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The crystal structure of the active domain of Anopheles anti-platelet protein, a powerful anti-coagulant, in complex with an antibody

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1. Introduction

Blood clotting is a vitally important process that must be carefully regulated to prevent blood loss on one hand and thrombosis on the other. Severe injury and hemophilia may be treated with pro-coagulants, whereas risk of obstructive clotting or embolism may be reduced with anti-coagulants. Nature provides a number of examples of anti-coagulant proteins produced by blood-sucking animals, which may provide templates for the development of new small molecules with similar physiological effects. We have therefore studied an anti-platelet protein (AAPP) from a malaria vector mosquito, and report its crystal structure in complex with an antibody.

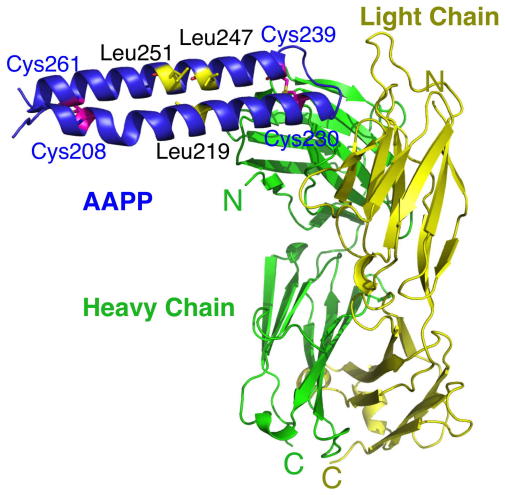
2. Experiment, Results and conclusion

AAPP was expressed in *E.coli*, and full-length AAPP can be readily expressed in a soluble form to a level of about 3 mg per liter of culture by overnight expression at 15ºC. A simple procedure to remove the affinity tag followed by gel filtration yields samples that appear pure by gel electrophoresis. A monoclonal antibody which made from mice was therefore raised against full-length AAPP and the AAPP-8H7 Fab complex was subjected to crystallization trials. Thin, in condition with 0.1 M HEPES pH 7.0, 15% PEG 20K, needle-shaped crystals were obtained. Crystals grew in space-group $P2_12_12_1$, with a=93.8 Å, b=99.4 Å, c=166.0 Å and contained two molecules in the asymmetric unit, and which diffracted to 1.8 Å resolution. Phases were obtained by molecular replacement the previously reported Fab structure as a starting model. This allowed a model of AAPP to be built from Tyr 202 to Glu 269, but no further ordered residues appear in the structure (Fig. 1). The 8H7 Fab structure shows the classical IgG domain structure of anti-parallel b-sheet sandwiches with an antigen binding pocket formed from loops on both the heavy and light chains. It interacts solely with the turn region of AAPP, so that the helical region points away from the antibody. Only AAPP residues from Glu 228 to Cys 239 make contact with the 8H7 Fab, but these turn residues make very close contact, including several salt bridges and hydrogen bonds. The SS bond formed between Cys 230 and Cys 239 also comes within van der Waals distance of Tyr 32 of the heavy chain. Experiments were carried out to determine whether the 8H7 Fab can block this interaction. To this end, AAPP was pre-incubated with the 8H7 Fab or whole IgG, and the binding ability of AAPP to immobilized soluble collagen type I was

assessed by a plate assay. Free AAPP effectively bound to soluble collagen in a concentration-dependent manner, whereas both 8H7 Fab and whole IgG significantly inhibited the interaction in a dose-dependent manner.

Overall the protein is extremely sensitive to proteolysis, but the crystal structure reveals a stable domain built from two helices and a turn, which corresponds to the functional region. Our work therefore opens new avenues to the development of both novel small molecule anti-clotting agents and anti-malarials.

A



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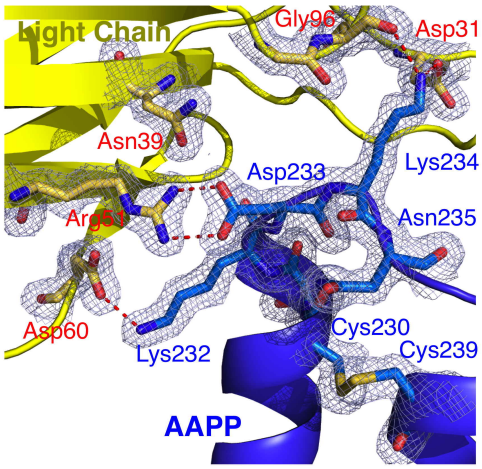

Figure 1. The structure of AAPP-Fab complex. (A) Cα trace of AAPP bound to the 8H7 Fab, with α-helices shown as coils and β-strands shown as arrows. The AAPP is shown in purple, with the cysteine residues marked. The disulfide bonds are shown in red. Light chain (yellow) consists of residues 1-218 and, heavy chain (green) consists of residues 1-215 of 8H7 Fab. (B) The 2mFo-DFc electron density map (contoured at 1σ) showing the interaction between AAPP and the 8H7 Fab. Residues involved in binding are shown as sticks, with carbon atoms colored blue for AAPP and yellow for the 8H7 Fab light chain. Hydrogen bonds are shown as red dotted lines.

3. Acknowledgement

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Reference

[1] Sugiyama K, Iyori M, Sawaguchi A, Akashi S, Tame JR, Park SY, Yoshida S. The Crystal Structure of the Active Domain of Anopheles Anti-platelet Protein, a Powerful Anti-coagulant, in Complex with an Antibody.

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