THE MECHANISM OF NEUTRALIZATION OF INFLUENZA VIRUS INFECTIVITY BY ANTIBODIES

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The Haemagglutinin (HA) is the influenza surface glycoprotein that interacts with infectivity neutralizing antibodies. As a consequence of this immune pressure, it is the variable virus component important in the antigenic drift that results in recurrent epidemics of influenza. We have determined the crystallographic structure of three complexes formed between the Fab fragments of infectivity neutralizing antibodies and HA and the mechanism of neutralization of virus infectivity by these antibodies. In addition, measurements of the affinities for an antibody of mutants that escape neutralization by this antibody show that a 1000-fold affinity decrease is associated to escape from neutralization of infectivity. The antibodies, which are representative of the range of antibodies that react with haemagglutinin, differ in their sites of interaction with haemagglutinin and in their abilities to interfere *in vitro* with its two functions of receptor binding and membrane fusion. Two of them overlap with the receptor-binding site and block access to the site while the third is distant from this site. The three antibodies also differ in their abilities to prevent the structural transition of HA that is required for fusion of virus and cellular membranes, one of them blocks this transition, the other two do not. We demonstrate that despite these differences all three antibodies neutralize infectivity by preventing virus from binding to cells. Neutralization occurs at an average of one antibody bound per four haemagglutinins, a ratio sufficient to prevent the simultaneous receptor binding of haemagglutinins that is necessary to attach virus to cells.

Keywords: VIRUS-NEUTRALIZATION INFLUENZA ANTIBODY

N-GLYCOSYLATION OF THE Fc-fragment of human IgG1 IS CRUCIAL FOR ITS STRUCTURAL INTEGRITY AND Fcg-RECEPTOR AFFINITY

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Fcg-receptors (FcgRs) are expressed on all immunologically active cells. Immune complexes consist of antigens coated with IgG-antibodys. They trigger immunological effects like endocytosis via binding of the Fc portion of the IgG to FcgRs. The Fc-fragment (Fc) is a homodimer which resembles the shape of a horse shoe. Both chains are associated via the CH3 domains. FcgR binds to the Fc-fragment at the glycosylated CH2 domains on the tip of the horseshoe. The structure of the FcgRIII-Fc complex shows that the carbohydrate moieties are located in the periphery of the FcgRIII-Fc interface and barely contact the receptor. However recent studies have shown that natural N-glycosylation of Fc is important for FcgR-binding. To investigate the role of individual monosaccarides for Fc conformation we determined the structures of wild type and consecutively truncated glycovariants of Fc. 9 sugars per chain were traceable in the electron density of native Fc. When only 6 sugars were present in the carbohydrate we found conformational changes in the carbohydrate and in the loop containing the N-glycosylation site. This affects the interface between Fc-fragment and FcgR and decreases binding affinity as previously shown by Isothermal Titration Calorimetry. Furthermore we observed that stepwise deglycosylation permits an approach of CH2 domains leading to a higher probability of closed states compared to open states which do favour FcgR binding. This investigation relates the glycosylation pattern of antibodies to the molecular basis of receptor affinity which may be applicable to optimise the activity of therapeutic IgGs.

Keywords: FC-FRAGMENT, GLYCOSYLATION, AFFINITY FOR FC-RECEPTOR

STUDIES OF NATURAL LIGANDS OF CHEMOKINE RECEPTOR CCR6

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Since their discoveries, chemokines and defensins have been associated with substantially different biological activities, and thus different natural functions. Chemokines are mostly recognized for their immunoresponsive properties during inflammatory processes, and their activities are invariantly linked to potent yet modestly specific interactions with helical, seven-transmembrane G-protein coupled receptors. Defensins, in turn, are members of an ancient, innate immunological aparatus and have strong antimicrobial properties. Both families of proteins are the subjects of numerous studies. Chemokines and their receptors are the targets of many therapeutic strategies while defensins are potentially excellent candidates to extend the endangered pool of therapeutically effective antibiotics. The major distinction between the biological role of both families of proteins has recently been questioned. It was shown that human β -defensins have strong chemotactic properties; comparable to chemokines, and that they specifically interact with chemokine receptor CCR6. Only one chemokine, MIP3a, has been identified as the ligand of CCR6, and this high specificity is quite unusual for chemokine receptors or chemokines. In order to investigate the possible structural basis of this unique similarity of properties of β-defensins and MIP3a, we solved the crystal structures of hBD1, hBD2, and MIP3a. We also studied the bacteriocidal and chemotactic properties of these three proteins, their aggregation state in solution and interactions with artificial lipid vesicles. The results of our work have allowed us to propose correlations between the structural features of these proteins and their multiple biological properties.

Keywords: DEFENSINS, CHEMOKINES, IMMUNOLOGY

CRYSTALLOGRAPHIC STUDIES OF DECAY-ACCELERATING FACTOR (CD55)

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Decay Accelerating Factor (DAF) is a cell surface complement regulatory protein that protects normal host cells from the destructive action of Complement. By binding many bacterial and viral proteins, DAF also provides a route by which pathogens can enter the cell. We have used X-ray crystallography to gain structural information about a DAF fragment encompassing all four sushi domains (DAF1-4). Crystals were obtained from E. coli expressed DAF1-4. Room temperature diffraction testing of crystals showed diffraction to 3.5Å. It was not possible to collect a full data set due to crystal degradation during the course of the experiment. It was anticipated that these problems might be resolved by cryoprotection. Crystals were cryoprotected by a variety of protocols using a wide range of cryo-protectants and data collected at a synchrotron source (SRS, Daresbury). All protocols proved to be detrimental to crystal quality as revealed by the presence of smeared diffraction spots. To circumvent this problem, crystals were grown in the presence of glycerol as a cryoprotectant. These crystals yielded data to a resolution of 2.8Å (ESRF, Grenoble). Data analysis revealed that several crystal forms had been obtained under identical crystallisation conditions. The crystal forms were of space group P1 with variable unit cell dimensions and variable numbers of molecules per unit cell. This multiplicity makes molecular replacement using the structure for the bottom two domains we have recently solved (see poster by Williams et al this meeting) difficult, but we are now pursuing structure solution using selenomethionine substitution.

Keywords: DECAY ACCELERATING FACTOR, COMPLEMENT REGULATOR, CRYSTALLISATION