Selection of Ankyrin Targeting HIV-1 Matrix and Identification of Its Binding Domain

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ABSTRACT

Ankyrin repeat protein is a novel class of non-antibody binding protein that can be applied as an alternative antiretroviral agent. Engineered ankyrin targeting the HIV-1 matrix (MA) would be a promising agent to interfere with HIV replication, since MA plays a major role in multiple processes of the viral life cycle. In this study, MA-specific ankyrin (AnkGAGG31) was isolated from an artificial ankyrin library using a semi-automated selection process with biotinylated MA-streptavidin magnetic beads. The AnkGAGG31-recognition site on MA was determined using both indirect and competitive ELISAs with overlapping MA tri-helical fragments and pentadecapeptides. The AnkGAGG31-recognition site on MA was determined using both indirect and competitive ELISAs with overlapping MA tri-helical fragments and pentadecapeptides. The AnkGAGG31 showed the highest binding signal to the MA-fragments covering helices 2-3-4 and peptides corresponding to helix 2 (residues 25-43), which were found as the target epitope. This finding was further analyzed by molecular modeling and docking. The rational models of AnkGAGG31-MA complex indicated that the strong binding interaction was shown on helix 2 at key residues K27MA, K30M, and K32MA. Taken together, the identification of the binding domain on the MA target improves our understanding of the AnkGAGG31-MA interaction and provides the information necessary to design innovative protein targeting of the MA protein.

Keywords: Ankyrin, Binding domain, HIV-1 matrix, MA protein, Phage-displayed panning