Crystal Structure of the Complex RNase Sa – Barstar at 1.7 Å Resolution

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Protein-protein recognition is fundamental for understanding of many biological processes. For those studies several types of complexes are used e.g. oligomeric proteins, antigen-antibody or enzyme-inhibitor complexes. Structural analysis of the interfaces and intermolecular contacts of proteins in complexes reveal variations between different types of complexes, which may reflect their different biological roles. The most information about enzyme-inhibitor complexes has been obtained from the study of proteases and their inhibitors. Another system suitable for study of protein-protein recognition is provided by ribonucleases and their inhibitors.

Microbial ribonucleases are secreted by various strains of microorganisms. They serve probably as digestive enzymes or bacterial toxins. Some of microorganisms produce an intracellular protein inhibitor of their ribonuclease to prevent its being active prior to the secretion, which would be lethal for the cell.

Bacillus amyloliquefaciens produces a ribonuclease barnase and also its specific inhibitor barstar (Hartleve et al 1972). Both are small single chain proteins. Barnase has 110 amino acid residues and no cysteines. Barstar has 89 amino acids from which two are cysteines. They are buried in the molecule and do not form a disulphide bridge (Lubienksi et al 1996). Barstar binds tightly to barnase, forming a one-to-one non-covalent complex with dissociation constant 6 x 10^-14 M (Hartlev 1993). The presence of cysteines caused some problems at crystallisation and therefore a double mutant barstar (C40,82A) was used for preparing crystals of barnase-barstar complex (Guillet et al 1993, Buckle et al 1994). The structure and activity of wild type and mutant barstar are similar. The mutant barstar binds to barnase slightly weaker than wild type barstar, with dissociation constant 2 x 10^-13 M. The crystal structure of the complex barnase-double mutant barstar was solved independently at 2.6 Å (Guillet et al 1993) and 2.0 Å resolution (Buckle et al 1994).

Barstar binds to and inhibits also a ribonuclease RNase Sa, a microbial ribonuclease produced by Streptomyces aureofaciens (Hartleve et al 1996). RNase Sa belongs to the same family of enzymes as barnase. Its polypeptide chain consists of 96 amino acids and contains one disulphide bridge. The 3-D structure of free enzyme and its complexes with nucleotide inhibitors has been solved at high resolution (Sevcik et al 1991, 1993a, 1993b, 1996). The sequence identity of RNase Sa and barnase is low (23%) but the active sites of the two enzymes are similar. The key residues of barnase which are responsible for barstar binding are present also in RNase Sa. The dissociation constant of RNase Sa-wild type barstar complex (K_D = 10^-10 M) is four orders of magnitude higher than that of barnase-barstar complex (Hartley 1996). Crystals of the complex of RNase Sa with wild type barstar were prepared, the structure was solved at 1.7 Å resolution and the contacts between the two proteins have been analysed in details. Streptomyces aureofaciens also...
produces a specific ribonuclease inhibitor. Expression of its gene is in hand (Krajcikova et al. 1998). For crystallisation of the RNase Sa-barstar complex for the first time, wild type barstar was used. Crystals were grown by vapour diffusion using a hanging drop. Both proteins were mixed in a one-to-one ratio without further purification of the complex. Final concentration of both proteins in the drop was 10 mg/ml in 0.1 M cacodylate buffer, pH 7.0-7.4. As a precipitant, ammonium sulphate at a concentration of 14–15% in the drop and 28–30% in the well solution was used (Urbánková & Ševčík 1998). Crystals grew in hexagonal space group P6_3. The asymmetric unit contained one molecule of the complex. X-ray data were collected with synchrotron radiation at EMBL Hamburg. The structure was solved at 1.7 Å resolution by molecular replacement. Refinement converged with R factor of 16.2% (Sevcik et al. 1998).

Mechanism of inhibition of RNase Sa by barstar is similar to that found in barnase-barstar complex by steric blocking of the active site. One of the barstar helices is positioned in the active site groove and together with its adjacent loop completely blocks the active site. As a result of complex formation, 1300 Å^2 of solvent accessible surface area is buried in the interface. 14 amino acid residues from barstar and 14 from RNase Sa form about 120 van der Waals contacts. Barstar residues involved in binding, except Glu 76, are located on continuous polypeptide chain (residues from 29 to 44). There are 14 hydrogen bonds (shorter than 3.25 Å) between RNase Sa and barstar. Seven of them are only "half" bonds as they are formed by residues with two conformations. All RNase Sa residues which are important in binding and cleaving substrate, except Glu 54, form hydrogen bonds with barstar.

The shape complementarity of the two proteins is not perfect, which results in a cavity which is filled with several water molecules. These form an additional network of hydrogen bonds. There are a total of 11 water molecules in the cavity involved in hydrogen bonds that link the two molecules. Similar network of hydrogen bonds mediated through water molecules was found also in barnase-barstar complex.

The area of the interface, the number of van der Waals contacts, direct hydrogen bonds and hydrogen bonds mediated through water molecules in RNase Sa-barstar complex were compared with those in barnase-barstar complex. These differences rationalise in general terms why the dissociation constant of the RNase Sa-barstar is larger than that of the barnase-barstar complex, i.e., the non-natural RNase Sa-barstar complex is less stable.

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**Ribonuclease Inhibitor from Streptomyces aureofaciens**

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An existence of natural protein ribonuclease inhibitor was discovered in 1952, when in guinea pig liver homogenates a latent RNase activity was observed (Pirotte and Desreux 1952) Ribonuclease activity could be revealed after acidification of the extract as a result of dissociation of RNase inhibitor complex. Since then it has been shown, that an occurrence of ribonuclease inhibitor seems to be widespread (Lee and Vallee 1993). It has been detected in various mammalian tissues, nonmammalian organisms, fungi and bacteria as its free form or in the complex with endogenous ribonucleases. In spite of its unambiguous role in RNA metabolism, a definite function for this protein is still not known with certainty (Hofsteenge 1997, Lee and Vallee 1952). Though a number of results have been obtained following different aspects of ribonuclease inhibitor occurrence, no experiments focused on its biological function have been published. Hofsteenge summarised...