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Anti-HIV Proteinase Monoclonal Antibody F11.2.32 that Inhibits Enzyme Activity

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Introduction

The hybridoma that produce inhibitory monoclonal antibody (mAb) termed F11 2 32 originate from mice immunized with recombinant proteinase of HIV-1 This mAb belongs to IgG1 isotype, and its binding and inhibitory properties are also preserved in the corresponding Fab fragment HIV-1 protease is a homodimeric enzyme belonging to the family of aspartyl proteinases The monomer comprises 99 amino acid residues containing a triplet AspThrGly which is located near the dimer interface Thus, in the functional homodimer the two amino acid triplets are adjacent to each other, forming a pepsine-like catalytic site at the bottom of a hydrophobic cavity

The catalytic site is covered by two flap regions, one contributed by each subunit, which undergo substantial movement during binding the substrate

Epitopes

Peptide segments 10-11 residues long, and spanning the whole HIV-1 protease sequence, were tested previously for their ability to inhibit the binding of IgG F11 2 32 to HIV-1 protease F11 2 32 mAb has been found to be reactive to peptide MSLPGRWKPKM (positions 36-46) of HIV-1 PR The F11 2 32 epitope relates to flap region of the enzyme This region is involved in the substrate binding and undergoes a substantial steric transition in each turn of the catalytic cycle To our knowledge, neither flap-reactive mAbs, nor flap-targeted inhibitors have been described up to now The inhibitory effects found for mAb F11 2 32 remain compatible with several candidate mechanisms (e g interference with the flap movement, indirect distortion of the active site, dissociation of protomers)

Inhibition

"Titration" experiment with the flap-specific Fab F11 2 32 was carried out in analogy with conventional (low molecular weight) inhibitors, but at conditions favourable for antibody binding The K_{inh} is 35 ± 2 4 nM, whereas K_d = 4 8nM was measured by surface plasmon resonance using the BIAcore system (Pharmacia Biosensor)

Structure

Crystallographic studies were successfully concluded with Fab F11 2 32 in free state and complexed with the proteinase epitope peptides (Lescar *et al* 1996, Lescar *et al* 1997)

Crystallographic data from measurements on crystals of Fab F11 2 32, Fab F11 2 32-(peptide 36-46) and Fab F11 2 32-(peptide 36-57) are in Table 1

Table 1.

Crystal form	Space group and unit cell	resolution	completeness and redundancy of data measurements
Fab F11 2 32	$P2_1$ a = 68 9 Å b = 96 4 Å c = 70 6 Å β = 105 4° Z = 4 V_m = 2 2 Å^3/Da	2 6 Å	90 9% 2 1
Fab F11 2 32/ Peptide 36-46	$P2_12_12_1$ a=82 4 Å b=96 3 Å c=105 8 Å Z=8 V_m= 2 1 Å^3/Da	2 2 Å	99 5% 4 7
Fab F11 2 32/ Peptide 36-57	$P2_12_12_1$ a=82 3 Å b=97 7 Å c=52 9 Å Z=4 V_m=2 1 Å^3/Da	2 6Å	99 7% 5 2

Specific clues for structural basis of the inhibition are provided, namely with the crystal structure of complex Fab F11 2 32 peptide 36-46 The refined model of the complex reveals ten well-ordered residues of the peptide (P36-P45) bound in a hydrophobic cavity at the centre of the antigen binding site The peptide adopts a β hairpin-like structure in which residues P38-42 form a type II β-turn conformation An intermolecular antiparallel β-sheet is formed between the peptide and CDR3-H loop of the antibody, additional polar interactions occur between main chain atoms of the peptide and hydroxyl groups from tyrosine residues protruding from CDR1-L and CDR3-H Three water molecules, located at the antigen-antibody interface, mediate polar interactions between the peptide and the most buried hypervariable loops CDR1-L and CDR3-H A comparison between the free and complexed Fab fragments shows that significant conformational changes occur in the long hypervariable regions, CDR1-L and CDR3-H , upon binding the peptide The conformation of the bound peptide, which shows no overall structural similarity to the corresponding segment in HIV-1 protease, suggests that F11 2 32 might inhibit proteolysis by distorting the native structure of the enzyme

Conclusions

The tested mAb is meant to serve as, "lead compound" for constructing alternative (non-active-site) inhibitors of lower molecular weight Several aspects of our findings are encouraging the observed inhibition is excellent, approach to its structural basis seems to be open in principle, and situation with the flap-specific mAb F11 2 32 is greatly simplified due to predominant involvement of a single CDR in the complex formation Even here, however, the development of mAb mimetics has proved to be far from trivial, since a simple peptide version (or cyclic peptide version) of CDR3-H does not display any inhibitory effects The main advantage of possible potent non-active site inhibitors could be seen in different mechanisms of development of resistance to them

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