CHAPTER 5

CONCLUSION

Since the HIV MA protein plays a key role in many steps of the HIV life cycle, the MA protein is considered as a target of choice for the design of anti-HIV-1 antibodies with high neutralization efficiency. Previously, the scFv-MA HB-8975 has been used to negatively interfere with the viral replication within HIV-1 infected cell *in vitro*. Therefore, this antibody could be useful in biotechnological and therapeutic applications. The aims of our study were: (i) to characterize the key amino acid residues involved in the binding interaction of scFv-MA HB-8975 with HIV-1 MA epitope, using a computational approach and a structure-based analysis; (ii) to engineer the display scFv-MA HB-8975 on the surface of baculovirus, using a novel leader peptide; and (iii) to apply the epitope specificity of the anti-MA mAb HB-8975 to the development of a simplified enzyme-linked immunosorbent assay (ELISA) for the HIV-1 protease (HIV-PR) activity.

(i) Computational assisted modeling and structure-based analysis were combined with experimental data obtained in our laboratory for an efficient investigation on the most important residues involved in the binding of scFv-MA HB-8975 to HIV-1 MA natural peptide substrates. The 3D structure of scFv-MA HB-8975 was built based on its X-ray structure homologue, and the complexes of scFv-MA HB-8975 with HIV MA natural epitope peptides were generated using a flexible docking method. The potential mean force (PMF) score calculated by molecular

docking method demonstrated the high efficacy since the PMF value correlated well with peptide ELISA results. Moreover, MM-PBSA and pairwise decomposition energies were calculated to determine the binding free energy of interacting residues in CDR regions of scFv-MA HB-8975 with peptide p17.1 The calculated binding free energies could indicate the key residues which exhibited the high binding affinity (decomposed energy >2 kcal/mol). Several important residues, MET100, LYS101, ASN169, HIS228, and LEU229, located in the CDR regions of scFv-MA HB-8975 were thus indentified, as they significantly contributed in the binding efficiency with natural HIV-1 MA epitope at the C terminus. Furthermore, site specific mutagenesis at position 100 in the scFv sequence provided evidence that the relevant data from computational study were trustworthy. This technique could be applied to determine which amino acids were the most important for the binding of scFv with its target molecules, and to modify the antibodies with optimized affinity and specificity by performing site-specific mutagenesis. Moreover, the generation and screening of efficient scFv from a large virtual combinatorial library based on the computational approaches could serve as a potential strategy for development of powerful therapeutic agents.

(ii) We identified a novel N-terminal octadecameric peptide sequence, N18E2, which mediated the plasma membrane addressing and anchoring of scFvE2/MA into the baculoviral envelope, and acted as a BV-envelope display signal. N18E2 could therefore be used in a general technology for BV-display of bioactive molecules such as scFv. In this study, the expression of scFvE2/MA containing novel leader sequence in Sf9 cells resulted in two different forms, soluble scFvE2/MA

protein and BV-displayed scFvE2/MA. Both scFvE2/MA formats retained the binding activity which was present in the parental antibody. Furthermore, we provided evidence that another scFv, scFv-M61B9, was also functional when displayed on the envelope of the BV vector. The baculoviral envelope glycoprotein GP64 is a low specificity attachment protein which allows BV to enter a wide variety of cells originating from mammalian or non mammalian species (Granio et al., 2009). Consequently, baculovirus display antibody would be applicable as a gene delivery vector for scFv that acts as intrabody in human gene therapy.

(iii) We showed that the particular interaction of anti-MA mAb HB-8975 with the free C-terminus of the HIV-1 MA requires the proteolytic activity of HIV-PR and cleavage of the peptide bound between the MA and CA domains. This perquisite could be applied to establish the HIV-PR activity assay, namely ELIB-PA. This assay provided an effective investigation on HIV-PR activity *in vitro* when two specific monoclonal antibodies, anti-MA mAb HB-8975 and anti-CA mAb G18, directed towards different motifs and domains in the HIV-PR substrate, H_6MA-CA, were successfully applied for detection. The complete inhibitory effect of LPV demonstrated the reliability of ELIB-PA for discriminating HIV-PR activity. Additionally, the efficacy of three HIV-PR inhibitors i.e. LPV, RTV and NFV could be evaluated using ELIB-PA by determining the IC_{50} and the acceleration of inhibition efficiency. Accordingly, the establishment of ELIB-PA could be valuable in high-throughput screening and identifying bunch of novel candidate HIV-PR inhibitors from the computerized drug-design. This will improve and accelerate the drug discovery process in the pharmaceutical-industry.