

タンパク質の構造転移と広角散乱: 浸透圧下における折畳みの研究

Structural transition of protein observed wide-angle scattering:

Study of protein folding under osmotic pressure

M. Hirai¹, T. Onai¹, G. Chin¹, & K. Inoue²

¹Department of Physics, Gunma University, Maebashi 371-8510, Japan.

²Japan Synchrotron Radiation Research Institute, Hyogo 679-5198, Japan

溶液散乱法(小角散乱法)の有用性

- 1) タンパク質の折畳み問題と疾病
- 2) 散乱法で得られるタンパク質の構造情報
- 3) 浸透圧下におけるタンパク質の熱構造転移の可逆性
- 4) 浸透圧下におけるタンパク質のアミロイド構造転移
- 5) まとめ

-1

散乱法で何を明らかにできるか。 (生体系の場合)

生体系の各構成要素の性質
と相互作用, **階層構造**
(molecule, cell, tissue...)



広い時間・空間スケールで
起きる**生体反応・機能発現**
の**多様性**

in vivo, in vitro...
*in situ*測定**の重要性**

様々な階層レベル(時空間)における
構造とダイナミクスに関する**情報**

SAXS, SANS, NSE,
SR-XR, NR...

多様な物理・化学的, 生理的な環境下
(溶液中)における**中性子・X線測定**

High-statistic data
& **Model analysis**



- 分子, 分子集合体の形状
- それらの内部構造, 表面電荷, 水和状態...
- 分子間相互作用, 高次構造形成・解離
-

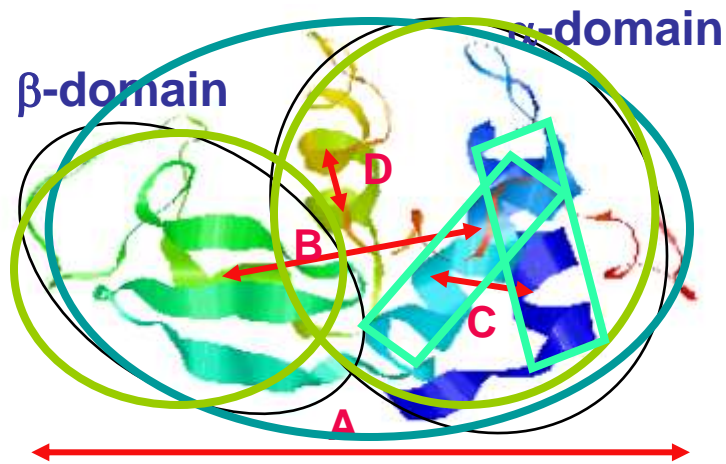
ナノスケールレベルでの**生体反応システム**の**解明**

-2

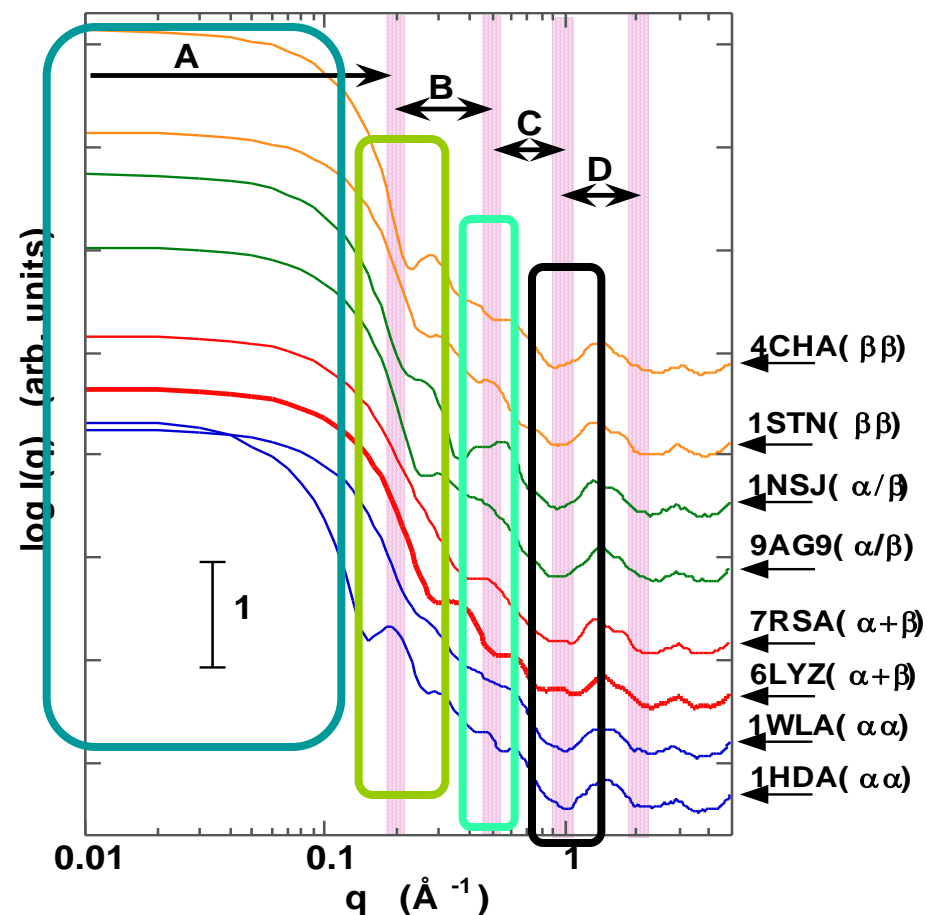
Schematic Correspondence of Scattering Curve of Proteins to Their Hierarchical Structures

Theoretical scattering profiles of proteins in solutions

3D structure of Lysozyme



広角散乱測定で得られる各領域のデータは、それぞれのタンパク質の階層構造を反映する。



Application of High-Resolution WAXS to Protein Folding Study

A: pH 2.2

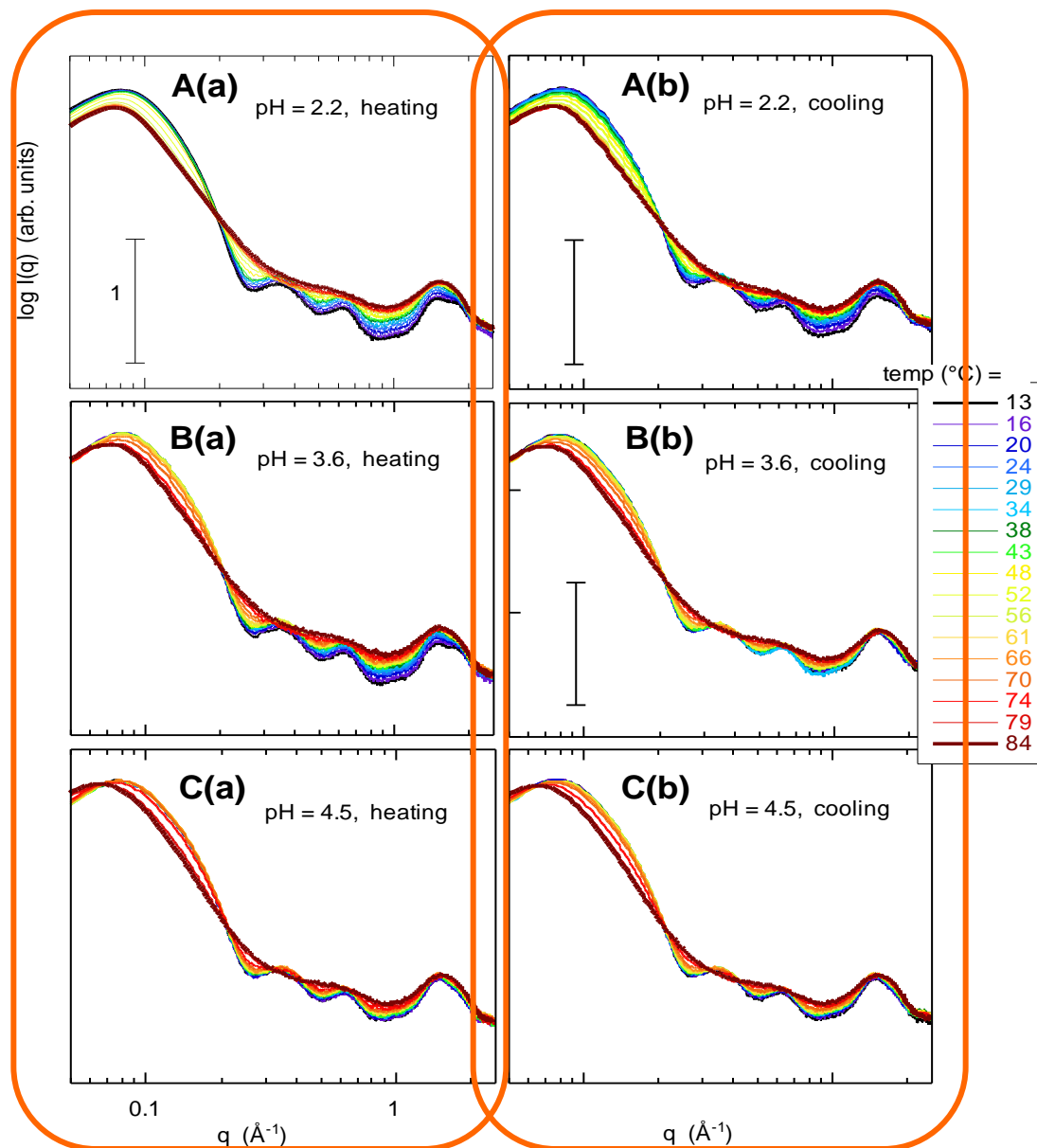
B: pH 3.6

C: pH 4.5

(a): heating (*unfolding*)

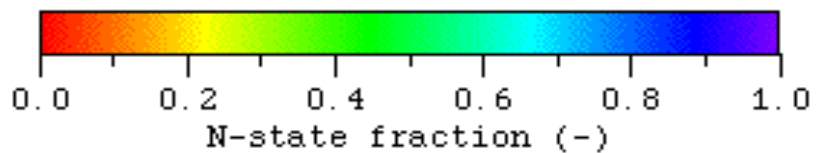
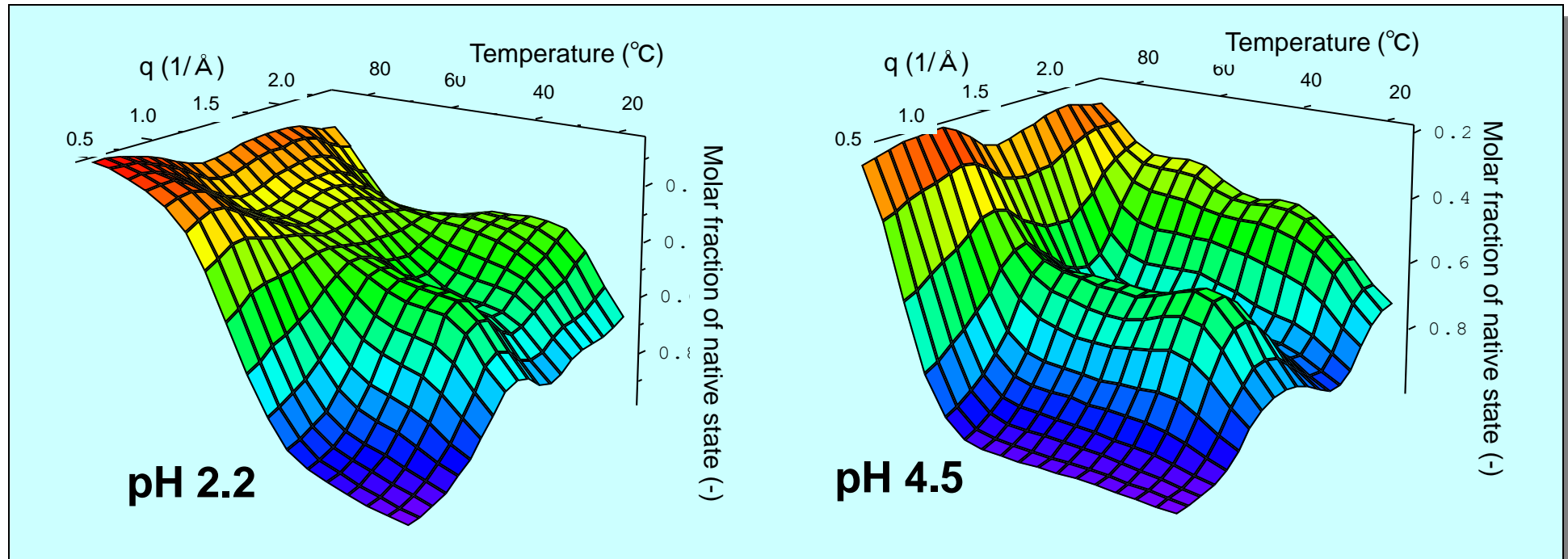
(b): cooling (*refolding*)

Temperature dependence of the WAXS curve of HEWL in heating (unfolding) and cooling (refolding) processes



Introduction-4

3D-Hierarchical Map of Molar Fraction of N-state protein against q -value and temperature

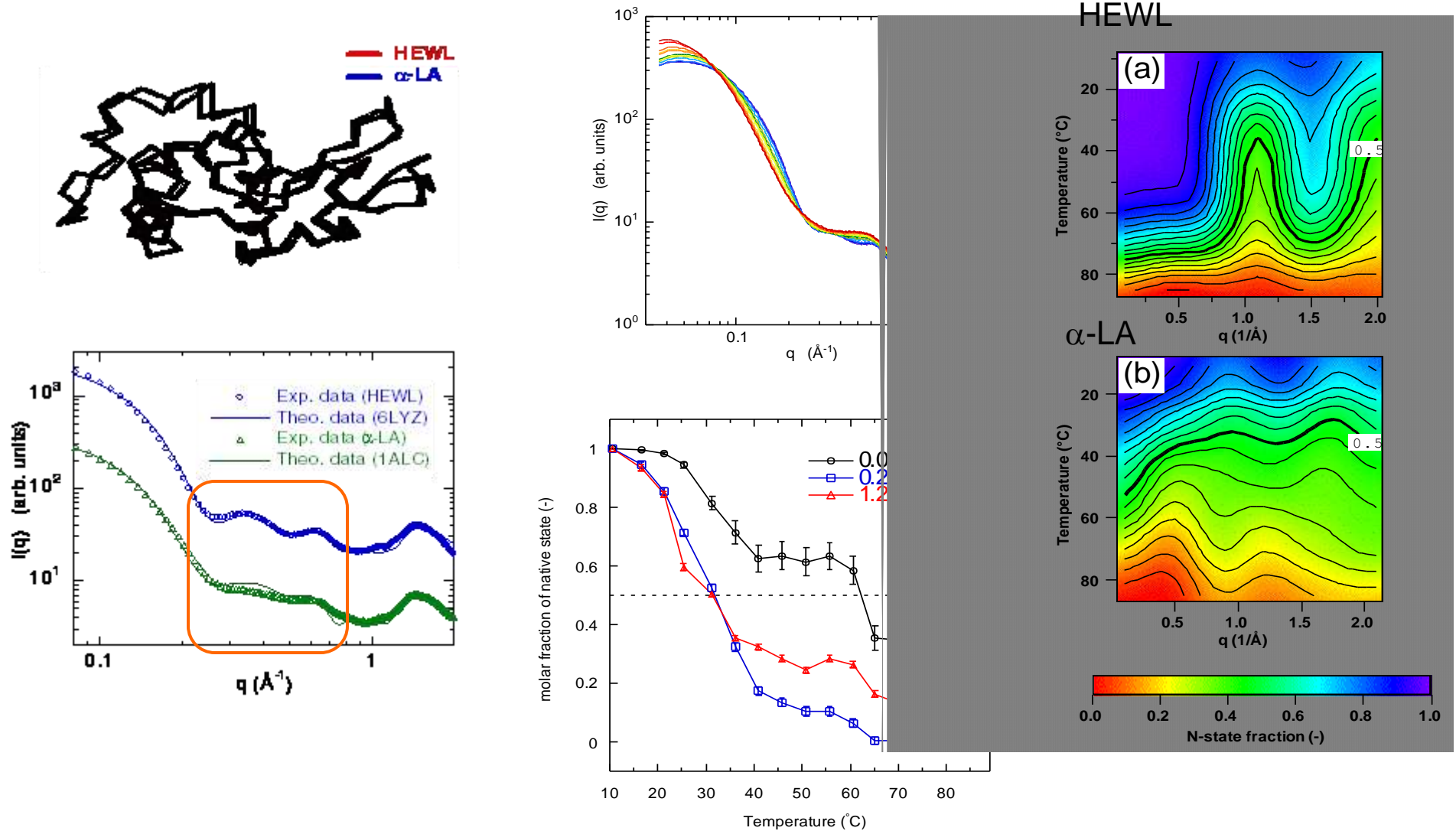


pH2.2 - transition proceeds less cooperatively in such as a multistate fashion.

pH4.5 - transition proceeds cooperatively for all hierarchical levels like a two-state transition.

Introduction-5

Comparison of experimental WAXS curves of HEWL and α -LA with theoretical ones: Thermal unfolding



Topics-1

Scattering curve of *lysozyme* depending on PVP concentration at different pH

PVP conc. (w/v)	P (N/m ²)	P (atm)
5	1.27E+04	0.125
10	4.30E+04	0.424
15	1.09E+05	1.08
20	2.28E+05	2.25
25	4.16E+05	4.11

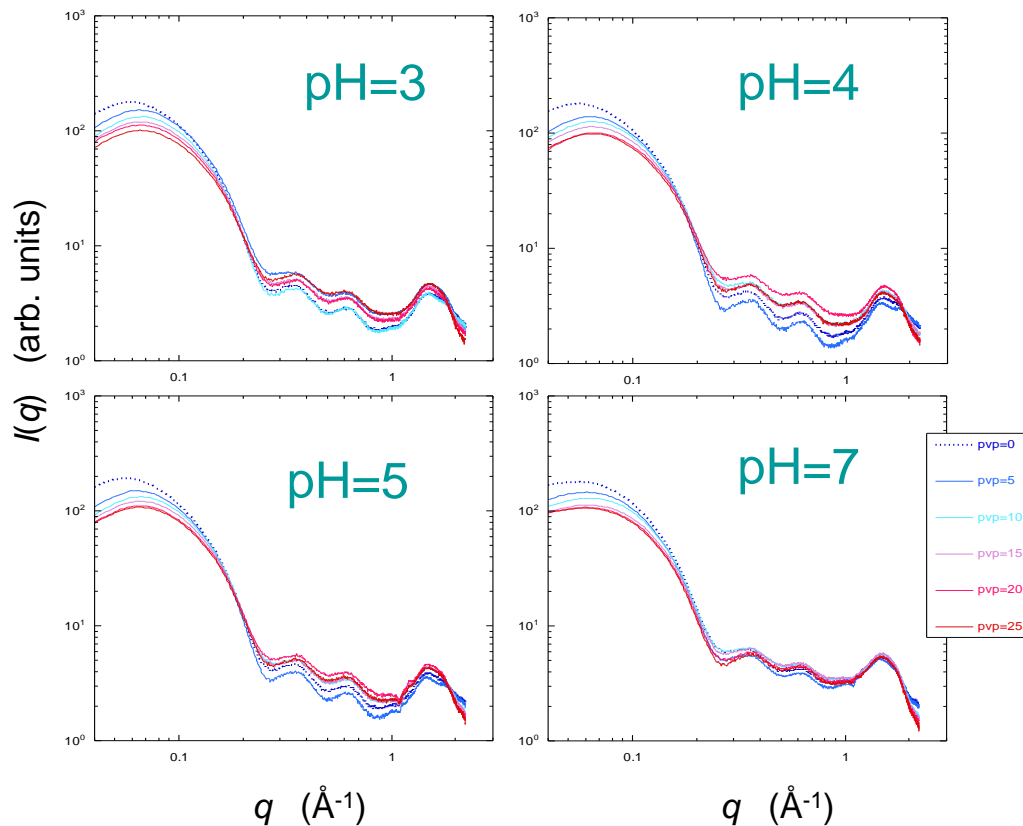
q -range : 0.1~0.2Å⁻¹

The slope becomes smaller with increasing PVP concentration for every pH.

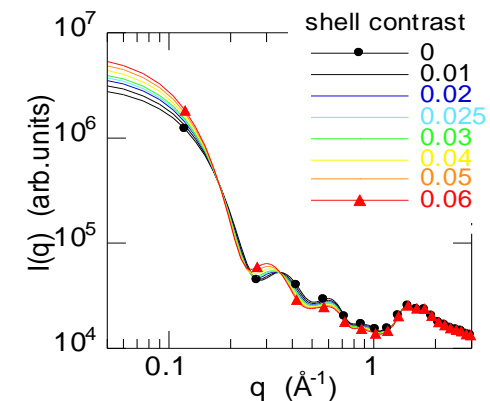
→ *Collapse of hydration shell*

q -range : >0.25Å⁻¹

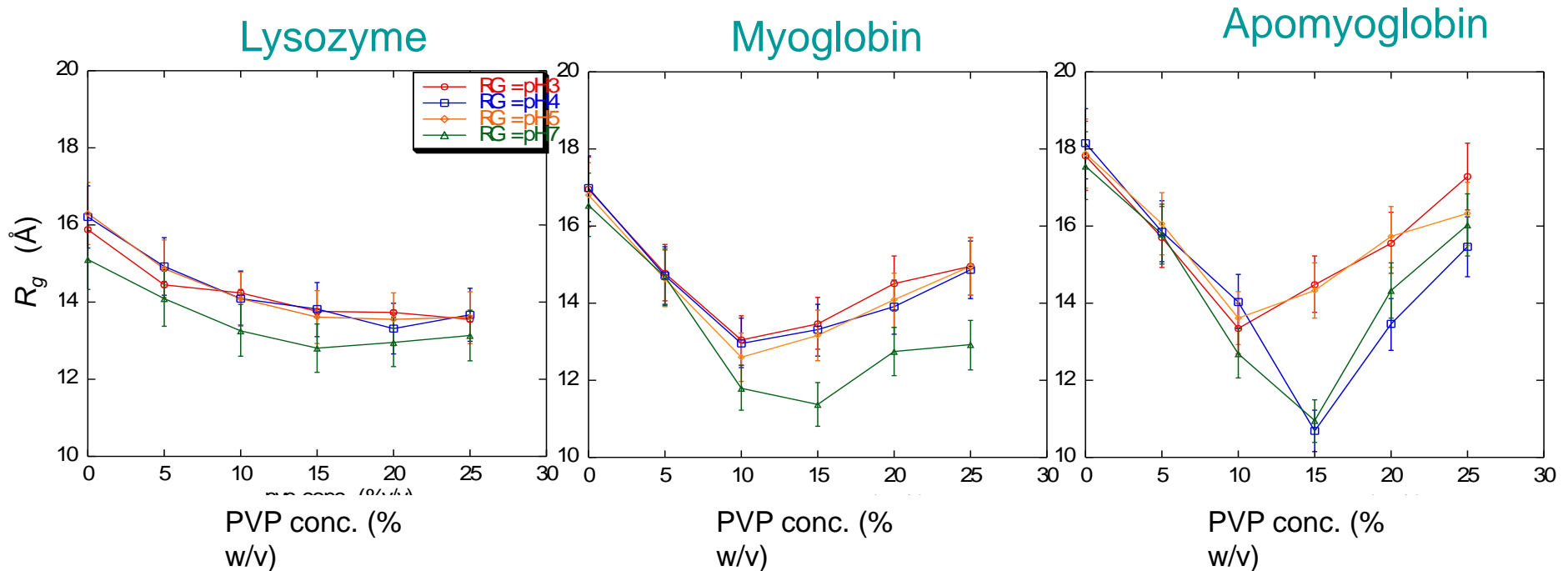
At pH7 the changes of the domain and secondary structures are smaller than those at other pH. With lowering pH the effect of the PVP tends to be more clear.



Theoretical scattering curve of lysozyme depending on hydration shell contrast obtained by CRY SOL



Comparison of PVP-concentration dependence of radius of gyration



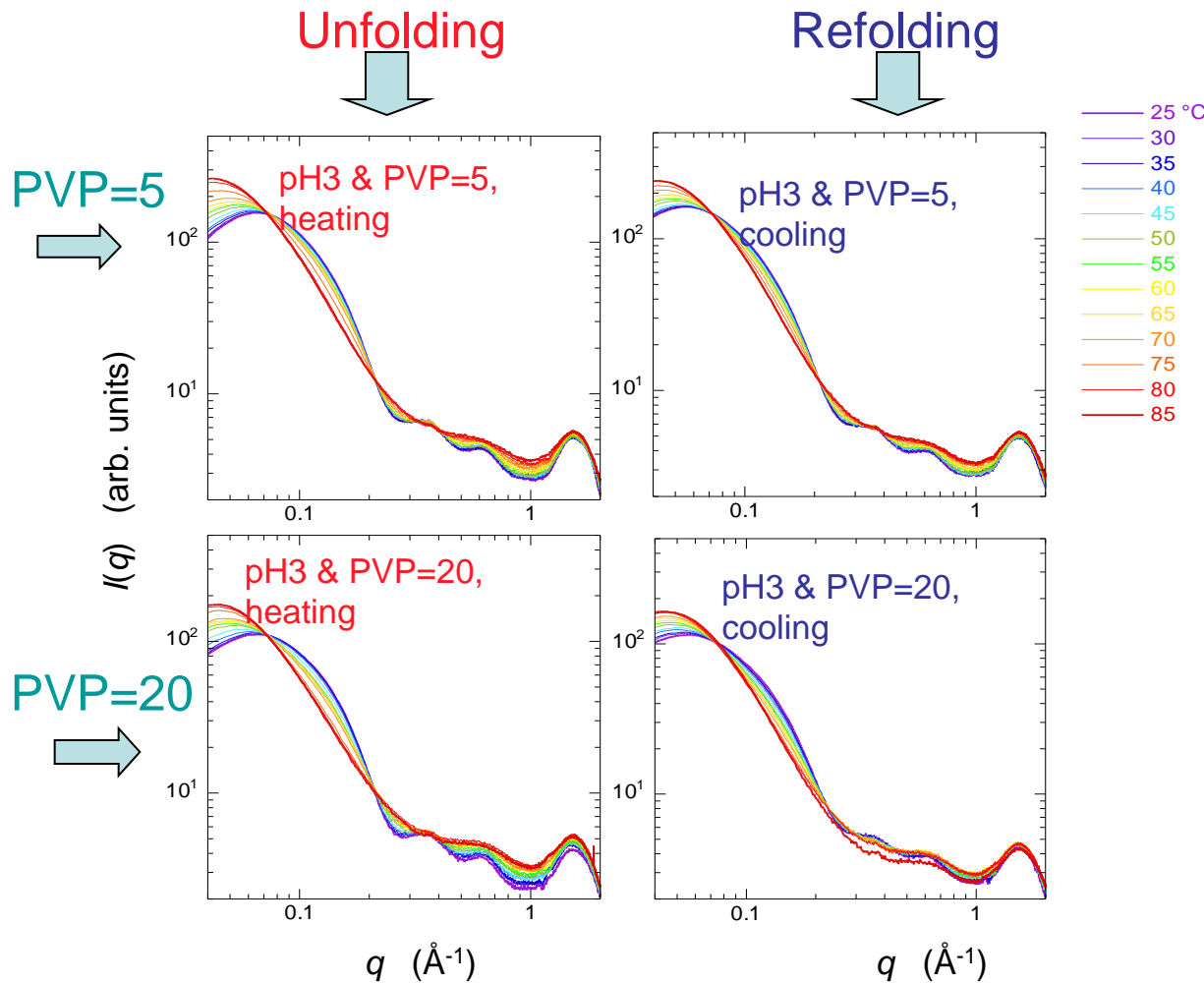
In the case of lysozyme, the R_g value show a decreasing tendency at every pH. The decrement becomes to be smaller above PVP \cong 15 %.

In the case of myoglobin, according to increase of the PVP concentration the R_g value becomes to increase after showing a minimum around PVP = 15 % at pH 7, and PVP = 10 % at other pH. The increase of the R_g value is ascribed to the oligomerization of the proteins.

Apomyoglobin shows a similar tendency as in myoglobin, which is much remarkable.

Topics-3

Reversibility of thermal unfolding-refolding of *lysozyme* under osmotic pressure at pH 3



PVP=5%w/v \rightarrow P=0.125 atm

PVP=20%w/v \rightarrow P=2.25 atm

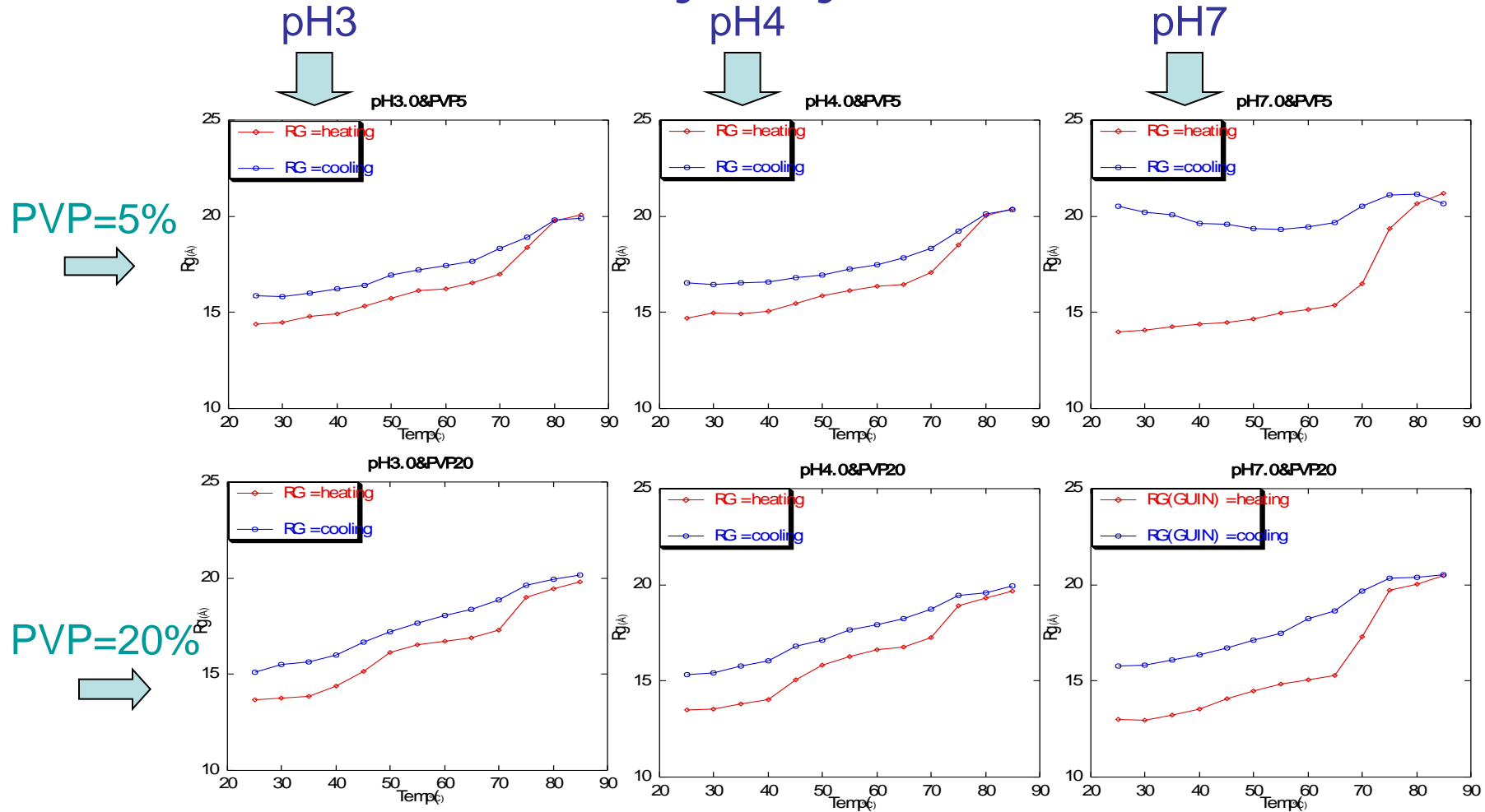
The change of the intramolecular structure becomes irreversible in the case of PVP = 20 in comparison with PVP = 5.

In addition, the change of the secondary structure is small.

The addition of the PVP obstructs the reversibility of the thermal structure transition of lysozyme that is originally reversible. This tendency is more remarkable than in the domain structure region (specific packing) of the molecule.

Topics-4

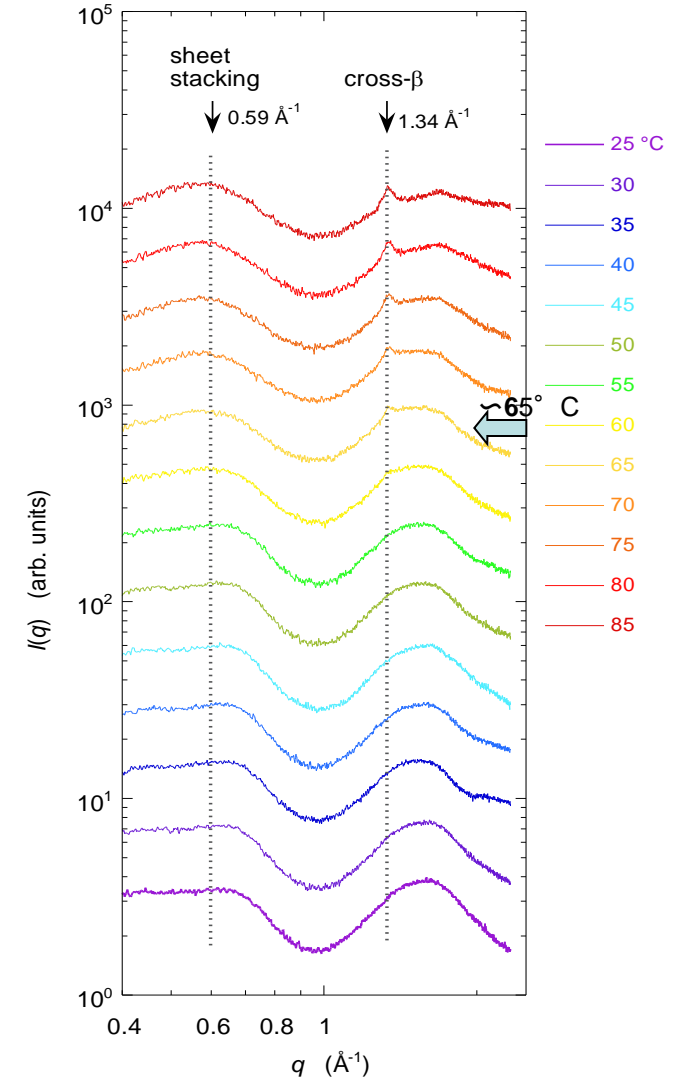
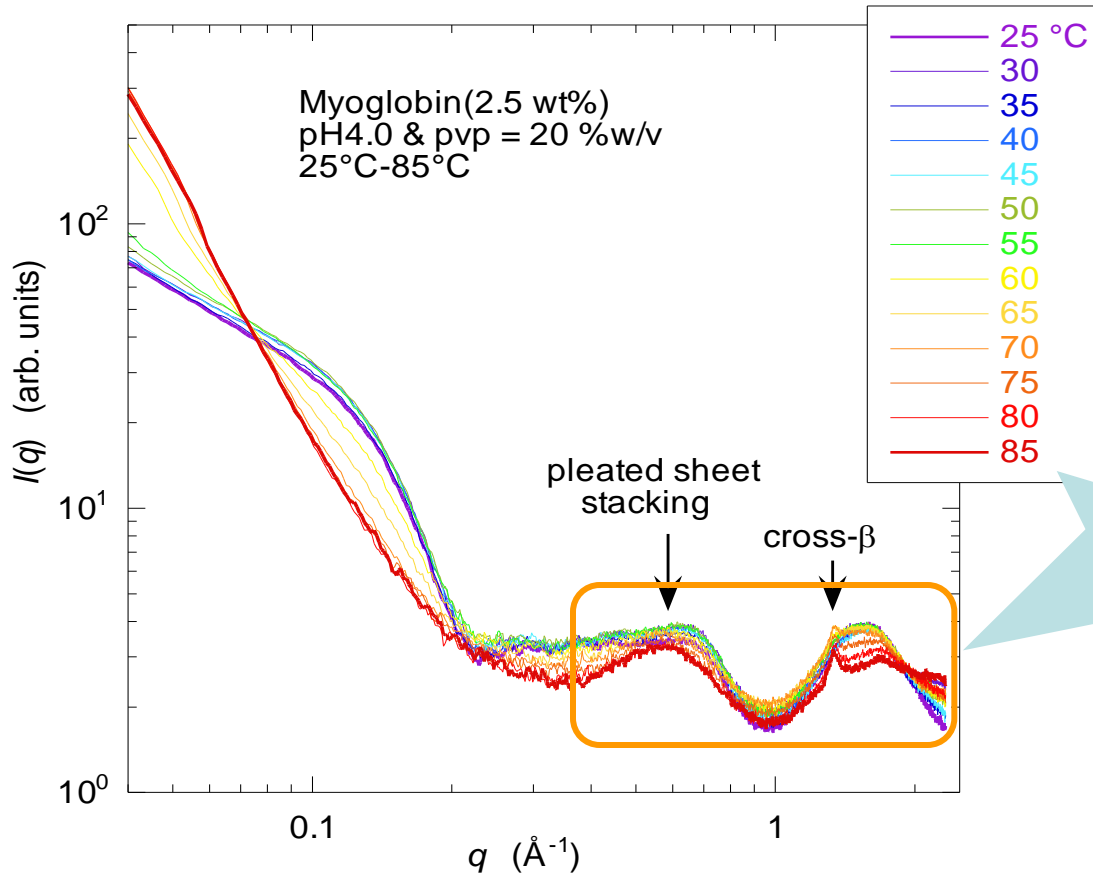
Comparison of temperature dependence of radius of gyration of lysozyme



*At every pH, thermal hysteresis is seen under the PVP existence.
In addition, under the presence of PVP, intermediate denatured state appears with the lowering of pH and the rise of PVP concentration in the unfolding process.*

Amyloid formation of *myoglobin* under osmotic pressure

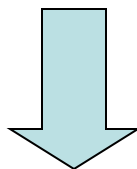
pH=4.0, PVP=20 %(w/v), P=2.25 atm



With elevating temperature, the peak at $q = \sim 1.34 \text{ \AA}^{-1}$ and the hump at $q = \sim 0.58 \text{ \AA}^{-1}$ appear, *indicating the $\alpha\beta$ transition (cross- β structure) and the pleated sheet stacking in amyloid formation.*

Summary

- (1) 浸透圧の増加は、タンパク質の水和シェルの変化(崩壊)を誘起する。
- (2) また、ミオグロビンにおいては、浸透圧の増加に伴う水和斥力変化が分子間相互作用を変えるため、多量体形成を誘起する。
- (3) 浸透圧の負荷は、リゾチームの熱構造転移の可逆性を抑制する。また、中間的な変性状態が安定化する。
- (4) 浸透圧下では、ミオグロビンの熱変性過程においても、アミロイドへの構造転移(ヘリックス-シート転移, シートのスタッキング)が起きる。



浸透圧の負荷は、*native* 構造を安定化させていた水和シェルの崩壊／不安定下を招き、そのことがミオグロビンのアミロイドへの構造転移を引き起こしたと考えられる。また、同じ条件下でも、リゾチームではアミロイド構造転移は起きない。

アミロイド凝集体形成においては、表面有効電荷や水和斥力などに起因する分子間相互作用が重要であるが、浸透圧の負荷によってもたらされるそれらの変化がタンパク質によって異なることを示唆する。