Conformational folding and stability of HET-C2 glycolipid transfer protein (GLTP)-fold: Does a molten globule-like state regulate activity?†

Roopa Kenoth[1,‡], Ravi Kanth Kamlekar[1,‡], Dhirendra K. Simanshu[2,‡], Yongguang Gao[1], Lucy Malinina[3], Franklyn G. Prendergast[4], Julian G. Molotkovsky[5], Dinshaw J. Patel[2,*], Sergei Y. Venyaminov[4,*], and Rhoderick E. Brown[1,*]

[1]The Hormel Institute, University of Minnesota, Austin, Minnesota;
[2]Structural Biology Program, Memorial Sloan-Kettering Cancer Center, New York, New York;
[3]Structural Biology, CIC bioGUNE, Derio, Spain;
[4]Mayo Clinic College of Medicine, Rochester, Minnesota;
[5]Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow, Russia

Supplemental Information for Publication

Table S1. Secondary structure calculations of HET-C2 at various pH values from far-UV CD spectra

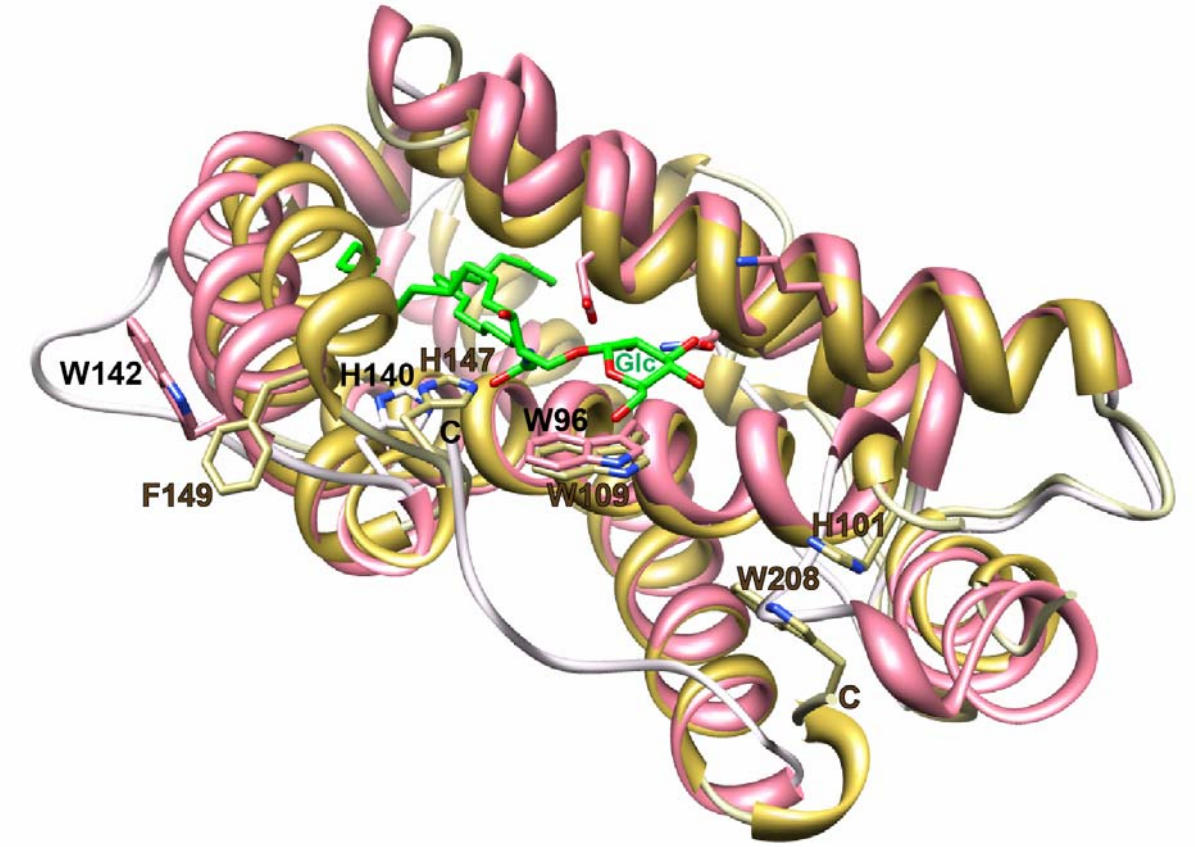
pH		2.0	3.0	4.0	5.0	6.0	7.4	9.0	10.0
Helix	*regular*	30.8 ± 3.7	33.3±5.3	36.0±4.1	36.4±4.1	35.6±4.0	37.4±4.6	37.0±2.2	34.9±4.5
	distorted	20.6 ± 0.9	21.6±2.4	24.4±2.4	24.0±2.0	23.0±2.4	22.7±2.7	22.5±2.2	21.4±2.9
	total	51.4 ± 3.9	54.9±6.9	60.4±2.6	60.4±2.6	58.6±3.1	60.1±4.3	59.5±4.9	56.3±5.6
β-strand	*regular*	3.7 ± 2.9	3.7±2.9	3.4±2.4	4.2±2.0	4.6±2.1	3.9±2.8	4.2±2.6	4.2±2.4
	distorted	4.5 ± 1.5	4.0±1.5	4.0±2.2	4.1±1.9	4.4±1.9	4.0±1.8	4.4±1.9	4.5±1.6
	total	8.2 ± 4.2	7.7±4.0	7.4±4.4	8.3±3.6	9.0±3.8	7.9±4.4	8.6±4.4	8.7±3.6
Turns		14.3 ± 3.5	13.0±3.3	13.1±2.9	12.7±3.3	12.6±3.9	11.1±3.6	11.7±3.9	12.5±3.8
Unordered		28.1 ± 1.5	24.9±4.0	20.5±3.2	20.6±2.2	22.7±2.5	22.9±4.1	22.2±3.3	24.5±4.2
# of helical segments		10.7 ± 0.4	11.2±1.2	12.7±1.2	12.5±1.1	12.0±1.2	11.8±1.4	11.7±1.2	11.2±1.5
Average helix length		10.0 ± 0.7	10.2±0.8	10.0±1.2	10.1±1.2	10.3±1.1	10.8±1.4	10.7±1.2	10.6±1.1
# of β-strands		4.7 ± 1.5	4.1±1.5	4.1±2.1	4.3±1.9	4.5±1.9	4.1±1.8	4.6±1.9	4.7±1.6
Average β-strand length		3.5 ± 1.2	3.7±1.0	3.6±0.7	3.6±0.8	3.6±0.9	3.9±1.0	3.9±0.9	3.8±0.9
Tertiary structure class		α + β	α + β	α + β	α + β	α + β	α + β	α + β	α + β

* *helix regular* or *β-strand regular:* fraction of residues (%) in central part of helical segments or strands; *helix distorted* or *β-strand distorted:* fraction of terminus residues (%) in helices (two at each end of helix, total of four per helical segment) or β-strands (one residue at each end of strand, total of two per strand); *total: regular + distorted*; *average length of helix* or *β-strand:* average number of residues per helix of β-strand, translation per one residue = 1.5 Å for α-helix and 3.3 Å for β-strand

Secondary structure was calculated from far-UV CD spectra as detailed previously (*18,25*) using the CDPro software package, which is a modified version of three methods: SELCON3, CONTINLL locally linearized approximation of CONTIN, and CDSSTR, using set of 48 reference proteins or 22 α+β reference proteins selected by the CLASTER program. Tertiary structure class was determined by CLASTER program. 6 values for each type of secondary srtructure (three methods of calculation × two sets of reference proteins) were averaged and the average values ± root-mean-square deviation are presented.

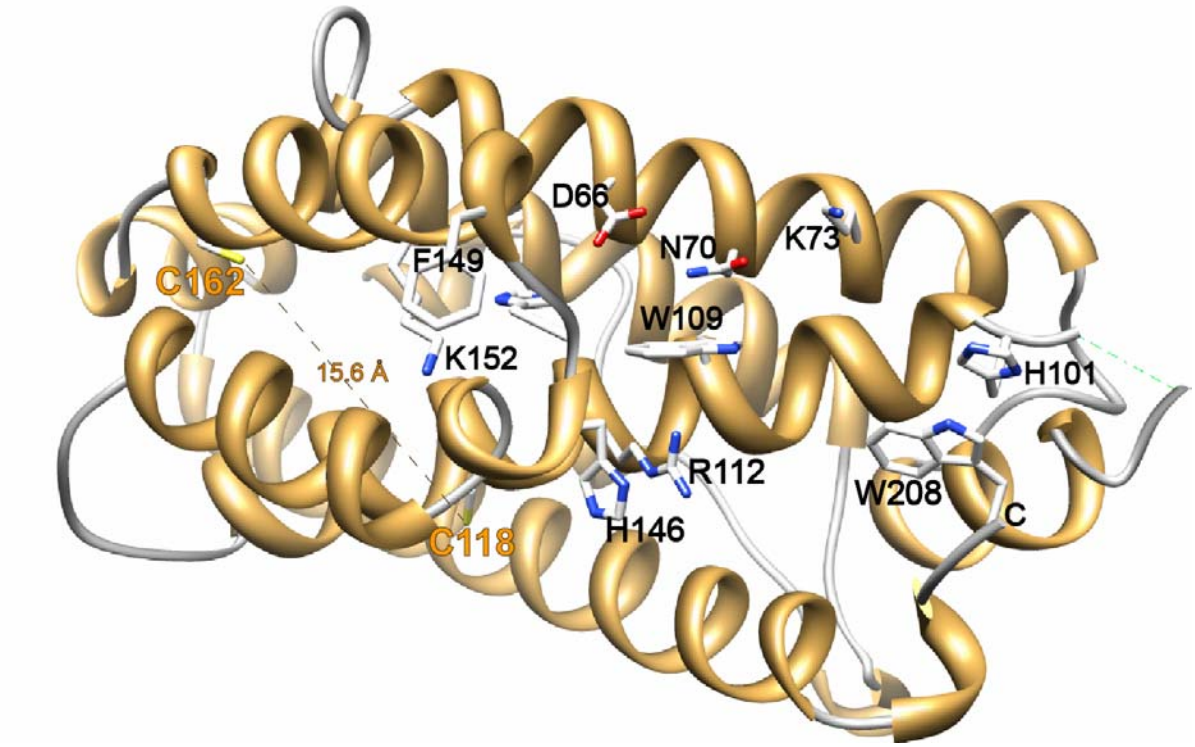
Secondary structure classification was determined with the DSSP program. Output included α-helix (H), 3_{10}-helix (G), β-sheet (E), β-bridges (B), turns (T), and bends (S), which were further grouped as follows: the α- and 3_{10}-helix were treated in CDPro as helix; β-sheet as β-strand; turns and bends were treated as turns; a minimum of two adjacent residues were required for such grouping for turns and bends. Single residues assigned to a structure (such as β-bridges, turns, and bends) were grouped as unordered, which contains residues that are not assigned to any defined structural class.

Figure S1



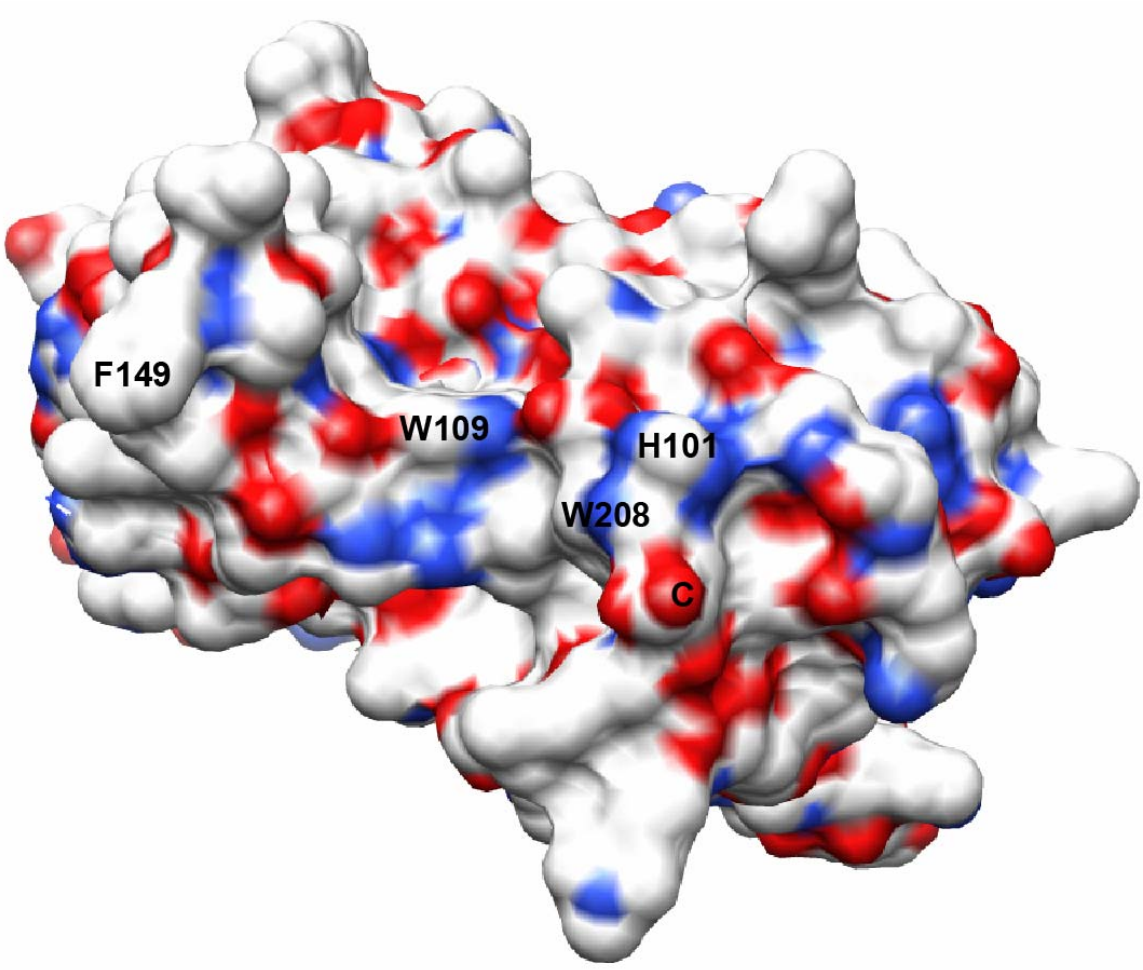
GLTP-folds of HET-C2 and GLTP. Superpositioning of HET-C2 (gold; PDB 3kv0) and human GLTP structures (cameo pink; PDB 1sx6) determined by X-ray diffraction. The glucosylceramide (green) in the GLTP-fold binding pocket is adapted from 1sx6. Outward displacement of helix-6 (left) occurs in GLTP during accommodation of glycolipid. Residues labeled in brown refer to HET-C2 while those in labeled in black refer to GLTP. Although the two core structures are very similar, the C-terminal (**C** and **C**) and N-terminal regions of each protein are strikingly different.

Figure S2



Cysteine localization in HET-C2 and GLTP. In HET-C2 (gold), the two cysteine residues (blue), Cys^{162} and Cys^{118}, are too far apart (~15.6 Å) to stabilize conformation via disulfide bridging, as revealed by the crystal structure [PDB 3kv0; (*18*)]. In GLTP (cameo pink), the three cysteine residues (blue) also are too far apart to undergo intramolecular disulfide bridging, e.g. Cys^{36}↔Cys^{112} = 14 Å; Cys^{112}↔Cys ^{176} = 8 Å; Cys36↔Cys176 = 9.3 Å [PDB 1swx; (*19*)]. Trp residues are green and key sugar headgroup anchoring residues are red and yellow highlighted.

Figure S3



The surface accessibility of the two intrinsic Trp residues of HET-C2. Space-filling rendition of HET-C2 derived from [PDB 3kv0; (*18*)]. Electrostatic surface charges are shown in blue for positive residues and in red for negative residues.