Ligand Binding Dynamics and Directionality in *Cellulomonas fimi* Family 4 Carbohydrate Binding Modules

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Abstract

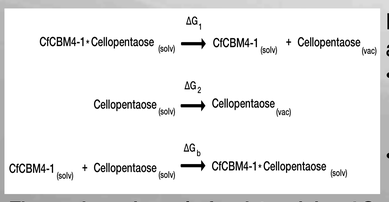
Enzymatic hydrolysis of biomass is often accomplished with multi-modular cellulase enzymes consisting of a catalytic domain appended to one or more carbohydrate binding modules (CBMs) by peptide linkers. CBMs frequently exhibit a limited range of specificity and appear to bind polysaccharide substrates in a directional fashion dictated by the position of the reducing end. The orientation of the ligand in binding cleft plays important role in the process of protein-carbohydrate recognition. Here, we use molecular modeling and free energy calculations to investigate protein-carbohydrate recognition mechanisms in two Family 4 CBMs, *Cf*CBM4-1 and *Cf*CBM4-2 and to elucidate the preferential ligand binding orientation. We evaluate four different cellopentaose orientations including that of the crystal structure and three others suggested by NMR. These four differ from each other based on position of reducing end of ligand and pyranose ring orientations. Using molecular dynamics, we find that the plausible orientations reduce to two cases. Ligand binding free energies calculated through free energy perturbation with Hamiltonian replica-exchange molecular dynamics indicate the two orientations are equally favorable. The calculated free energies are in excellent agreement with isothermal titration calorimetry measurements of binding free energy. Comparison of dynamics from MD simulations further suggests the approximate structural symmetry of the oligosaccharides relative to the amino acids along the binding cleft plays a role in the promiscuity of ligand binding.

Introduction

- β-1,4-glucanase CenC from *Cellulomonas fimi* has two Type B CBMs attached in tandem, *Cf*CBM4-1 and *Cf*CBM4-2. Both of them show β-jelly roll fold forming a binding cleft which binds soluble cello-oligosaccharides like cellopentaose along with amorphous cellulose.
- Experimental binding studies of various cello-oligosaccharides to *Cf*CBM4-1 present discrepancies regarding the orientations of the ligand
  - NMR spectroscopy (3) suggests cellopentaose binds bi-directionally.
  - X-ray crystallography (1) has captured one ligand orientation.
- Our objective is to obtain insights as to how the orientation of the ligand affects the binding properties and which orientations are preferred; at the same time, these results provide a general understanding of the mechanism of the oligomeric-carbohydrate recognition process.

Molecular Dynamics and FEP/REMD

- Molecular dynamics simulations were constructed in CHARMM from PDB structures.
  - Force fields: CHARMM36 w/ CMAP correction for proteins; CHARMM 36 carbohydrates for cellopentaose, and modified TIP3P for water
  - Minimization, heating to 300 K, and equilibration in the *NPT* ensemble for 100 ps
  - 250 ns *NVT* ensemble simulations in NAMD (~27000 atoms)



Thermodynamic cycle for determining ΔG with FEP/REMD. "Solv" refers to the solvated state, and "Vac" refers to the gas-phase state.

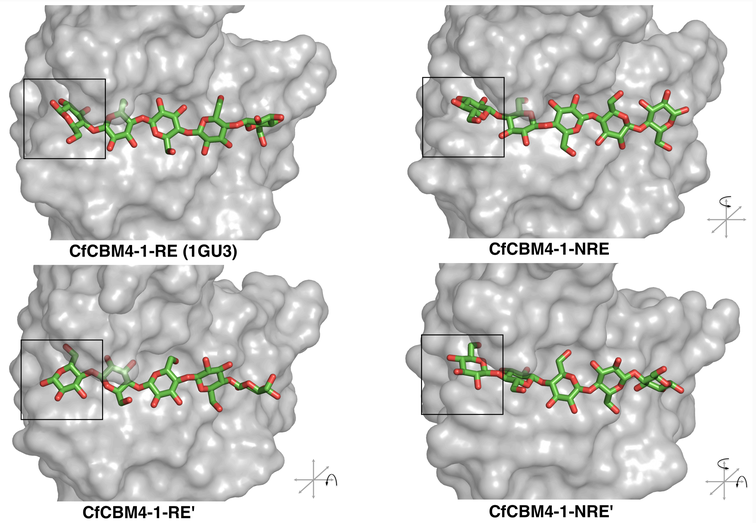
FEP/H-REMD protocol as described by Jiang and Roux (2) in NAMD

- Couples Hamiltonian Replica-Exchange MD to Free Energy Perturbation to improve sampling.
- Potential energy expressed independently as Weeks-Chandler-Anderson repulsion, dispersion, electrostatics, and restraints – scaled by thermodynamic coupling parameters.

Different Cellopentaose Orientations

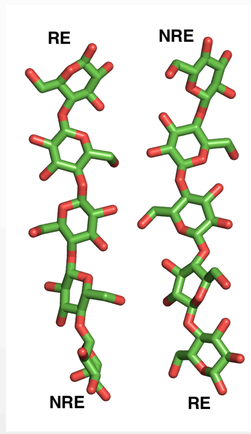
The cellopentaose may bind to *Cf*CBM4-1 in three possible orientations in addition to the orientation of the ligand in the crystallographic structure (1GU3). These four orientations differ from each other based on –

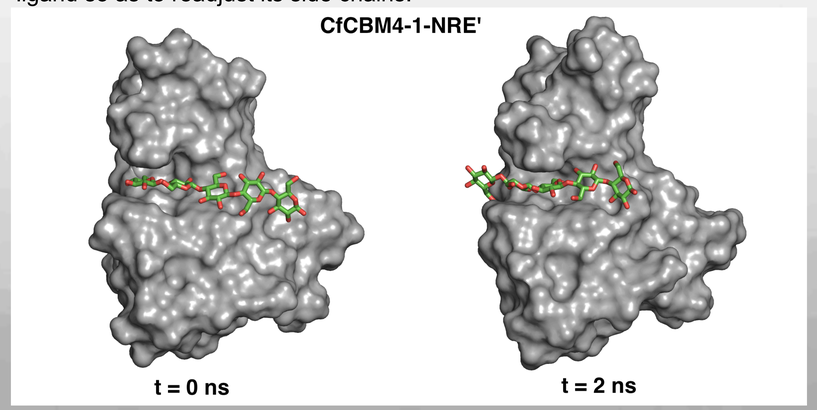
1. The position of reducing end (RE) of the ligand in the binding cleft
2. The orientation of pyranose ring determining where the side chains are.



Symmetry of Cellopentaose

- The approximate structural symmetry of oligo-saccharides accounts for the ability of the protein to bind the cello-oligomer regardless of directionality. (i.e. position of reducing end of ligand in the cleft).
- Reversing the direction of cellopentaose to put the RE on the other side of cleft does not change the structural symmetry, while rotation of pyranose ring along C1-C4 axis puts the hydroxymethyl groups on the opposite side of chain than that of the structural orientation thus disrupting symmetry.
- Two systems *Cf*CBM4-1-RE' and *Cf*CBM4-2-NRE' result in the cellopentaose off-register by one binding subsite compared to the structurally bound ligand so as to readjust its side chains.

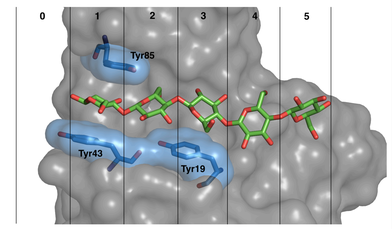




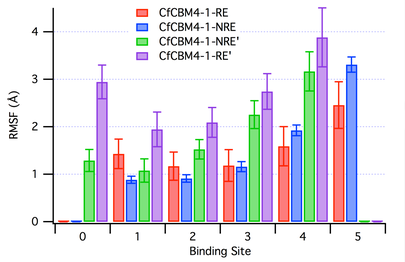
Thermodynamic Favorability and Dynamics

	ΔG_b^o	ΔG_{repu}	ΔG_{disp}	ΔG_{elec}	ΔG_{rstr}
	kcal/mol	kcal/mol	kcal/mol	kcal/mol	kcal/mol
Cellopentaose	-	68.04 ± 0.38	-61.78 ± 0.13	-66.25 ± 0.34	-
*Cf*CBM4-1-RE	-4.51 ± 1.30	73.83 ± 1.09	-78.87 ± 0.20	-59.18 ± 0.54	- 0.28
*Cf*CBM4-1-NRE	-5.86 ± 1.51	74.21 ± 1.16	-78.86 ± 0.28	-61.25 ± 0.61	0.06
*Cf*CBM4-1 Experimental	-5.24 ± 0.9	-	-	-	-

- The binding free energies, ΔG_b^o, of cellopentaose to *Cf*CBM4-1-RE and *Cf*CBM4-1-NRE are within error and are consistent with the experimental value (4). The above table illustrates the contributions of all components in the free energy calculation.



- Root Mean Square Fluctuation (RMSF) of each binding site, study of hydrogen bond formation of the cellopentaose to the surrounding protein and interaction energy of each binding site with the protein residues corroborates that the cellopentaose can bind *Cf*CBM4-1 bi-directionally in a similar fashion.



Study of Pairwise alignment with *Cf*CBM4-1 (PDB ID – 1GU3)

- 26 of the 69 CBM families demonstrate the similar β-sandwich fold
- 9 of these 26 families have glycan bound structures available in PDB database (27 structures in total)
- 16 structures observe the ligand in the same direction as the 1GU3 structure
- 11 structures observe the ligand in the opposite direction of the 1GU3 structure

Conclusions

- The absolute binding free energies of *Cf*CBM4-1-RE and *Cf*CBM4-1-NRE to cellopentaose suggest that *Cf*CBM4-1 does not preferentially bind cellopentaose in the orientation captured in 1GU3. Rather, *Cf*CBM4-1 likely binds in two orientations irrespective of position of reducing end.
- MD simulations immediately suggest the hypothesized orientation of cellopentaose in *Cf*CBM4-1-NRE' and *Cf*CBM4-1-RE' is unlikely given the lack of structural symmetry and general instability of the ligand.
- Bi-directional binding of cellopentaose to *Cf*CBM4-1 is a potential mechanism for increasing the proximity of glycoside hydrolases to amorphous cellulose. With fewer specificity limitations, these CBMs have an evolutionary advantage in polysaccharide deconstruction.

References and Acknowledgements

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