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THE STRUCTURE OF C-TERMINAL MEROZOITE SURFACE PROTEIN 1, A MALARIA VACCINE CANDIDATE, IN COMPLEX WITH A MONOCLONAL ANTIOBODY

G. A. Bentley[1] J. C. Pizarro[1] V. Chitarra[1] D. Verger[1] S. Longacre[2] I. Holm[2] F. Nato[3] S. Dartevelle[3] S. Petres[3]
[1]Unite DImmunologie Structurale, Institut Pasteur 25 Rue Du Dr. Roux PARIS 75724 FRANCE [2]Unite dImmunologie Moleculaire des Parasites, Institut Pasteur [3]Plateux Technique 5 - Genomique Structurale, Institut Pasteur

The search for a malaria vaccine has been directed mainly towards combating *Plasmodium falciparum*, the species responsible for most of the morbidity and mortality arising from the disease in man. Proteins exposed on the surface of the *Plasmodium merozoite*, the erythrocyte-invasive form of the parasite, are prime candidates for vaccine development since many of these proteins are targets of the humoral immune response from persons with naturally acquired immunity. The most abundant surface protein of *P. Falciparum* is Merozoite Surface Protein 1 (PfMSP1), a 195 kDa molecule attached to the membrane by a glycosyl-phosphatidylinisotol (GPI) anchor. The protein undergoes proteolytic maturation that ultimately leaves only the C-terminal 11 kDa segment, PfMSP1-19, attached by the GPI moiety to the surface of the invading parasite. Vaccination trials in animal model systems have shown that immunisation with PfMSP1-19 can lead to protective immunity. These observations, and the limited polymorphism of PfMSP1-19 (less than 5% between different strains), have made this naturally occurring polypeptide fragment a leading vaccine candidate. In order to gain further understanding of the nature of immune protection given by this antigen, we are engaged in structural studies of PfMSP1-19 and its complexes with specific monoclonal antibodies (mAb). Here, we report the crystal structure of the complex formed between PfMSP1-19 and the Fab fragment of the murine monoclonal antibody G17.12, determined by molecular replacement and refined at 2.9 Å resolution. The mAb recognised a discontinuous epitope on the first EGF domain of PfMSP1-19.

Keywords: MALARIA VACCINE CANDIDATE FAB COMPLEX IMMUNOLOGY

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SOLVENT CONTENT IN THE ANTIGEN-ANTIBODY INTERFACE AS FOUND IN THE LYSOZYME-HyHEL-5 COMPLEX

G. H. Cohen D. R. Davies
[1]National Institutes of Health

The high resolution, 1.7 å structure, of the complex of lysozyme with the fab hyhel-5, r(work) = 0.189, r(free) = 0.231, has been determined. We have examined the interactions between the components of the complex and the contribution made by solvent molecules bound in and around the antigen-antibody interface. A total of 29 solvent molecules are incorporated in the interface and its immediate surroundings. The solvent molecules within the interface bridge the few imperfect contacts between the protein surfaces. The solvent molecules surrounding the interface contribute to the sealing of the edge of the interface and may also constitute a small extension of the interface areas. These 29 solvent molecules appear to represent a loss of solvent molecules in comparison to the uncomplexed components of the interface whose solvent content is estimated at 40 or more. These results may be compared with the findings of willson in a thermodynamic study of the complex of bobwhite quail lysozyme with hyhel-5 [Xavier, K. A., Shick, K. A., Smith-Gill, S. J. & Willson, R. C. 1997. Biophys. J., 73:2116-2125]. Their work suggests that an uptake of a minimum of about 6-12 water molecules may occur on complex formation.

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CASPASE ACTIVATOR HUMAN GRANZYME B, CRYSTAL STRUCTURE AND IMPLICATIONS IN APOPTOSIS

E. Estebanez-Perpina P. Fuentes-Prior D. Belorgey K. Maskos R. Kiefersauer M. Braun W. Bode H. Rubin R. Huber
Max Planck Institut Fuer Biochemie, Martinsried Structural Biology (Abt. Prof. Robert Huber) Am Kloferspitz 18A , D-82152 Martinsried Bei Planegg, Munich MUNICH D-82152 GERMANY

Granzyme B, together with the caspases and the Bcl-2 family members, plays an important role in eliciting apoptosis in virus-infected and tumor cells. The substrate specificity of Granzyme B is unusual for a serine proteinase, as it cleaves peptide bonds after aspartyl residues. The major structural element responsible for such substrate specificity is Arg226, which is anchored at the back of the S1-specificity pocket. The architecture of the substrate binding site of Granzyme B nicely explains the cleavage of hexapeptides such as the sequences Ile-Glu-Thr-Asp'-Ser-Gly and Ile-Glu-Ala-Asp'-Ser-Glu present in the activation site of pro-caspase-3 and Bid, respectively, proven physiological substrates of Granzyme B. Our crystal structure of recombinant human Granzyme B unexpectedly revealed a dimer, mediated by the interdigitation of oligosaccharide chains attached to Asn65 in the two monomers. This structural finding, together with observations that binding and uptake of Granzyme B in target cells is effected by the cation-independent mannose-6-phosphate (M6P) receptor, and that receptor dimerization is an essential element of the internalization mechanism, suggest that the glycosylated Granzyme B dimer would be the form preferentially recognized by its receptor. To investigate the probable binding mode of Granzyme B to its cell receptor we have modelled the binding of the Granzyme B dimer to the M6P-receptor domains -3 and -9 that mediate M6P-recognition.

Keywords: GRANZYMES, CRYSTALLOGRAPHY, APOPTOSIS

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STRUCTURE OF THE ACTIVE FORM OF THE CATALYTIC REGION OF THE COMPLEMENT PROTEASE C1R

V. Harmat[1] J. Kardos[2] P. Gal[2] O. Barabas[1,2] L. Graf[3] G. Naray-Szabo[1] P. Zavodszky[2]
[1]Lorand Eotvos University Department of Theoretical Chemistry Pazmany Peter Setany 1A BUDAPEST H-1117 HUNGARY [2]Institute of Enzymology, Hungarian Academy of Sciences, Budapest, Hungary [3]Lorand Eotvos University, Dept. of Biochemical Chemistry, Budapest, Hungary Budapest, Hungary

The complement system is a major effector arm of the immune defense. Its activation results in the destruction and clearance of invading pathogens. The first component of the complement system (C1) plays an essential role in the molecular immune response as it triggers the classical pathway of the activation of the complement system. C1 is a supramolecular complex, consisting of one C1q, two C1r and two C1s subunits. C1r and C1s are modular serine proteases, responsible for the enzymatic activity of C1. C1r is the key component of the complex since it makes a dimer, forming the core of the C1r(2)-C1s(2) tetramer and it is capable of self activation. The structure of the proenzyme form of C1r was determined to 2.9 Å resolution, however the detailed structural bases of the unique autoactivation and the very narrow substrate specificity of this serine protease are still unknown. We collected a 2.3 Å resolution dataset at ESRF from a crystal of the catalytic region of C1r (space group $P2_1$). The phase problem was solved with molecular replacement. The asymmetric unit contains two dimers of C1r. Both dimers represent head to tail packing, however they differ in the relative orientations and contact surfaces of the monomers. The observed interdomain flexibility at the junction of the CCP1 and CCP2 domains of C1r in its dimer can have an essential role both in the autoactivation mechanism and in the C1s activation.

Keywords: COMPLEMENT ACTIVATION, MULTIDOMAIN SERINE PROTEASE, INTERDOMAIN FLEXIBILITY