A Conformation of the Loop Region in the *De Novo* Designed Helix-loop-helix peptide

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Phage-displayed library is a powerful method to obtain peptides binding to enzymes and receptors. The high affinities might be at least partially caused by the interaction between conformationally constrained peptides and the target proteins, due to a low loss of entropy upon binding [1]. We have constructed a *de novo* designed helix-loop-helix peptide as a novel scaffold for phage-displayed peptide libraries. Amino acids on the helix were randomized to give a library, which was screened against cytokine receptors (e.g. G-CSF). In addition, we randomized amino acid residues on the loop region to construct a phage-displayed library (AELAALEAELAALE-GX_5G-KLAALKAKLAALKA; X is the randomized position). However, repeated selections of the library against integrin$\alpha_5\beta_1$ resulted in no enrichment of phage particles displaying RGD (Arg-Gly-Asp) motif; the RGD motif is often observed in the binding peptides obtained from the other phage-displayed libraries of liner or cyclic peptides. Consequently, it is inferred that the loop region was too constrained to form β-turn conformation for RGD motif in binding to integrin$\alpha_5\beta_1$ [2]. In this work, we examined to elongate the loop region to construct new libraries.

We constructed three novel loop libraries on the coat protein pⅧ of filamentous phage: L-lib9 (AELAALEAELAALE-GX_7G-KLAALKAKLAALKA), L-lib11 (AELAALEAE LAALE-GX_9G-KLAALKAKLAALKA) and DR-library (AELAALEAELAALE-GX_5G-KLXXLKXKLXXLKA). At first, we examined to isolate well-known consensus sequences, for instance, RGD motif for Integrin and HPQ (His-Pro-Gln) for Streptavidin [3]. A screening with the DR-library, which is potentially most flexible among three libraries, only succeeded in isolating HPQ motif. Second, we synthesized two RGD-containing loop peptides, L-lib9-RGD and L-lib11-RGD, determined the secondary structure by circular dichroism (CD) spectroscopy, and assayed their binding affinities to integrin$\alpha_5\beta_1$ by surface plasmon resonance (SPR) analysis. The CD showed two peptides had α-helical structure, but the SPR showed no binding of peptides to integrin$\alpha_5\beta_1$. These observations suggested that a conformation of the loop region of the libraries, L-lib9 and L-lib11, are highly constrained.

References

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