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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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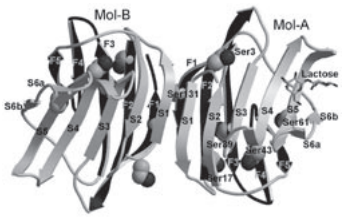
Galectin-1 (Gal-1) is a member of the beta-galactose binding animal lectin family, having a wide range of biological activity. We prepared a mutant Gal-1 (CSGal-1) where all Cys residues were replaced by Ser, because Gal-1 is susceptible to oxidation at Cys residues. To elucidate how the substitutions 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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