The Thermal Unfolding of b2-Glycoprotein I

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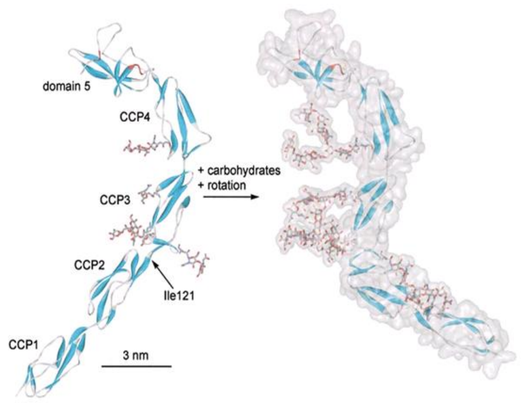
β2- Glycoprotein I (B2GPI) is an abundant glycoprotein of human plasma. It participates in blood coagulation processes and the clearance of phosphatidylserine exposing- apoptotic cells. B2GPI is also the major antigen involved in the aberrant immune response that characterizes the anti-phospholipid syndrome.

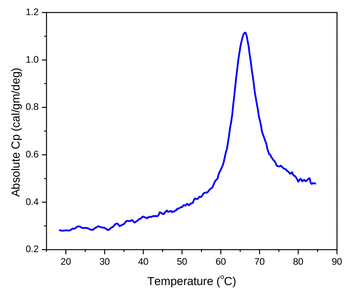
B2GPI consists of a single chain of 326 aminoacids arranged in five domains. Four of them are the so called sushi or short consensus repeat (SCR) domain with its conserved beta sandwich- arrangement. The fifth domain is larger and it has a lysine rich region that was shown to participate in the interaction of B2GPI with anionic lipids. B2GPI has four N-glycosilation sites with complex bi and triantennary complex glycans containing sialic acid that comprise ~20% of its total weight.

To study the folding behavior of this multiple domain glycoprotein we performed differential scanning calorimetry at various pH and salts conditions. We found that at pH 7.0, in the absence of salt, the protein unfolding proceeds with a pre-transition at lower temperatures. In order to characterize this earlier intermediate we perfomed circular dichroism studies of the thermal unfolding of B2GPI in the far and near UV region. The far UV signal shows a two state unfolding transition centered at the same temperature of the calorimetric one. In contrast, the near UV signal shows complex behavior. These results would indicate that the protein folding intermediate has a different tertiary structure while conserving its secondary one.

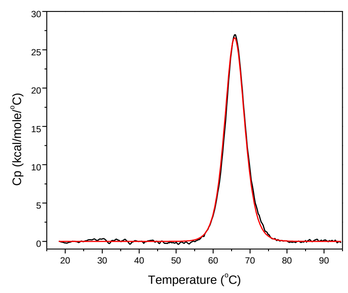


Crystal structure of B2GPI (left) compared with the **atomic model containing the carbohidrate chains as modeled based on SAXS experimental data** (right).
(Hammel et al. *J. Mol. Biol.* (2002)85-97)

A



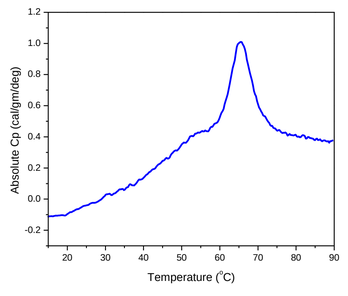
B



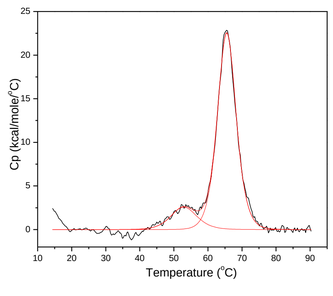
C

Tm	**65.99 ±0.1 C**
ΔH	**191 ± 6 kcal/mol**
ΔH_v	**124 ±4 kcal/mol**

D



E

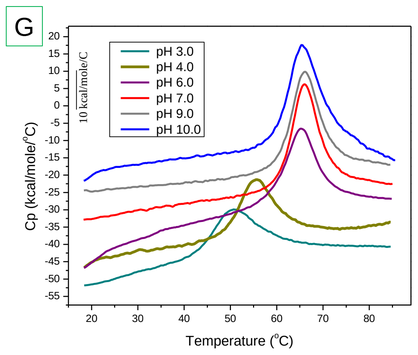


F

Tm_1	**52.95 ± 0.2 C**
ΔH_1	**34 ± 2 kcal/mol**
Tm_2	**65.55 ± 0.02 C**
ΔH_2	**163 ± 1 kcal/mol**

Differential scanning calorimetry of B2GPI in the presence (A,B,C) **or absence of salt** (D,E,F).
Figures A and D show the absolute heat capacity function after subtraction of the heat capacity of the solvent. Figures B and E show the fitting of the normalized heat capacity functions after baseline substraction, in tables E and F the Tm and enthalphy of the thermally induced protein denaturation calculated for the fitted functions is stated. B2GPI (2- 5 μM) was dialyzed 16 hs in phosphate buffer 10 mM (pH 7.0) with or without 0.1 M NaCl and filtrated (0.22 μm) before obtaining the thermogram in a VP-DSC Microcal calorimeter. The Origin Microcal VP-DSC package was used for data procesing.

G

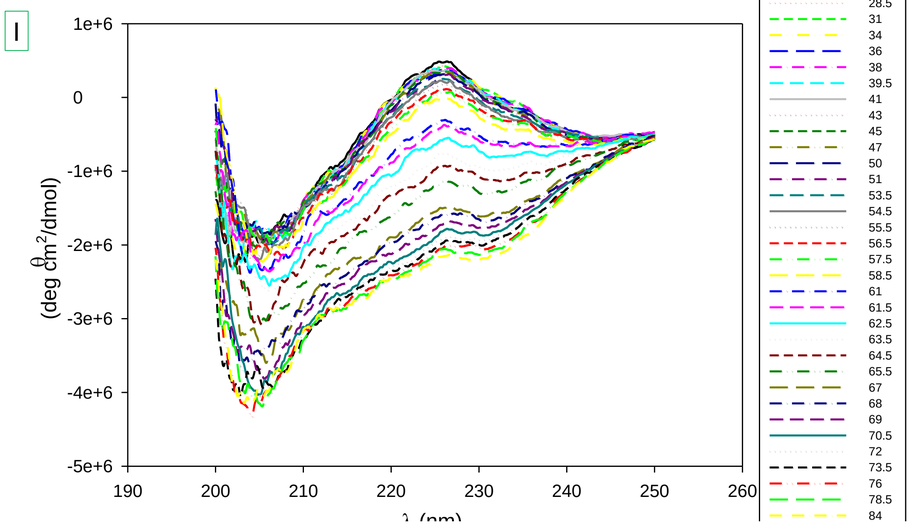


H

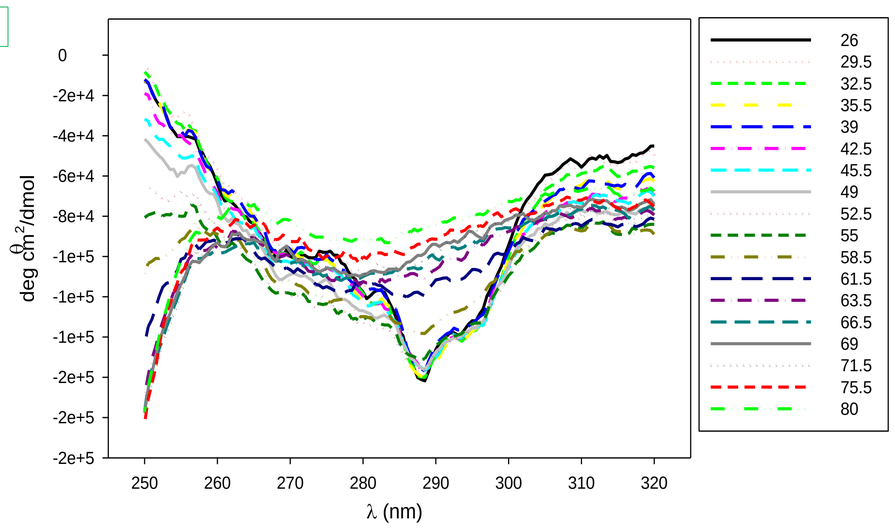
pH	Tm (C)	ΔH (kcal/mol)	ΔHv (kcal/mol)
3.0	50.9 ± 0.5	123 ± 1	70.4 ± 0.5
4.0	55.20 ± 0	125 ± 1	98 ± 1
6.0	65.90 ± 0.01	222 ± 1	131 ± 1
7.0	66.0 ±0.1	191 ± 6	124 ± 4
9.0	65.88 ± 0.01	188 ± 1	121.5 ± 0.5

Differential scanning calorimetry at different pH.
Figures G shows the normalized heat capacity function after subtraction of the heat capacity of the solvent for different pH. In table H , Tm and the enthalphy of the thermally induced two state protein denaturation are shown.
pH 3.0 and 4.0, acetate buffer; pH 6.0 and 7.0, phosphate buffer, pH 9.0 and 10.0 glycine/NaOH buffer.

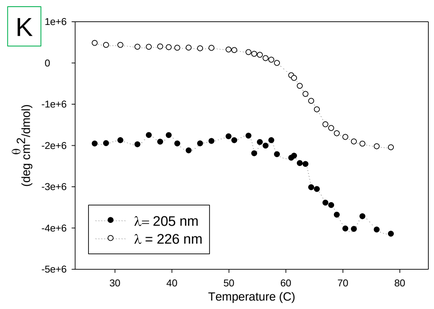
I



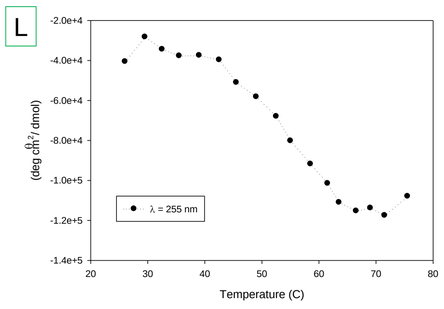
J



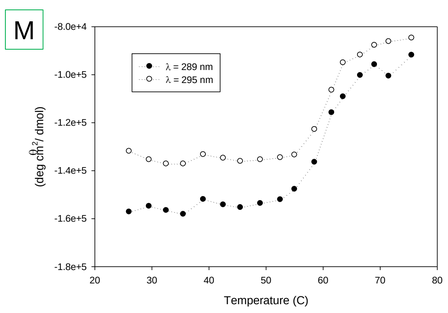
K



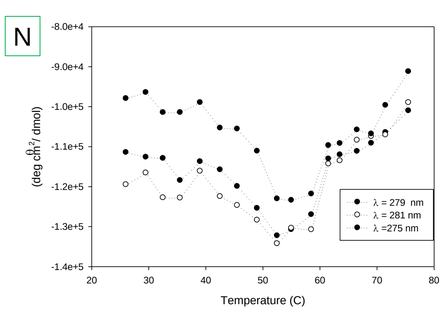
L



M



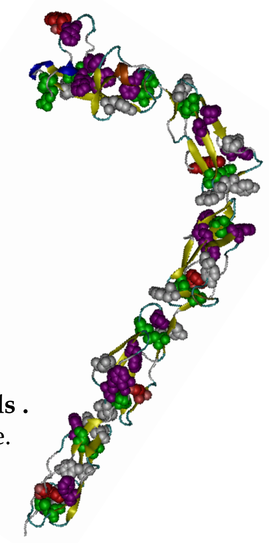
N



O

λ (nm)	Tm (C)
205	65.2
226	63.8
255	54.9
289	59.4
295	60.5

Thermal denaturation of B2GPI in the absence of salt followed by circular dichroism.
Figures I and J show the far and near UV spectra of B2GPI at different temperatures.
In figures K to N the intensity of the polarized light absorption as a function of temperature for different wavelenghts is depicted. In table O the transition temperature for the fitted two state transition functions is shown.
B2GPI was dialyzed 16 hs in phosphate buffer 20 mM (pH 7.0) and centrifuged (13.000 rpm, 10 min). For near UV CD spectra (250-320 nm) a 1 cm quartz cuvette was used and 5 spectra were acquired at 50 nm/min with a 2 second response and a bandwidth of 2 nm. For far UV CD spectra (200-250 nm) a 0.2 cm quartz cuvette was used and 3 spectra were acquired at 50 nm/min with a 8 second response and a bandwidth of 2 nm. The temperature was controlled using a circulating water bath. A Jasco 810 discograph was used in these experiments.



Crystallographic structure of B2GPI depicting aromatic aminoacids and disulfide bonds .
In green, disulfide bonds; in red triptophans; in grey tyrosine and in purple phenilalanine.
Image generated by VMD program, based on the 1C1Z.pdb structure.