2P055 抗体を用いた抗原蛋白質の揺らぎの検出
Detection of conformational dynamics of protein antigen by antibody

We showed that an anti- hen egg lysozyme (HEL) monoclonal antibody could distinguish Cys6-Cys127 alkylated lysozyme (CM6-Cys127-HEL) from native HEL whose crystal structures are similar. The decreased binding affinity to CM6-Cys127-HEL was mainly due to the decreased association rate constant (k_a), indicating that k_a is an indicator of proportion of the native format determinant in equilibrium. It should also be noted that the antibody could recognize the difference of conformational dynamics. In order to analyze the dynamic property of HEL, especially the effects of the reduction, in detail, we expressed HEL and C6A/C127A mutant in E. coli, and purified them. Based on the analysis using Biacore and NMR, we discuss the conformational dynamics of HEL detected by antibody.

2P056 天然変性タンパク質 HIV-1 Tat と転写コアクティベータ CBP の KIX ドメインとの相互作用
Interaction of the intrinsically disordered HIV-1 Tat protein with the KIX domain of the transcriptional coactivator CBP
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HIV-1 transactivator of transcription (Tat) is an intrinsically disordered protein, which interacts with multiple target proteins during HIV-1 gene expression. One of the targets of Tat is the KIX domain of the transcriptional coactivator CREB-binding protein (CBP). However, the structure and mechanism of the Tat-KIX interaction is poorly understood. To investigate the structure of the bound form and the mechanism of coupled folding and binding of Tat, we constructed the expression systems of the full-length Tat (86 residues) and KIX, and purified them with the Ni-affinity chromatography and gel filtration. We have measured CD spectra of the free and bound forms of Tat and KIX. NMR measurements of the Tat-KIX interactions will be presented at the meeting.

2P057 ニワトリオムポモイドにおける変性中間状態の構造と熱力学
Structure and thermodynamics of the unfolding intermediate of hen egg ovomucoid
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Chicken ovomucoid (OVM) is a potent allergen from egg white, the thermodynamic characterization of which is an unsolved, but important issue in reducing its allergenicity. We studied conformational stability of OVM, at pH 8.0, in the wide pressure (3~700 MPa) and temperature (5~50°C) range using tyrosine/tyrosinate fluorescence and 1H-NMR spectroscopy. We found no full unfolding is possible for OVM, but a stable intermediate is present under certain conditions of P and T, allowing us to draw a P-T phase diagram between the folded state (N) and the intermediate state (I). 1H-NMR at 0.1 MPa, and the proteolysis reaction under pressure, suggest that in state I only the 3rd-domain is folded. Such a stable I state is likely to be the major cause of the allergenicity.

2P058 变温変圧核磁気共鳴とDNAバインディング部位変異体を用いたSOD1オリゴマー形成の抑制
Variable temperature and pressure NMR studies on flexible conformation of c-Myb DNA-binding domain
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The main purpose of this study is to find details of the dynamic conformational state of the c-Myb DNA-binding domain, R2R3, under the physiological conditions. Both 1D 1H and 2D 15N/1H HSQC NMR spectra of R2R3 showed that, with increasing temperature in the range below the thermal denaturation, most peaks lost their intensities rather heterogeneously over residues, and nearly diminished at the physiological temperature. The extensive conformational fluctuations are rather unusual for proteins like enzymes, but would be rather normal for DNA binding proteins, as conformational disorder is often advantageous in its function. We also show the results obtained using high-pressure NMR experiments, and discuss the flexible property in correlation with the function.

2P059 ジスルフィド結合のシャッフルを標的とした異常なタンパク質オリゴマー化の抑制手法
Disulfide shuffling in Cu,Zn-superoxide dismutase is a key to develop potential drugs for neurodegeneration
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Dominant mutations in Cu,Zn-superoxide dismutase (SOD1) cause a familial form of amyotrophic lateral sclerosis, of which a pathological hallmark is the formation of abnormal SOD1 oligomers in affected spinal cords. Our group has recently revealed that mutations facilitate the disulfide shuffling within and among SOD1 proteins and thereby form the disulfide-crosslinked oligomers. Here, we have identified several drugs that can effectively and selectively inhibit the formation of SOD1 oligomers and found that those drugs act on the disulfide-shuffling process between specific cysteine residues without affecting structural stability of mutant SOD1 proteins. Further investigation on roles of cysteine residues in the SOD1 oligomerization is now in progress.