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Influenza A viruses are important human pathogens resulting in periodic pandemic threaten, while nonstructural protein 1 of influenza A virus (NS1A) shields the virus against host defense as an immunosuppressor. Mutational inactivation of the dsRNA binding activity of NS1A highly attenuates virus replication. This study investigated the structural principles of dsRNA recognition by NS1A protein. The complex crystal of NS1A RNA binding domain (NS1A RBD) with dsRNA diffracted X-rays to 1.7 Å and was in space group *C*2 with unit cell dimensions of *a*=60.707 Å, *b*=57.218 Å, and *c*=83.709 Å. The crystal structure revealed that NS1A RBD forms a dimeric six-helical fold and used a dimeric anti-parallel helices $\alpha 2/\alpha 2'$ to recognize the major groove of the dsRNA as a sequence-independent mode. The RNA helix adopted 40°bending towards the NS1A RBD at both ends of the helix to facilitate the RNA-protein interactions. The highly conserved residues within a positive patch, including R35, R37, R38, and K41 played the primary roles for dsRNA binding by hydrogen bonds and electrostatic interactions. Outside this positive patch, conserved residues, such as T5, D29, D34, S42 and T49, also contributed for dsRNA binding through hydrogen bonds directly or via water bridges. The significant conformational change of invariable residue R38 before and after NS1A RBD binding to dsRNA indicated that R38 played a key role for dsRNA binding by penetrating its side chain into dsRNA helix. The protein-RNA interactions observed from the crystal structure were further supported by the isothermal titration calorimetry assay of NS1A RBD and its mutants binding to dsRNA. Moreover, Agrobacterium co-infiltration assay suggested that arginine 38 may also play important roles for dsRNA binding *in vivo*.

Keywords: RNA-protein interactions, viral proteins, X-ray crystallography of biological macromolecules

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X-ray structure of a cysteine-less mutant galectin-1

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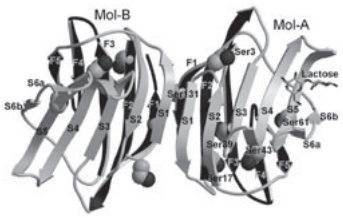
Galectin-1 (Gal-1) is a member of the beta-galactose binding animal lectin family, having a wide range of biological activity. We prepared a mutant Gal-1 (CSGal-1) where all Cys residues were replaced by Ser, because Gal-1 is susceptible to oxidation at Cys residues. To elucidate how the substitutions of amino acid residues affect the three dimensional structure of the protein, the X-ray structure of CSGal-1/lactose complex has been determined at 1.86 Å resolution. The monomer of GSGal-1 adopts a beta-sandwich structure formed by two anti-parallel beta-sheets. By dimerization of Mol-A and Mol-B, pairs of the same beta-sheets are connected to give two large anti-parallel beta-sheets, and a lactose molecule occupies the carbohydrate-bindindg site of Mol-A, as shown in a figure. The r.m.s. deviations for C-alpha atoms is 0.41 Å between CSGal-1 and the wild-type human Gal-1, and the interactions between protein and the bound lactose molecule are equivalent to each other, showing that two structures are almost identical. The substitution of six Cys residues for Ser does not affect the overall structure and the carbohydrate-binding site structure of the protein.



Keywords: X-ray structure, lectins, galactose-binding protein

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Far-red fluorescent protein mKate reveals pH-induced *cis-trans* isomerization of the chromophore

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The far-red monomeric fluorescent protein mKate (Lex = 588 nm, Lem = 635 nm), originating from wild-type red fluorescent progenitor eqFP578 (sea anemone *Entacmaea quadricolor*), is characterized by the pronounced pH dependence of fluorescence, relatively high brightness, and high photo-stability. The protein also demonstrates “kindling” phenomena- the increase of fluorescence brightness upon irradiation by excitation light. The protein has been crystallized at pH ranging from 2 to 9 in three space groups and four structures have been determined by X-ray crystallography at the resolution of 1.75 - 2.6 Å. The phenomenon of pH-dependent fluorescence of mKate has been shown to be due to reversible *cis-trans* isomerization of the chromophore phenolic ring. In the non-fluorescent state at pH 2.0, the crystal structure of mKate shows the chromophore in the *trans*- isomeric form. The weakly fluorescent state of the protein at pH 4.2 is characterized by a mixture of *trans* and cis isomers. The chromophore in a highly fluorescent state at pH 7.0/9.0 adopts the *cis* form. Three key residues, Ser143, Leu174, and Arg197, residing in the vicinity of the chromophore, have been identified as being primarily responsible for the far-red shift in the spectra. Structure-based single S158A amino acid mutation destabilizes the trans conformation of the chromophore, causing the annihilation of the kindling effect, with the concomitant increase of pH stability and brightness of the mKate_S158A variant. Analysis of the stereochemistry of the intermonomer interfaces has revealed a group of residues consisting of Val93, Arg122, Glu155, Arg157, Asp159, His169, Ile171, Asn173, Val192, Tyr194, and Val216, as being most likely responsible for the observed monomeric state of the protein in solution.

Keywords: fluorescent proteins, cis-trans isomerization, pH dependence of fluorescence

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Two threonyl-tRNA synthetases with complementary functions; Crystal structure of ThrRS-1

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To maintain high fidelity in translation, twenty kinds of aminoacyl-tRNA synthetases (ARSs) exist in general for twenty kinds of amino acids, each ARS being specialized to recognize only the cognate amino acid (A) and the cognate $tRNA^A$. Some organisms, however, have two genes for ThrRS, and considered that their proteins (ThrRS-1 and ThrRS-2) are complementary to each other in functions, one for catalysis and the other for editing. In order to clarify their three-dimensional structures, we started X-ray analyses of putatively assigned ThrRS-1 (APE0809) and ThrRS-2 (APE0117) from *Aeropyrum pernix* (*Ap*), and those (ST0966 and ST2187) from *Sulfolobus tokodaii* (*St*). These proteins were overexpressed in *E. coli*, purified, and crystallized. The crystal structure of *Ap*-ThrRS-1 has been successfully determined at 2.3 Å resolution, as the first example. *Ap*-ThrRS-1 is a dimeric enzyme composed of the two identical subunits, each containing two domains for the catalytic reaction and for the anticodon-binding. The essential editing domain is, however, completely missing as expected. These structural features are consistent with that ThrRS-1 catalyze only the aminoacylation of the cognate tRNA, and suggest the necessity of the second enzyme ThrRS-2 for editing. Since the N-terminal sequence of *Ap*-ThrRS-2 is similar to the sequence of the editing domain of ThrRS from *Pyrococcus abyssi*, *Ap*-ThrRS-2 is expected to catalyze de-aminoacylation of the misacylated serine moiety at the CCA terminus.

Keywords: aminoacyl-tRNA synthetase, crenarchaea, aeropyrum pernix

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The structure of archaeal ribosomal stalk complex

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Ribosome has a highly flexible lateral protuberance called the stalk at the GTPase-associated center on the large subunits. This ribosomal stalk is universally conserved in all domains of life and plays a crucial role for replenishing translation factors which catalyze translation reaction in a GTP-dependent fashion. The detailed studies of the archaeal ribosomal stalk complex of *Ph*P0(*Ph*L12)$_2$(*Ph*L12)$_2$(*Ph*L12)$_2$ from the hyperthermophilic archaean *Pyrococcus horikoshii* showed that it has the ability to access to eukaryotic elongation factors at the levels comparable to that of eukaryotic stalk (Nomura, 2006). Therefore, the archaeal heptameric stalk complex and the eukaryotic pentameric stalk complex apparently share conserved functional structures. In order to understand detailed structural and functional characteristics of archaeal ribosomal stalk complex, we solved the crystal structure of the archaeal ribosomal stalk complex from *P. horikoshii* at 2.3 Å resolution by Se-MAD method. This archaeal ribosomal stalk complex is composed of *Ph*P0(*Ph(*L12)$_2$(*Ph*L12)$_2$(*Ph*L12)$_2$ whose flexible C-terminal parts are truncated. Comparing with bacterial ribosomal stalk complex L10(L12)$_2$(L12)$_2$(L12)$_2$, the *Ph*P0 has longer and more flexible C-terminal helical spine composed of three helices with linker loops (helix-loop-helix-loop-helix). The manner by which the L12 dimers bind to the C-terminal spine are also very different.

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Keywords: structures of macromolecules, ribosomes, translation factors

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Successful cryocooling of protein microcrystalline samples for powder diffraction

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Modern developments of the powder diffraction technique have allowed the investigation of systems with large unit cells such as proteins [1]. Protein powder specimens consist of a large number of randomly oriented diffracting micro-crystals. These micro-crystals are usually formed rapidly by batch crystallization. Frequently, the resolution and quality of the data are limited mainly by rapid deterioration of the protein crystal structure during exposure to the intense synchrotron X-ray beam. In a typical single crystal diffraction experiment radiation damage can be minimized by collecting diffraction data under cryocooled conditions (typically 100 K) which requires the addition of a cryoprotecting agent to the protein sample to prevent freezing of the mother liquor. In this study, we succeeded in obtaining various cryocooled samples of human insulin at 100 K avoiding ice formation. Powder diffraction data were collected at both room temperature and under cryocooled conditions (ID31, ESRF, Grenoble, France). As expected both the cryoprotectant and the sample container have a remarkable impact on the data quality. Significant variation of the lattice parameters and peak widths with the type and concentration of cryoprotecting agent has already been observed and will be presented for the case of insulin. Preliminary data interpretation correlating these changes with the structural and microstructural characteristics of the systems under study will be shown.

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Keywords: protein, powder diffraction, cryocooling

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New development of frozen buffer-free crystal mounting method for the longer wavelength SAD phasing

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Using longer wavelength X-ray for the SAD phasing is one of the trends in macromolecular crystallography. At longer wavelength, the anomalous intensity difference or the Bijvoet ratio of almost all sulfur containing protein crystals is around 1% of total reflection intensity.