High pressure SAXS studies

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Outlook

- Thermodynamics of protein unfolding by pressure
- High pressure SAXS: Technique
- Pressure induced protein unfolding
- Pressure effects on the protein-protein interaction
- Pressure effects on other soft matter systems
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Why Pressure?

- Important thermodynamic quantity
- Only affects volumetric properties (Le Chatelier)
- „biomimetic“: deep sea animals
- Effect on protein stability
- Technological relevance (food industry)
Which pressures induce protein unfolding?

- The (pressure-) unfolded state has a smaller volume than the folded state (~ 1% effect)
- **Pressure 1 – 7 kbar**: effect on **non-covalent** bonds: Changes of the **tertiary** structure
- **Pressure > 10 kbar**: effect on **covalent** bonds: changes of the **primary** and **secondary** structures
Hawley theory

Description of unfolding for a two-state system (folded ↔ unfolded) in terms of the Gibbs free energy change $\Delta G(p, T)$:

$$\Delta G(p, T) = \Delta G_0 - \Delta S_0 (T - T_0) - \Delta C_p \left[ T \left( \log \frac{T}{T_0} - 1 \right) + T_0 \right]$$

$$+ \Delta V_0 (p - p_0) + \frac{\Delta \kappa}{2} (p - p_0)^2 + \Delta \alpha (p - p_0) (T - T_0)$$

(Expansion in $p, T$ up to second order)
Hawley theory

$\Delta G(p, T)$: elliptical shaped phase diagram

- Heat-denaturation
- Cold-denaturation
- Pressure-denaturation
- (Stress-denaturation)
Heat-denaturation

- Simultaneous change of total energy and volume
- Entropy driven
- Highly unfolded state (lacking nearly residual secondary order)
- Random coil – like state
- Aggregation-prone
Cold-denaturation / Stress-denaturation

Cold
• Volume driven
• Highly dependent on the water structure
• Not as unfolded as heat
• Often beyond the freezing point of water

Stress (negative pressure)
• Hardly studied (complicated experiments, computer studies)
• Methods: Berthelot tubes, ultrasonic
Pressure-denaturation: Mechanisms

- pressure induced decrease of the protein volume only
- depends highly on the solvent (water)
- reversible

- Collapse of internatal cavities
- Electrostriction
- Hydrophobic interaction: Water-mediated interactions become favored
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Method’s to study high pressure effects

High pressure environment (in situ)

• Fluorescence (Trp residues)
• NMR
• FT-IR spectroscopy (secondary structure)
• Circular dicroism (secondary structure)
• SAXS & SANS (tertiary structure)
• Optical microscopy (cell structures)
High pressure SAXS: HP sample cell

High pressure sample cells

• Pressure range: 1 bar – 4….7 kbar

• X-ray windows: two flat diamonds

• Pressurizing medium: water

High pressure SAXS

Experimental set-up

• 1) HP sample cell
• 2) Pressure sensor
• 3) Manual spindle pump
HP windows

• Diamond windows for sealing
  ⇒ High flux (synchrotron)
  ⇒ Higher photon energy due to X-ray absorption
  ⇒ Pseudo-Kossel lines
HP buffer

- Stand buffer samples:
  - $\text{HPO}_4^{2-} + \text{H}_2\text{O} \rightleftharpoons \text{PO}_4^{3-} + \text{H}_3\text{O}^+$
  => number of charges increases

- HP buffer
  - $\text{TrisH}^+ + \text{H}_2\text{O} \rightleftharpoons \text{Tris} + \text{H}_3\text{O}^+$
  => number of charges constant

Pressure favors charged state -> change in pH for standard buffers
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Staphyloccocal nuclease (SNase)

• 149 amino acids, $M_w = 16.8$ kDa
• Globular protein
• No disulfide bonds $->$ destabilized
• Standard protein for high pressure studies
• Several mutants available
HP SAXS – SNase

High pressure effects

- Decrease of $I(0)$
  $\Rightarrow$ reduced contrast as water gets compressed

- Increase of radius of gyration
  $\Rightarrow$ unfolding

SNase – $p$ vs $T$

- $p$ - unfolding at $p \sim 2 - 3$ kbar
- Two-state folding
- Pressure-induced unfolded state is more compact than the heat induced one

SNase - $p$ - $T$ phase diagrams

Experimental phase diagram of WT Snase and destabilized mutant.

G. Panick et al., Biochem. 38, 4157 (1999).

Effect of crowding (self and Ficol crowding)

SNase kinetics – \( p \) – jump

Rapid pressure increase / release
⇒ Unfolding / Folding reaction
⇒ Activation volumina (hydration) of the transition state

- Two-state kinetics
- Positive activation volume
⇒ dehydration, transition state close to native state

J. Woenckhaus et al., Biophys. J. 80, 1518 (1999).
SNase - (de-)stabilization by cosolvents

Cosolvents can change the pressure – effect

• Kosmotropic: stabilizing
• Chaotropic: destabilizing

Urea destabilizes, TMAO stabilizes

Roughly: Pressure + additional perturbations are additive

SNase mutants

Single AA exchange in **hydrophobic** core of a hyperstable SNase variant

- Change in protein stability
- Large + charged: destabilize
- Change of charged state: change of stability

Other HP SAXS studies

**T4 lysozyme mutants**

- Penetration of water into hydrophobic core


**Urate oxidase**

- $\mu$-induced dissociation of tetramers


**Ankyrin repeat domain**

- Two-state folding also for non-globular proteins

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Concentrated protein solutions

- Thermodynamics of protein solutions
  (temperature density phase diagram) fabrication of crystals
- "biomimetic": cytosol ($c_p > 300 \text{ mg/ml}$)
- investigating the protein-protein interaction

Temperature – density phase diagram


Scheme of a cell's interior

Lysozyme

- 129 amino acids, $M_w = 14.3$ kDa
- from hen egg white => cheap, high purity, large amounts
- breaks glycopeptide chains of bacterial cell walls
- present in various secrets such as saliva
- often used “model protein”

solutions used
- diluted: $c_p = 10$ mg/ml
- concentrated: $c_p = 100; 200$ mg/ml
- pressure insensitive buffer (bisTris 25 mM) at pH 7
SAXS – Structure factor $S(q)$

SAXS intensity of concentrated solutions

$I(q) \sim P(q) \, S(q)$

hard sphere for different particle numbers $\eta$

- type of interaction
- strength of interaction
Modeling the interaction potential

- form factor of an ellipsoid of revolution

- DLVO potential

\[
V_{DLVO}(r) = \frac{e^2}{4\pi\varepsilon_0\varepsilon_r} \frac{Z_{eff}^2}{(1 + 0.5 \frac{\sigma}{\lambda_D})^2} \frac{\exp\left(-\frac{r - \sigma}{\lambda_D}\right)}{r} \exp\left(-\frac{r - \sigma}{d}\right) - J\sigma \frac{\exp\left(-\frac{r - \sigma}{d}\right)}{r}
\]

\( e \): elementary charge, \( \varepsilon_0 \): vacuum permittivity, \( \sigma \): diameter, \( Z_{eff} \): effective charge, \( \varepsilon_r \): permittivity, \( \lambda_D \): Debye screening length, \( J \): attraction strength, \( d \): range of attraction

- use of Mean Spherical Approximation (MSA) to determine \( S(q) \)
- \( Z_{eff} \) and \( d \) kept constant; \( p \) dependence of \( \varepsilon_r \)
- free parameter for refinement is \( J \)

Concentrated solutions & the structure factor $S(q)$

Nonlinear shift of the correlation peak with increasing pressure

- $p < 2.0$ kbar: shift to higher $q$
- $p > 2.0$ kbar: shift to smaller $q$

Attraction strength $J$ & interaction potential $V(r)$

minimum at $J = 2.0$ kbar (c$_p$ = 100 mg/ml)

pressure-induced change of repulsion barrier

$\sigma = 2.99$ nm, $d = 0.27$ nm, $Z_{\text{eff}} = 8$


Effect on protein concentration and salt added

$S_{\text{eff}}(q)$ for 10 wt.% at $T = 25^\circ \text{C}$ with 100 mM NaCl

Minimum present for different
- Protein concentration
- Temperature
- NaCl amount

Reentrant liquid-liquid phase separation

- 1 phase -> 2 phases
  (low & high concentrated)


Results of the SAXS measurements

Measurements on lysozyme in buffer solution

• nonlinear pressure dependence of the interaction potentials

• present for different temperatures, concentrations, and NaCl

• no unfolding within the pressure range studied
  (form factor, FTIR spectroscopy)

• no change of the proteins’ effective charge as pressure-insensitive buffer is used

➢ influence of water


M.A. Schroer, M. Tolan, R. Winter.
Properties of water

• transport properties of water (diffusion coefficient, viscosity) have extrema at 2 kbar

⇒ collapse of the second hydration shell of water starts at 2 kbar
⇒ change of the water moderated interaction (“effective screening”)

To study this effect in more detail the influence of **osmolytes** was investigated!
Osmolytes: Small biologically relevant molecules

Different small molecules affect proteins
- kosmotropes: stabilizing
- chaotropes: destabilizing

Biologically relevant are:

**Urea** \(((\text{NH}_2)_2\text{CO})\)
- degradation product in organisms
- destabilizes proteins

**TMAO** (Trimethylamine N-oxide, (CH$_3$)$_3$NO)
- stabilizes proteins => counteracts urea
- TMAO concentration in fishes increase with sea depth

2:1 mixtures (urea:TMAO) are present in deep sea organisms

SAXS on lysozyme in osmolyte solutions

SAXS curves show different pressure dependence for the two cosolvents

- TMAO: strong effect
- urea: like in buffer

no unfolding present

⇒ change of the interaction

Different pressure dependence

**TMAO**: strong influence
- minimum at 2.5 kbar
- steep decay
- weak nonlinear effect

**urea**: as in buffer solution

**urea/TMAO mixtures**: compensation
- 4:1 & 2:1 as buffer solution
- 1:1 TMAO dominates

Interpretation

- collapse of the 2. hydration shell in buffer and urea
- no collapse for TMAO in solution
- the effects are cancelled in case of mixture

urea
- fits perfectly into the water network

TMAO
- enhances the hydrogen bonding ("water structure maker")
- 2. hydration shell is located more outwards) contrary to pressure


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High pressure lipids & membranes

Structural transitions in lipids and membranes by pressure

⇒ Complex pressure – temperature phase diagrams

Can be perfectly probed by high pressure SAXS

High pressure lipids & membranes

• Mixtures of model: DMPC / DHPC (3.2 : 1) at 61.9 °C:
  • Decrease of Bragg reflection
  • Increase of broad peak
  ⇒ Lamellar to nematic transition

• Pressure jump: time-resolved SAXS
  ⇒ Kinetics of the phase transition

Nanoparticles

- Gold NP coated with PEG-ligands
  - Stable, low dispersity, functionalization
  - Applications in medicine, catalysis, sensors, ...

- Diluted:
  - Only the form factor of the gold core
  - Radius: 6.15 nm, polydispersity: 7%

- Concentrated:
  - Structure factor
  - Pressure induced changes

Nanoparticles

- Compression of the ligand shell
- Decrease of the second virial coefficient $b_2$

$\Rightarrow$ Collapse of the ligand shell
$\Rightarrow$ Transition from repulsive to attractive by pressure

Summary

• Pressure induces structural transitions in biological and soft matter
• HP SAXS allows to probe these transitions
• Pressure effect on structure and interactions
• Alternative / different effects in comparison to e.g. $T$
• More studies are nowadays performed but still a lot to be explored
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