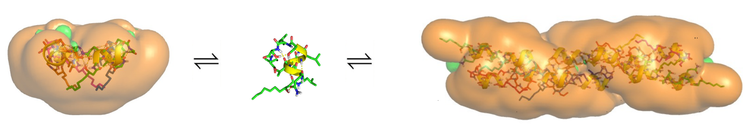


Fig 8 : Models for the supramolecular assembly.

7. Perspectives

- Study of Pseudodesmin mutants : Leu7, Ala2, DA, …
  Diffusion analysis have been already carried on by NMR
  They present different ability to self assemble
  Analysis from the proposed model
  Simulation of mutants in chloroform
- Interaction between Pseudodesmin and membranes

8. References

1. Sinnaeve D. *et al*. 2012 *Chemical Science* 3, 1284
2. Sinnaeve D. *et al*. 2009 *Tetrahedron* 65, 4173-4181
3. Sinnaeve D. *et al*. 2009 *Chemistry – A European Journal* 15, 12653-62

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