Effects of hydration on β-lactoglobulin dynamics and stability

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Outline

- β - lactoglobulin and water

- Water slaved β-lactoglobulin dynamics ?

  DSC and Neutron Scattering

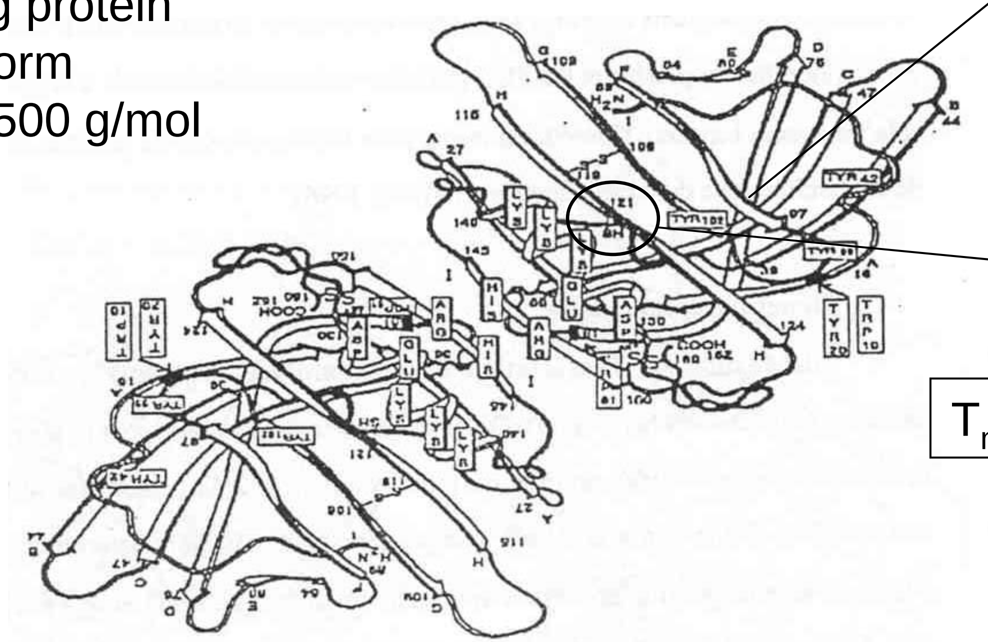
- Water and β-lactoglobulin structure?

  BLG stability and function

β-lactoglobulin

Globular protein
Lipocalin family
Retinol binding protein
pH 7 dimeric form
Monomer : 18500 g/mol
2 S-S / 1 SH
β-sheet

BLG native structure
Sawyer et al.1985



Hydrophobic central cavity

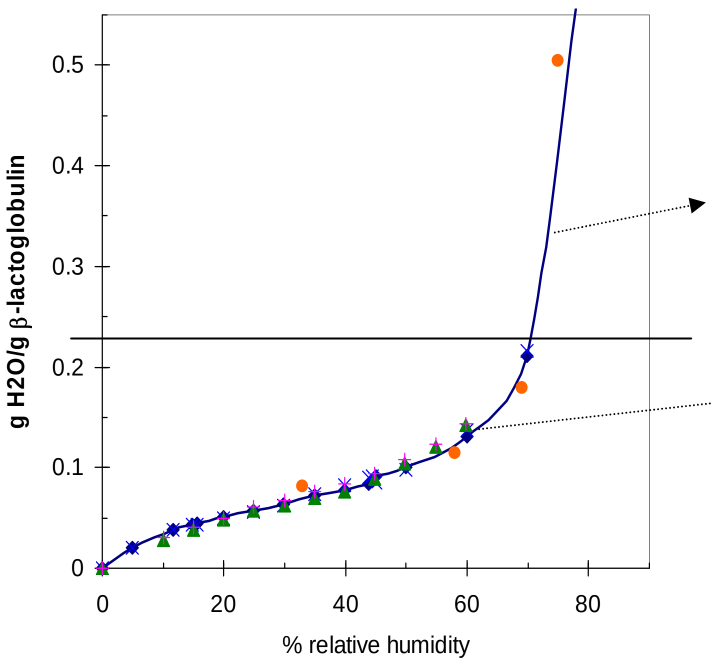
SH/S^-

$T_m = 75°C$

BLG purified according to Fox et al (1967) from raw milk

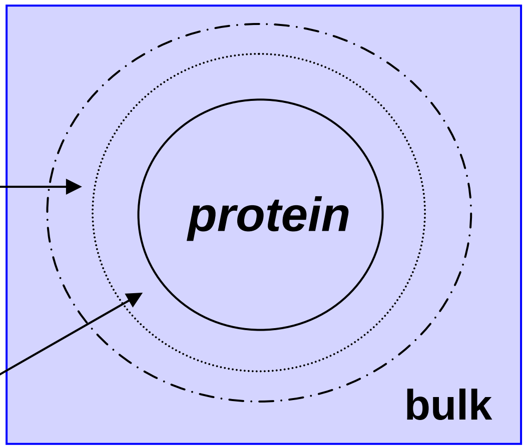
Food industry context: whey protein powders processing and storage
Functionalities: gel and emulsion stability

β-lactoglobulin and water ?



Second, third shells
Freezable water
Different from
Bulk water

Monolayer and
Unfreezable water
first shells



Autosorb, Biosystems, France
Automatic sorption balance
Or equilibration against saturated salts (red)

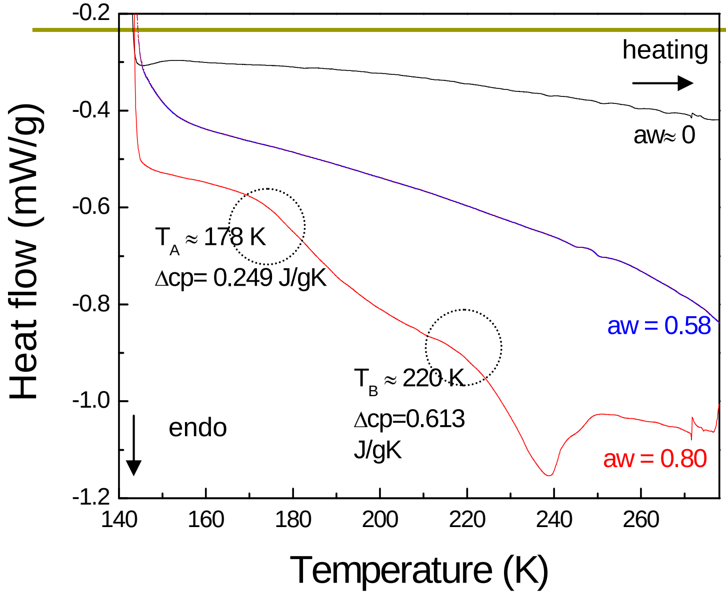
In agreement with Ruegg et al., 1975

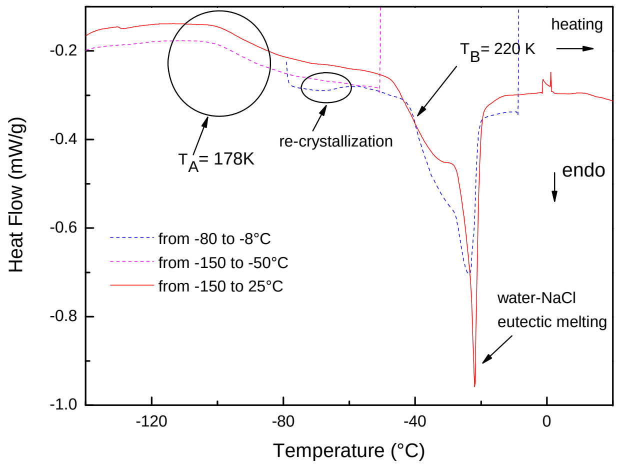
Water and dynamics ?

Differential Scanning Calorimetry: glass transition

Neutron Scattering: picosecond dynamics

Water and glass transition





DSC Q100 TA Instruments 10°C / min

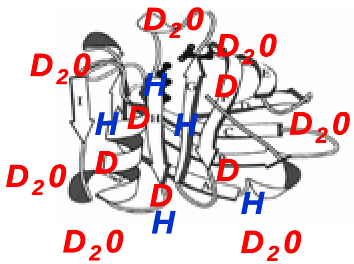
• aw = 0 or 0.58
No freezable water
No glass transition at low temperature

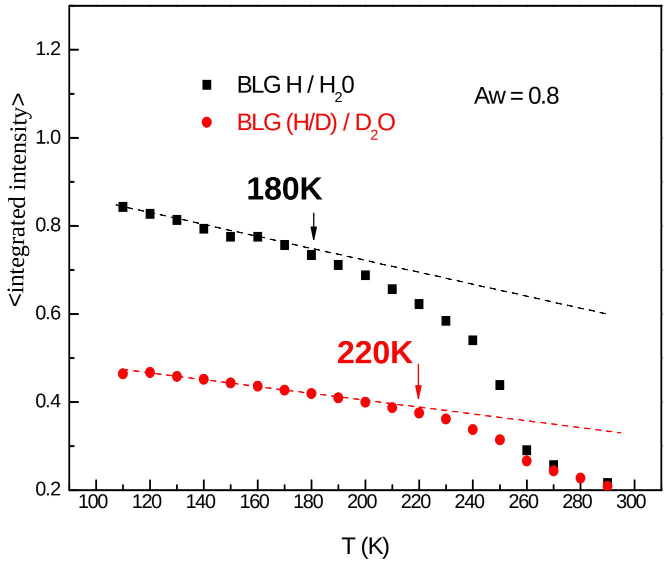
•aw = 0.8
freezable water
two thermal events = cp jump:
T_A = 178K
T_B = 220K

What is the origin of these two thermal events T_A and T_B? Associated to water or protein dynamics ?

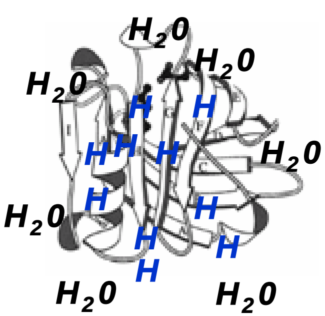
Water and picosecond dynamics

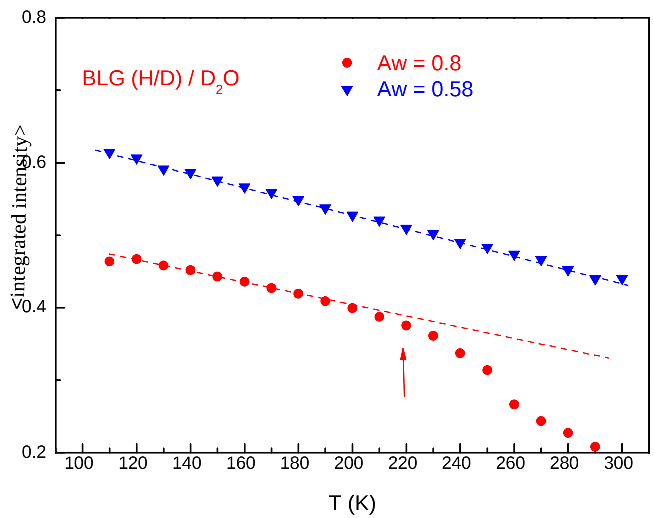
***protein** dynamics*
BLG (H/D) in D_2O





***water** dynamics*
BLG (H) in H_2O





With freezable water
(aw = 0.8):

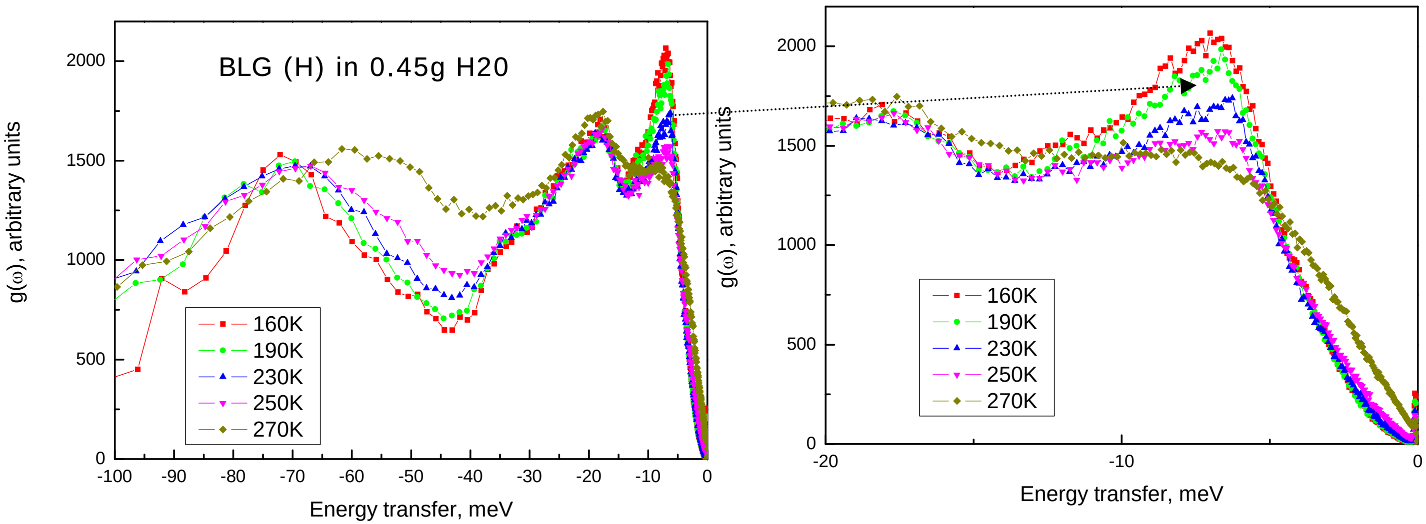
BLG (H/D) in D20
(protein dynamics)
dynamical transition
temperature = **220 K**
= T_B (DSC)

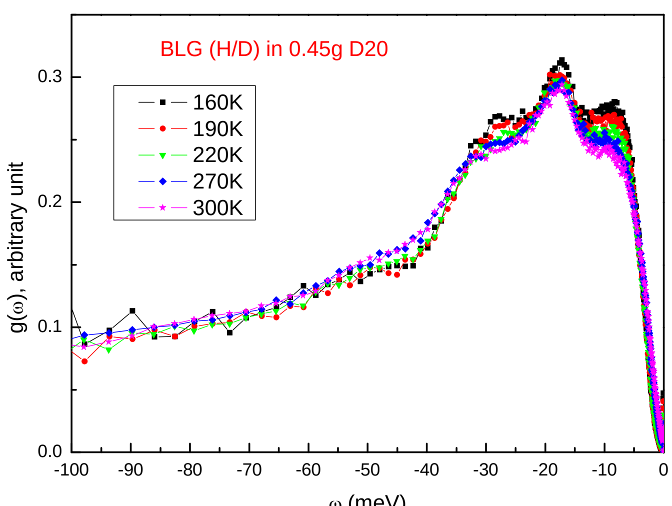
BLG (H) in H20
(water dynamics)
Dynamical transition
temperature **180 K = T_A**

No dynamical transition without freezable water
(aw = 0.58) (in agreement with DSC results)

Elastic neutron scattering,IN13, ILL, Grenoble

Water and dynamical transition





- **Water vibrational density of states Change after 190 K= dynamical transition = T_A**

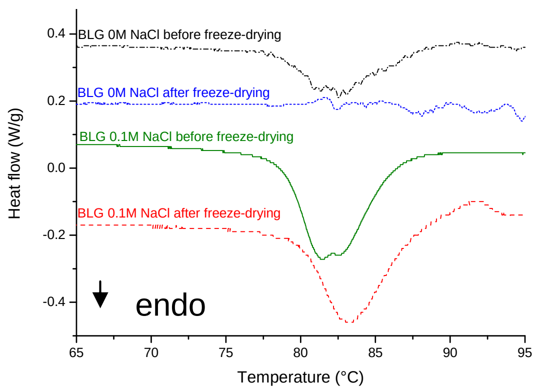
- **No strong change in protein vibrational modes at T_B = 220K**

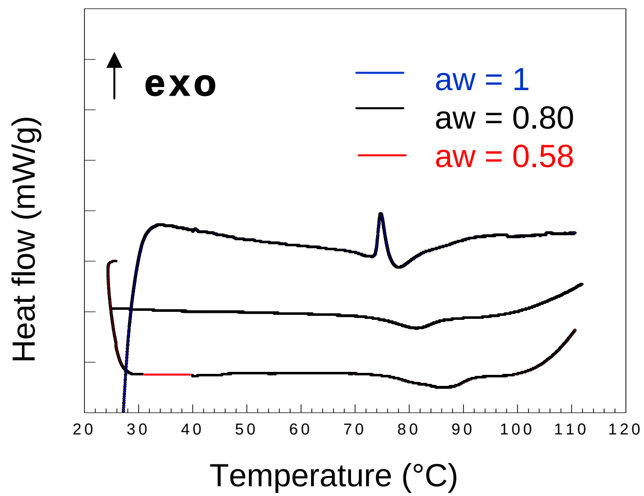
Water slaved protein dynamics

Inelastic neutron scattering, Mibémol, LLB, Saclay

Water and protein structure

Protein structure melting
Tm : melting temperature
ΔH : enthalpy of unfolding





aw	Tm (°C)	ΔH (J/g)
1	76	0.98
0.80	81	1.81
0.58	94	2.80

Purified protein (reference)
= freeze-dried protein in solution
(aw =1) in presence of NaCl

•aw 0.58 and 0.8 :
folded protein but not native structure
melting peak ≠ reference

•aw ↓ => ↑ Tm
• aw ↓ => ↑ ΔH
higher BLG stability
(better protein powder stability)

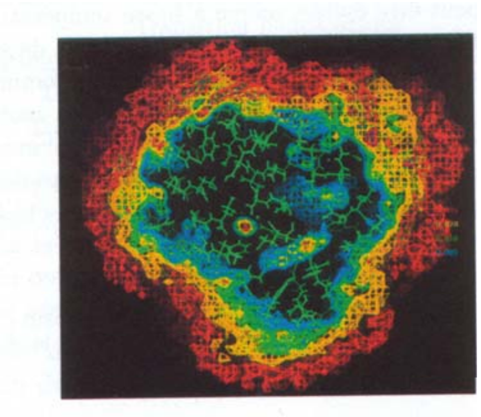
DSC III Setaram 1°C / min

Water and protein function

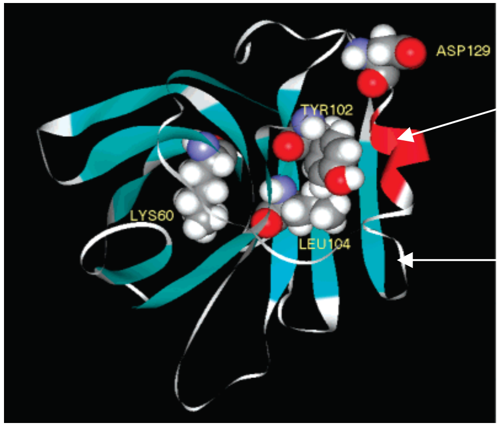
BLG activity: retinol binding

• without freezable water
  - no dynamics, partly folded
  - Stability : OK
  - Function : ?   probably not active

• with freezable water
  - Dynamics slaved by hydration water
  - folded but not the native structure
  - Stability : decrease (compare to aw = 0.6)
  - Function: OK



BLG and water stability



BLG and function

Conclusion

- **Water slaved β-lactoglobulin dynamics**

- **Water and dynamics**

Controlled β-lactoglobulin structure

Low water content = protein folded => not the native structure

Increase protein stability (↑Tm and ΔH)

Protein powders storage = pharmaceuticals and food industries

Function (Retinol binding) ? Only with freezable water => Dynamics

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