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Crystal structure of the ankyrin repeat domain of human ribonuclease L

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Introduction

In mammals, viral infections initiate an innate immune response predominantly mediated by type I IFNs. Type I IFNs regulate the transcription of a number of genes that inhibit or block viral replication through diverse mechanisms. There are three well-established antiviral pathways for interferon action: these involve the double-stranded RNA (dsRNA)-dependent protein kinase, PKR, the Mx proteins, and the 2-5A system, respectively.

In the 2-5A system, treatment of cells with IFN activates genes encoding several 2',5'-linked oligoadenylate synthetases (OASs) and a single gene encoding ribonuclease L (RNase L). The OASs are activated by binding to dsRNA, a frequent by-product of virus infection. The activated OASs generate 5'-triphosphorylated, 2',5'-phosphodiester-linked oligoadenylates (2-5A) from ATP. The 2-5A molecule functions by binding to RNase L, changing from an inactive monomer to a catalytically active homodimer. The activated RNase L cleaves RNA containing dyads of UU, UA, AU, AA, and UG. The RNA degradation inhibits protein synthesis and thus inhibits viral replication.

RNase L is an unusual ribonuclease in that it requires the activator molecule 2-5A to catalyze the hydrolysis of single-stranded RNA. 2-5A is itself very unusual, comprising a type of oligoadenylates with 2',5' internucleotide linkages, in contrast to the typical 3',5' linkages found in RNA and DNA. The human form of RNase L is a 741-amino acid protein with a molecular mass of 83,543 Da. RNase L consists of three domains, namely the N-terminal ankyrin repeat, the protein kinase homology, and the C-terminal ribonuclease domains. The N-terminal ankyrin repeat domain (ANK), a region containing nine ankyrin-like macromolecular recognition repeats (the ninth ankyrin repeat is incomplete), is responsible for 2-5A binding, and the C-terminal domain is responsible for catalytic activity. RNase L was thought at first to act to prevent virus replication, and recently the enzyme was proposed as a candidate risk factor for hereditary prostate cancer. Among the naturally occurring mutants of RNase L examined, only the Arg462Gln variant showed less RNase activity, and this variant also showed a significant association with prostate cancer. Therefore, elucidation of all the amino acid residues that could influence RNase L activities, i.e. 2-5A binding, homodimerization and catalysis, is urgently required.

Experimental

Crystallization of ANK was carried out in a way similar to the method described for non-tag ANK [1]. The data collection was performed by rotation method at 100 K using an ADSC Q210 CCD detector with synchrotron radiation (λ = 0.978 Å at beam line NW12 of the PF-AR, Japan). The crystals belong to an orthorhombic space group $P2_12_12_1$ with cell dimensions of a = 63.20 Å, b = 72.83 Å, and c = 82.63 Å ($\alpha = \beta = \gamma = 90°$). An assumption of one molecule per asymmetric unit leads to an empirically acceptable V_M value of 2.38 Å^3/Da, corresponding to a solvent content of 48 %. The best diffraction data from a native crystal were collected up to 1.8 Å resolution

Results and Discussion

We determined the crystal structure of the ANK / 2-5A complex by the molecular replacement method using the coordinate of "consensus ankyrin repeat protein" (PDB code: 1MJ0) as the template for constructing a search model and refined the resulting model to an R-factor of 0.202 (R_{free} of 0.230) at 1.8 Å resolution. The final model consists of residues 21-305, 220 water molecules, and one 2-5A molecule.

ANK folds into eight ankyrin repeat elements and forms an extended curved structure with a groove running across the long concave surface. Previous primary structure analysis suggested that RNase L had nine ankyrin repeats, but the ninth ankyrin repeat is incomplete. However, this prediction for RNase L differs from the present crystal structure of ANK, which consists of eight ankyrin repeats. Residues 306 to 333, corresponding to the incomplete ninth repeat, are disordered. As in other ankyrin repeat proteins, each repeat is formed by ~33 amino acid residues and consists of pairs of antiparallel α-helices stacked side by side and connected by a series of intervening β-hairpin motifs. The 2-5A molecule is accommodated in the concavity and directly interacts with ankyrin repeats 2 to 4. The structural detail for the recognition of 2-5A by ANK will be described elsewhere [2].

References

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