REANALYSES OF PRESSURE-DEPENDENT CHEMICAL SHIFT DATA OF VARIOUS PROTEINS BY PCA PROCEDURE

Takayuki Nakano [1] and Kazumasa Sakurai [2]

Abstract

According to the preceding studies, two kinds of changes in protein conformation occur upon pressure application. One is (i) mechanical compression and the other is (ii) transition between thermodynamically distinct states. The conformational change via mechanism (i) corresponds to a change within a thermodynamic subensemble of conformer, and that via mechanism (ii) corresponds to the transition between different subensembles of conformers. It is suggested that intermediate states of protein accumulating in the conformational change via mechanism (ii) is relevant for its function or folding process. In order to investigate these states for understanding the structure-function relationship of proteins, we examined the previously formulated method, CS-PCA, to the various pressure-induced chemical shift change data. This method seemed to successfully dissect the data into the two contributions from (i) and (ii). Relatively small proteins showed less significant contribution from mechanism (ii). It is also confirmed that, for relatively large proteins, whose contribution from mechanism (ii) were significant, the positions of the residues with large $\Delta\delta$ values through the mechanism (ii) are near to the internal cavities and/or trapped water molecules. These observations indicated that the internal cavities and trapped water are relevant to the conformational change to functional or folding intermediate states.

Key words : NMR, protein, pressure-induced unfolding, chemical shift data, principal component analysis.

1. Introduction

Pressure application induces decreases in the partial molar volume of the protein molecules, leading to a shift of equilibrium of the protein conformational states. It is now widely accepted that two kinds of changes in protein conformation occur upon pressure application. One is (i) mechanical compression and the other is (ii) transition between thermodynamically distinct states. The conformational change via mechanism (i) corresponds to a change within a thermodynamic subensemble, or an energy-landscape basin, of conformer (e.g., the folded conformer), whereas that via mechanism (ii) corresponds to a shift of conformational population between different subensembles of conformers separated by a significant energy barrier (Fig. 1).[1] This view is originated from the attractive observation, the non-linear chemical shift changes ($\Delta\delta$), in the first high-pressure NMR measurement by Akasaka and co-workers,[2,3] and have been improved by them with a plenty amount of supporting data.[1,4] Biological significance of the pressure-induced states by mechanism (ii) remains controversial. Akasaka and co-workers suggested that the pressure-induced intermediates via mechanism (ii) is relevant for its function. This view is distinct from the classical ones, where the native structure itself is relevant to its function. Other reports suggested that the pressure-induced states correspond to the intermediate states of the folding process.[5,6] In either case, it is important to analyze the structures of these states for understanding the structure-function relationship of proteins.

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1. Department of Biological Engineering, BOST, Kindai University, Wakayama 649-6493, Japan

2. High Pressure Protein Research Center, Institute of Advanced Technology, Kindai University, Wakayama 649-6493, Japan

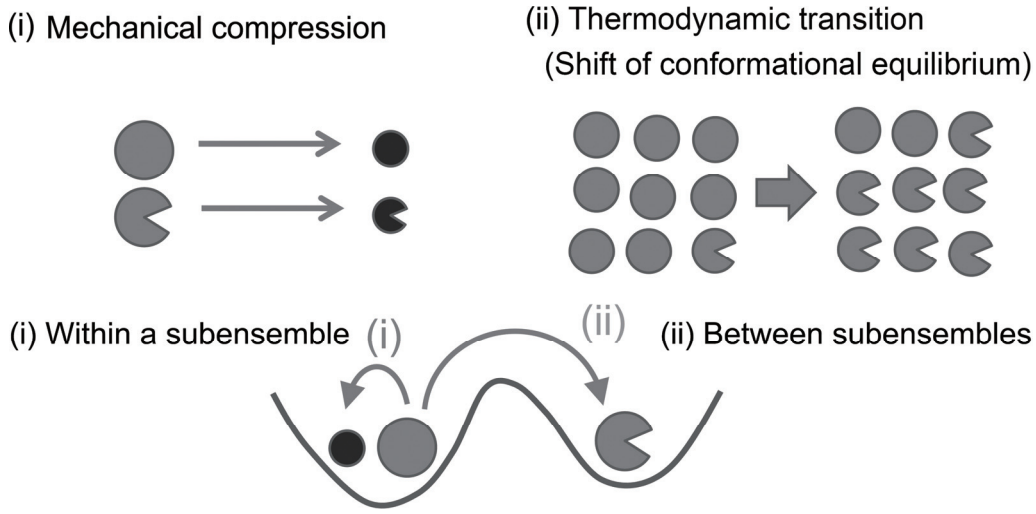


Fig. 1. A simplified illustration of two kinds of pressure effects on protein conformation in solution. Upon pressure application, (i) mechanical compression and (ii) thermodynamic transition, or shifts of conformational equilibrium, occur concurrently. The former and latter correspond to conformational change within a conformational subensemble and transitions between subensembles, respectively.

At present, pressure-dependent chemical shift changes (Δδ) of individual residues can be measured by using high-pressure NMR apparatus. A practical problem is how to decompose the obtained Δδ values into these two effects. Conventionally, such pressure-dependent Δδ data were fitted to a quadratic equation to be decomposed into the contributions from (i) and (ii). On the other hand, we have suggested a distinct analytical method named as chemical shift-principal component analysis (CS-PCA).[7,8] In our previous report, we tried a CS-PCA method to the pressure-dependent chemical shift data from bovine β-lactoglobulin (βLG), as a sample data set, to decompose them into contributions from (i) and (ii) too, and compared the obtained results to those from the conventional quadratic analysis. It was found that the extracted Δδ values for mechanism (ii) by the conventional method was not completely clean because the information from mechanism (i) was also involved therein. On the other hand, CS-PCA provided information of the (ii) contribution completely uncorrelated from that of the (i) contribution.[9] Thus, for the analysis of pressure-dependent Δδ, the CS-PCA procedure is thought to be adequate compared to the conventional method using the quadratic equation. In order to confirm the validity of this method, and to make further characterization of the pressure-induced states, we attempted to apply this method to pressure-dependent Δδ data from various proteins.

2. Materials and methods

The pressure-dependent chemical shift data on protein G, the HPr H15A mutant (HPr mut), apo myoglobin (apoMb), and p13 were kindly provided from Prof. Kitahara and Prof. Akasaka. These data can be seen in the review of the history of high pressure NMR technique written by them and their co-workers.[4] The data from βLG is the same ones as those reported in the last year.[9] The original papers where these data have first appeared and the conditions where the data were acquired are listed in Table 1.

Table 1. The source of the data used as sample data for the present CS-PCA analysis.

Protein	Original paper on which the data appeared	Measurement conditions			# of observable residues	# of measurement points (pressure range)
		Temp.	pH	Buffer		
protein G	Wilton et al.[10]	25°C	5.6	MES	52	6 (5~200 MPa)
HPr mut	Kalbitzer et al.[11]	25°C	7.1	Tris-HCl	67	5 (5~200 MPa)
p13	Kitahara et al.[12]	25°C	7.0	Tris-HCl	82	9 (3~300 MPa)
apoMb	Kitahara et al.[5]	35°C	6.0	MES	65	5 (3~150 MPa)
βLG	Sakurai[9]	25°C	2.0	None (HCl)	139	6 (5~125 MPa)

We performed a PCA of the provided data using Igor Pro with house-made semi-automatic program. Details of the data processing were previously described.[7,8]

3. Results and discussion

3.1 Dissecting the pressure-induced changes (i) & (ii) by CS-PCA for various proteins

Kitahara et al.[4] have reviewed the analytical results with the conventional method on pressure-dependent chemical shift data from various proteins investigated so far. We also examined the CS-PCA method on some of the data sets, including those from Protein G, the HPr H15A mutant (HPr mut), apo myoglobin (apoMb), and p13, as well as our original data from βLG. As a result of the singular value decomposition, we obtained a number of principal components (PCs) and corresponding contribution ratios for respective proteins. The number of PCs is the same as that of the number of measurement points (see Table 1). Table 2 shows the accumulative contribution ratio of the first three PCs for respective data sets. Accumulative contribution ratio is an indicator for how many PCs should be considered to describe the original data. It is practically considered that the including the PCs whose accumulative contribution ratio reaches 85% is enough to describe most contributions of the signal changes. In addition, the contributions of the 2nd PCs for Proten G and HPr mut are significantly less than those of the 1st PCs, whereas the contributions of the 3rd PCs for p13, apoMb, and βLG are significantly less than those of the 1st and 2nd PCs. These results suggest that only the 1st PC is almost enough for descriptions of the conformational change of protein G and HPr mut whereas two PCs are needed for descriptions of those of p13, apoMb, and βLG. Furthermore, the score plots, or variations of the fractions of each PCs, of the 1st and 2nd PCs for all proteins were found to change smoothly depending on the pressure, whereas those of the 3rd PCs were fluctuating independently of pressure (data not shown), supporting that the first two PCs contain the information about the pressure-dependent structural changes whereas the 3rd and higher PCs reflect the measurement noises of the chemical shift values. With these discussions, we concluded that considering at least two PCs are enough to retain the important information about the pressure-dependent conformational changes.

Table 2. Accumulative contribution ratios for the 1st, 2nd, and 3rd principle components from the CS-PCA for respective proteins analyzed.

	Protein G	HPr mut	p13	apoMb	βLG
1st PC	85.0%	87.3%	82.9%	79.6%	82.9%
2nd PC	90.3%	93.0%	90.6%	89.4%	92.3%
3rd PC	94.4%	96.6%	93.5%	95.2%	95.2%

Figure 2 shows the plots of the scores of the 1st PC against those of 2nd PC. Hereafter, the PC2-PC1 plane is called as “PC plane”. Basically the traces of these plots are curling, though the degree of the curvatures is different from each other (Fig. 2, the black points). A certain direction on the PC plane corresponds to a certain conformational change of protein molecule, characterized by a certain Δδ profile. The curvature of the plot indicates that at least two kinds of conformational changes are included in the pressure-induced changes. We interpreted that these transitions are (i) mechanical compression and (ii) thermodynamic transitions.

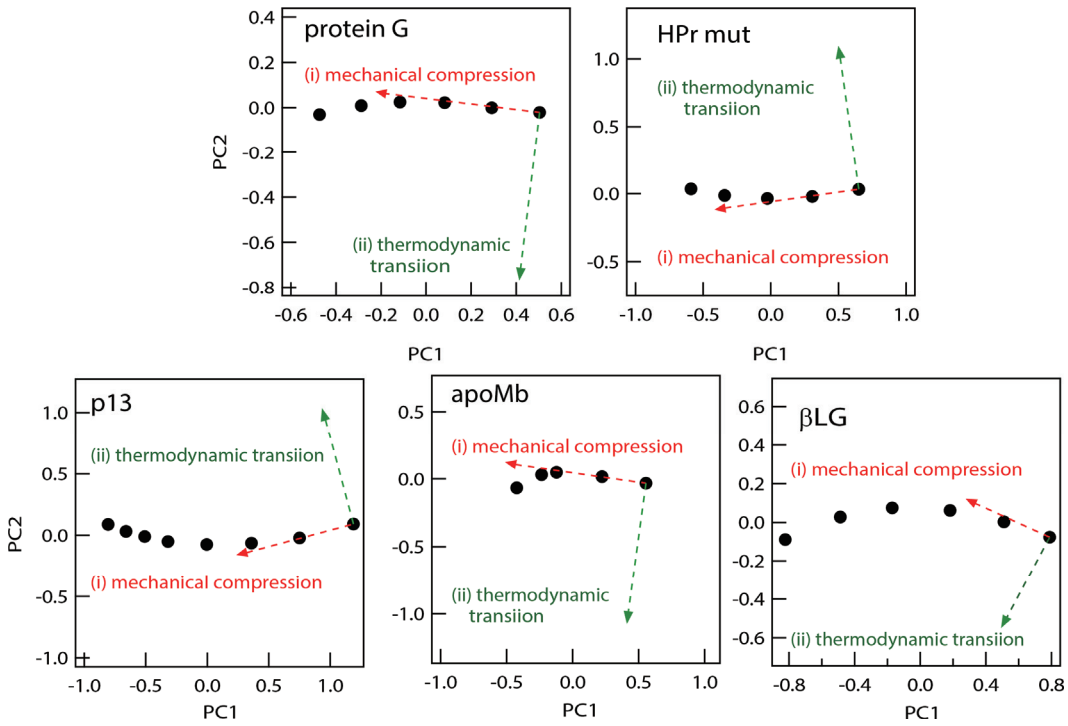


Fig. 2. Extracted PCs for each protein. One black point on the PC plane corresponds to one spectrum at each pressure point. As the pressure increased, the position of the point changed from right to left. The direction, along which the spectral change at the lowest pressure region occurred, is supposed to correspond to the (i) mechanical compression (indicated by red arrows) (see text). On the other hand, the direction, along which the spectral point is moving away from the red arrows, is supposed to correspond to the (ii) thermodynamic transition (indicated by green arrows).

From such a point of view, the plots on the PC planes for protein G and HPr mut are almost linear, indicating that only one kind of conformational change during the increase in pressure is dominant for these proteins. On the other hand, the plots for apoMb, p13, and βLG showed significant curvatures, suggesting that two kind of conformational changes are included.

Since (i) mechanical compression is an elastic response of protein molecule, the $\Delta\delta$ will be linear against the pressure at any pressure points. Thus, the direction of the plot from the first point (corresponding to the spectrum obtained at the lowest pressure) should reflect the mechanical compression. On the other hand, the thermodynamic transition is a cooperative process, where the change will abruptly start at a certain pressure point, drawing a sigmoidal curve. Thus, the direction corresponding to (ii) thermodynamic transition should be distinct from that of (i) mechanical compression. We hypothesized that the direction of (ii) is orthogonal to that of (i).

It should be noted that obtained information of the (ii) contribution completely uncorrelated from that of the (i) contribution (Fig. 3), indicating that the present procedure successfully separated the original data into the two contributions.

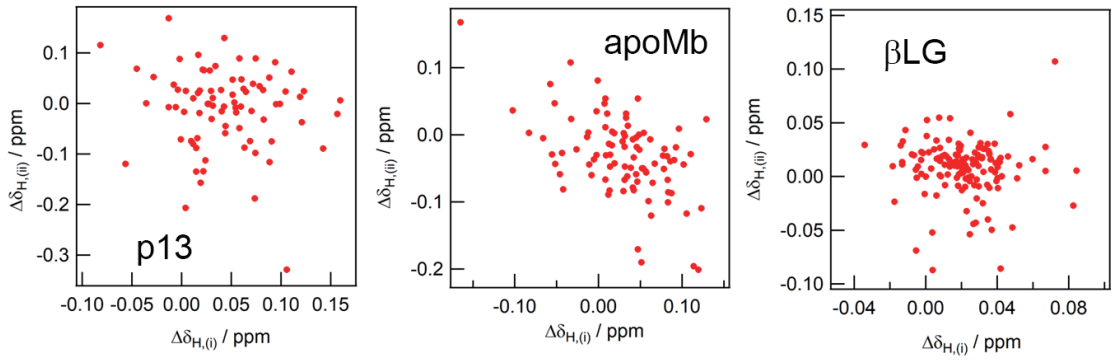
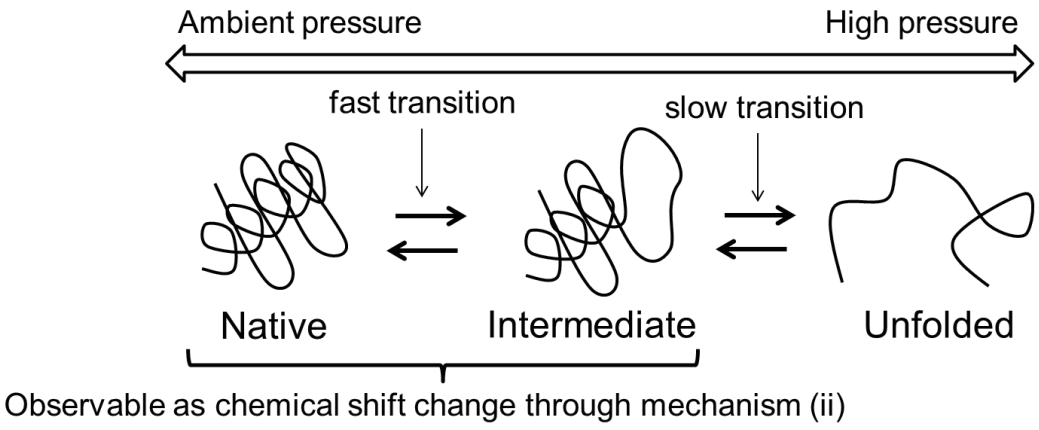


Fig. 3. Correlation plots of obtained $\Delta\delta_H$ values for each residue of (i) mechanical compression against those for (ii) thermodynamic transition, for p13, apoMb, and βLG. It is confirmed that there are no significant correlations between these values, indicating that the contributions from (i) and (ii) were successfully decomposed.

3.2 Interpretation for the observed conformational change from mechanism (ii)

As mentioned above, only 1st PC is enough for the description of the spectral changes of protein G and HPr mut. It indicates that most change is brought from conformational change by mechanism (i). On the other hand, first two PCs are needed for the description of the spectral changes of p13, apoMb, and βLG, indicating that significant contribution from the conformational change by mechanism (ii) is included in the Δδ data.

We interpreted that the observed chemical shift changes result from pressure-dependent cooperative conformational change between the native state and certain intermediate states, rather than that between the native and globally unfolded states (Scheme 1). It is noted that chemical shift changes should be attributed to the conformational change in a relatively "fast" time scale with respect to the NMR time scale (~tens of microseconds). The application of high pressure to the protein solution ultimately induces global unfolding of protein molecules. In general, however, the global unfolding is relatively "slow" conformational change with respect to the NMR time scale. Instead, the observed chemical shift change should be attributed to conformational change to "partially" perturbed states, because the conformational change between the native and this states likely occur in a fast time scale. Preceding papers also made the same interpretation (e.g., such partially perturbed state is called as "low-lying excited state" in ref (2)). This conformational change will occur at the same or a lower pressure than that where the global unfolding occurs.



Scheme 1. Schematic presentation of process of pressure-induced conformational changes.

There are other various methods to detect the pressure-induced conformational changes of protein than NMR. For example, pressure-dependent conformational changes of βLG were investigated by using FT-IR[13] and X-ray crystallography[14]. However, neither reports mentioned the conformational change via mechanism (ii) explicitly; the changes in secondary structure detected by FT-IR were attributed to the global unfolding, whereas the changes in the crystal structures results from a mixture of the mechanisms (i) and (ii). Thus, measurements of pressure-induced chemical-shift changes and subsequent analyses is an only way to detect the conformational changes through the pressure-induced thermodynamic transition of protein.

We inspected the positions of the residues which show large Δδ values in (ii) thermodynamic transition. With the PDB structure, we also investigated the positions of the trapped water molecules and the internal cavities within the protein structures of the investigated proteins, and compared them to the residues with high Δδ values mentioned above. For p13, apoMb, and βLG, the residues with large Δδ through mechanism (ii) were found to locate near the trapped waters or the internal cavities (Fig. 4). These observations indicate that the pressure-induced transition between native and intermediate states might be initiated by the residues close to trapped water molecules or cavities. This suggestion is supported by some reports; Eriksson et al.[15] reported that some cavity-creating mutants of T4 lysozyme decreased their thermal stabilities, whereas Lassalle et al.[16] reported that a cavity-filling mutant of c-Myb R2 domain raised its stability. It is plausible that these transitions induce a local conformational change around these residues through immersion of water molecules and/or enhanced fluctuation brought by the (trapped or immersed) water. On the other hand, the CS-PCA results showed that the conformational changes though mechanism (ii) are less significant in protein G and HPr mut. Figure 4 showed that these proteins has less water molecules and internal cavities probably because they are small proteins and have less defects in the structural packing. Thus, the structural fluctuation will not be enhanced before the global unfolding.

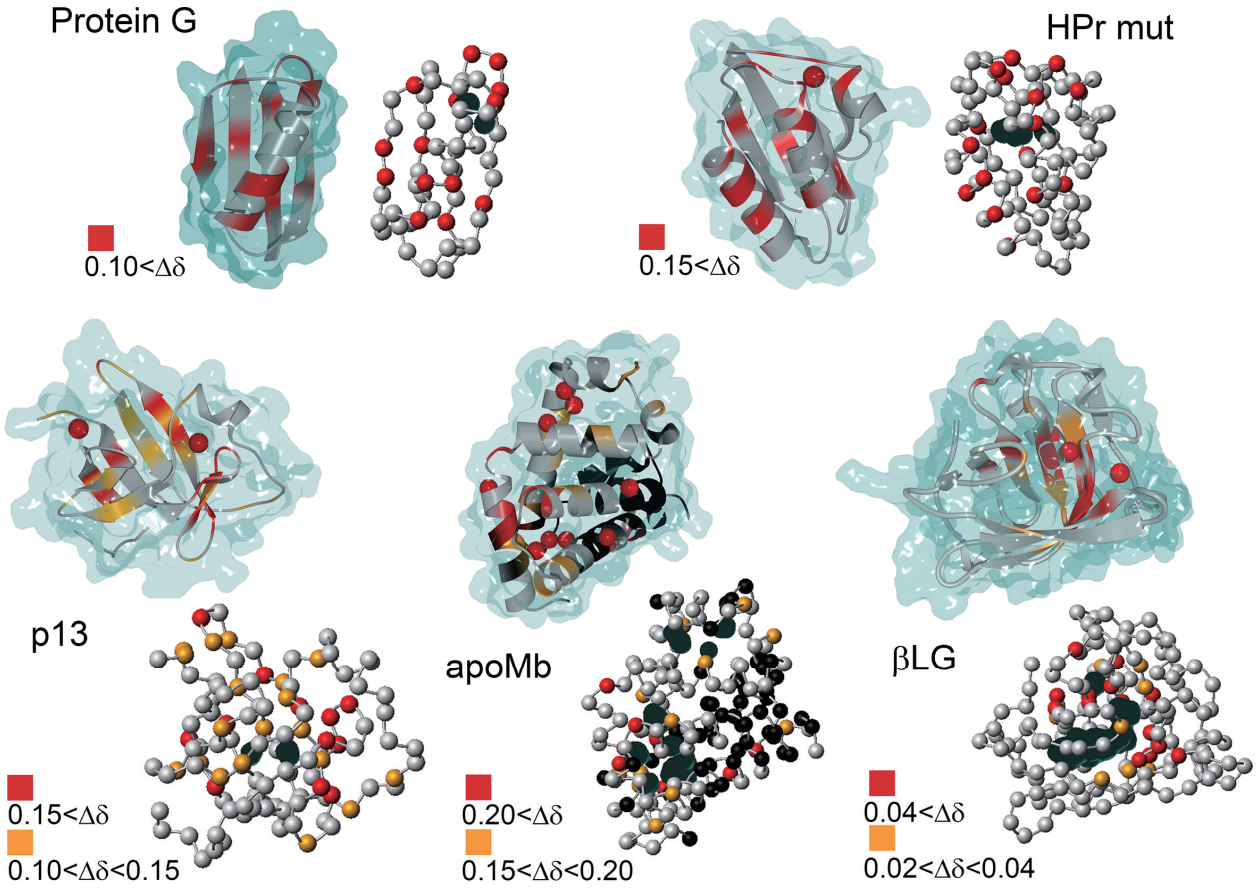


Fig. 4. Comparisons of the position of the trapped water molecules or the internal cavities with the positions of the residues relevant to the (ii) thermodynamic transition. The residues with significant Δδ values for (ii) thermodynamic transition are mapped on the crystal structures of respective protein, which are shown in the ribbon models and tube models. Both models for each protein are depicted in the same angle. In both models, the residues with significant Δδ values (in ppm) are colored red and orange. In the ribbon models, the water molecules trapped within the protein molecules are shown as red balls, and the molecular surfaces are also depicted as transparent cyan surface. In the tube models, the positions of Cα atoms are indicated by balls, and the internal cavities, which calculated by MOLMOL[17] with a 1.4 Å solvent probe except for p13 (1.3 Å), are shown as navy-blue orbs. The ribbon models and tube models were depicted by Molfeat (Fiatlux, Tokyo, Japan) and MOLMOL[17], respectively. The IDs for PDB data used for these graphics for protein G, HPr mut, p13, apoMb, and βLG are 1GB1, 1PTF, 1A1X, 1BMD, and 1BEB, respectively.

Similar discussion was made from other proteins. For example, Maeno et al.[18] suggested that pressure-induced conformational change between ground (native) state and slightly modified structures is initiated by residues near the water molecule trapped in the internal cavities through enhanced fluctuation brought by the trapped water. In addition, from the results of the preceding studies, such pressure-induced intermediate states are suggested to be high-energy states.[4] In these states, the protein molecules assume native-like structure with locally unfolded/modified potions. Its energetic state is slightly higher than its globally stable “native” state, and the high-energy state and the ground “native” state are separated by a low energy barrier. Although such a high-energy state of ubiquitin was reported to correspond to a functional state [19], there are no reports suggesting that the pressure-induced conformational change is relevant to its function for the proteins analyzed in this paper. Instead, interesting agreements between the pressure-induced intermediate state via mechanism (ii) of apoMb and βLG and their folding intermediates were found: In previous studies, the structures of the initial folding intermediates of apoMb and βLG were investigated by using the quenched-flow pulse-labelling method.[20,21] Their results suggested that α-helices A, G, and H of apoMb and β-strand F, G, and H of βLG have already been structured in the initial folding intermediate states. It is interesting that these protected residues agree well with the residues whose Δδ values for the conformational change via mechanism (ii) were found to be significant in the present analysis (Fig. 4). These observations indicate that the pressure application enables protein molecules to sample various conformational state, including functional state and/or folding intermediate. However, what kinds of intermediates can be induced by pressure is to be clarified in future studies.

In conclusion, we examined the previously formulated method, CS-PCA, to the various pressure-induced chemical shift change data. This method seemed to successfully dissect the data into two contributions from (i) mechanical compression and (ii) thermodynamic transition. Relatively small proteins showed less significant contribution from mechanism (ii). It is also confirmed that, for relatively large proteins, whose contribution from mechanism (ii) were significant, the positions of the residues with large Δδ values through the mechanism (ii) are near to the internal cavities and/or trapped water molecules. These observations indicated that the internal cavities and trapped water are relevant to the conformational change to important intermediate states for biological function or folding process. In future, further analysis with this method will provide comprehensive knowledge for the pressure-induced conformational changes.

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和文抄録

様々な蛋白質の圧力依存化学シフトデータに対する主成分解析

中野　隆行[1]、櫻井　一正[2]

これまでの研究から、圧力の増加に伴い(i)力学的圧縮と(ii)熱力学的転移の２つのタイプの蛋白質構造変化が同時に進行することが分かっている。(i)力学的圧縮とは蛋白質が天然状態や中間体状態などの状態のまま圧縮される構造変化であるのに対し、(ii)熱力学的転移とは蛋白質が異なる状態へ転移する際の構造変化のことである。ここで、(ii)の過程によって現れる中間体が機能や折り畳み過程に重要であると指摘されており、この過程による構造変化を詳細に調べることが重要である。高圧 NMR という手法により、圧力依存的な構造変化の過程を、各アミノ酸残基の化学シフトの変化として調べることができる。昨年の報告で我々は、化学シフトー主成分解析（CS-PCA）という化学シフトデータの解析法を用いて圧力依存的な構造変化の解析を行ったところ、(ii)の情報と(i)の情報が明確に分離されていることが分かり、本方法の有効性が示された。そこで、様々な蛋白質の圧力依存化学シフトデータに対し本解析法を適用した。その結果、①小さな蛋白質では(ii)の寄与が少なかった、②大きな蛋白質で(ii)の影響の大きかった残基は分子内キャビティや分子内結合水に近いものが多かった、ということが分かった。分子内キャビティや分子内結合水のもたらす揺らぎが、機能や折り畳みに重要な状態への構造転移に重要であることが示唆された。

キーワード：NMR、蛋白質、圧力変性、化学シフト、主成分解析

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1 近畿大学生物理工学部　生物工学科，〒649-6493 和歌山県紀の川市西三谷 930

2 近畿大学先端技術総合研究所　高圧力蛋白質研究センター，〒649-6493 和歌山県紀の川市西三谷 930