1P014 IgG 結合ドメインの立体構造の違いについて 一残基間平均距離統計に基づく解析
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3D structural Differences in the IgG Binding Domains -Analysis based on the Interresidue Average Distance Statistics-
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We have shown that the predicted subdomain regions with interresidue average distance statistics reflect well to the residues with high ϕ values. In this study, we apply the present method to the IgG binding domains. Recently, He *et al.* Biochemistry, 44, 14055(05)) demonstrated that the variants of the IgG binding domains A and G (A 219 and G 311 respectively) share the 59% sequence homology but have different 3D structures, i.e., 3 α structure and α β structure respectively. We try to analyze the relationships between the differences in 3D structures and their sequences using the interresidue average distance statistics. First of all, we construct the predicted contact maps (Average Distance Map, ADM) of these proteins, and then we make simulations of random conformations of these sequences with the interresidue potential derived from the average distance statistics. In the simulations, contact frequency, $g(i,j)$ are calculated. $g(i,j)$ is defined when a distance between Ca atoms $R_{ij} < R_{cut}(=10\text{\AA})$. Furthermore, we calculated a value $Q(\mu, \nu) = (g(\mu, \nu) - \sum_{i|j=m} g(m)/\sum_{i|j=m} g(i,j))/D(m)$ where $g(m) = \sum_{i|j=m} g(i,j)/\sum_{i|j=m} D(m) = \sum_{i|j=m} (g(m) - g(i,j))/\sum_{i|j=m} D(m)^{1/2}$. Finally we define $p(\mu) = \sum_{\nu} Q(\mu, \nu)$. The $p(\mu)$ values for two proteins show the characteristic profiles respectively. We also compare the present results with other techniques.

1P016 セルフタイマーを内蔵するタンパク質 EA4
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1aE05 EA4 is a protein with a built-in self-timer
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Many living organisms have time-measuring devices that regulate various biological activities. Recent advance in research on circadian clock systems has revealed the core circadian oscillation mechanism at the molecular level. On the other hand, however, little is known about the mechanisms that govern the timing and duration of developmental events that happen only once in a lifetime. One of the most remarkable examples of such a non-oscillatory timer is the silkworm embryonic glycoprotein EA4. EA4 has long been thought to measure the duration of developmental arrest during winter, since it exhibits a transient spike of ATPase activity a set number of days after onset of the cold period. This ATPase activity occurs at the time that development restarts. The countdown to activity is known to start with the dissociation of an inhibitory peptide called PIN under cold conditions, but the time measuring mechanism is unknown. Here we present the crystal structures and functional properties of EA4 with and without glycosylation. We show that EA4 is a homodimeric, copper-dependent ATPase, with each subunit consisting of a copper-zinc superoxide dismutase fold, with a short additional N-terminal sequence capable of binding an additional copper ion. The sugar chain of native EA4 appears to reinforce the binding of PIN. A model is proposed for the mechanism by which ATPase activity appears days after being triggered.

1P015 ロングレンジの構造情報を利用したMusashi タンパク質と標的RNA との複合体の構造解析
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Structural analysis of Musashi protein complexed with the target RNA with long-range structural information
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Musashi1 protein regulates differentiation of central nervous system through translational inhibition of the target gene. Musashi1 has two RNA binding domains (RBD1 and RBD2). We have already determined the structures of each single RBD. Here, we have tried to determine the arrangement of the two linked RBDs in the complex with the target RNA. In order to obtain the long-range structural information needed to determine the arrangement, residual dipolar couplings (RDCs) and paramagnetic relaxation enhancement (PRE) were utilized. The structures of each RBD in the complex with target RNA were determined by NOE-based structure calculations. Then we determined the relative orientation of two RBDs in the complex on the basis of RDCs. RDCs were produced by partially aligning the Musashi1-RNA complex with stretched polyacrylamide gel. Incorporation of another kind of long-range structural information deduced from PRE was also intended. For this purpose, all Cys residues of Musashi1 were replaced by Ala residues and then the replacement by a Cys residue was carried out for four selected residues, respectively. MTSL carrying NO radical was attached to the single Cys residue for four mutant Musashi1 proteins as the paramagnetic center, and PRE measurements were tried. These analyses have suggested the way how two tandem RNA-binding domains of Musashi recognize its target RNA.

1P017 ATP 依存性プロテアーゼ FtsH の mobile 領域の役割
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The role of the mobile regions in the ATP-dependent protease activity of FtsH
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The ATP-dependent protease FtsH digests misassembled membrane proteins and short-lived soluble proteins in order to maintain membrane integrity and control their cellular regulation. The enzyme has an N-terminal transmembrane segment and a C-terminal cytosolic region consisting of an AAA+ ATPase domain and a protease domain. Previously we determined the whole cytosolic region of FtsH and proposed that a part of FtsH forms a tunnel to send the substrate to the protease catalytic site (Suno R., et al., Mol Cell 2006). The structural analysis suggested that several mobile regions play an important role in the operating mode of FtsH. Here, we report about the effect of mutations in these mobile regions. Based on the structural information, it is conceivable that a α -hairpin and a lid-helix, which form the tunnel, are involved in translocating the substrate. Stepwise deletion of the α -hairpin showed a similar ATP-dependent protease activity as the wild type, but a concurrently decreased ATPase activity. The ATPase activity was switched off completely by the addition of a casein. This suggests that the α -hairpin determines the optimal position of the ATPase domain. Besides, the lid-helix covering the protease catalytic site can kink at the position of the highly conserved Glycine448. Substitution of this residue by other amino acids resulted in a complete loss of ATP-dependent protease activity. It is considered that these mutations obstruct the flexibility of the lid-helix, and leading to a more rigid FtsH with impaired functionality.