An orthogonal partial cleavage approach for solution-phase identification of biologically active peptides from larger chemically synthesized peptide libraries

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Introduction

Peptide library screening represents a new method to rapidly discover peptide ligands with biological activity, thus greatly facilitating the traditional drug discovery process. We have previously described a synthetic peptide library approach to screen for peptide ligands that bind to specific macromolecular targets (Selectide Technology) [1]. This method is based on the "one-bead one-peptide" concept where each individual peptide-bead expressed only one peptide entity. Because the peptides were not in solution, these libraries could not be used in solution-phase bioassays. We have now broadened the applicability of the technology to permit solution phase assay with releasable peptide libraries [2]. In this system, each bead contains only one peptide entity, but the peptides are attached to each bead via three different types of chemical linkers. Two of these linkers can be cleaved orthogonally and sequentially under very mild but differing chemical conditions. The remaining linker cannot be cleaved under these conditions thereby leaving sufficient peptide on the bead for microsequencing. The peptide library was initially distributed into 96-well plates with 500 beads/well. The peptides were then released into solution phase followed by biological assay. Approximately 100 pmole peptide was released from each bead into solution. Based on volume/well the concentration of each individual peptide in solution was approximately 1μM. The beads from the wells with positive response were then redistributed into individual wells with 1 bead/well. Subsequent release of peptides and biological testing allowed us to physically isolate the bead-of-origin for microsequencing. The general scheme of the methodology is shown in Figure 1.

Results and Discussion

This method has been successfully applied to identification of peptide ligands specific for (i) an anti-β-endorphin monoclonal antibody (MoAb) and (ii) the platelet-derived gp IIb/IIIa membrane fibrinogen receptor.

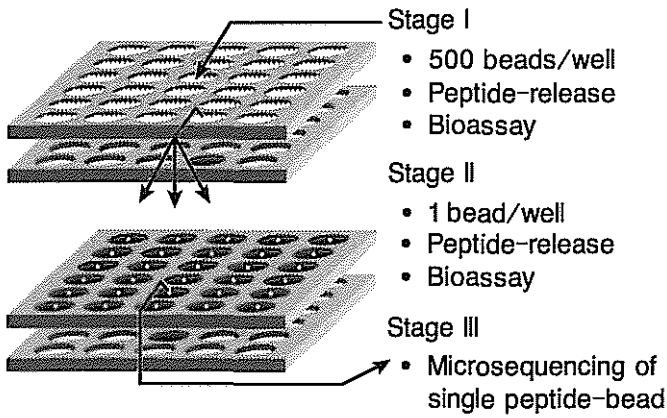

Fig 1. General scheme of the two-stage release peptide library screening methodology.

A linear tetrapeptide library XXXXG—bead was used in the screening of anti-β-endorphin MoAb (clone 3-E7). This MoAb has been studied extensively [1, 3]. The native epitope is YGGF and the motif identified from library screening was YG_F. The peptides were released into solution and competitive ELISA assay was performed. Four reactive beads were sequenced: YGGF (identified twice), YGVF and YGAF. In another experiment, purified platelet-derived gpIIb/IIIa receptor was used to screen a disulfide cyclic tripeptide library (CXXXC—bead). Again, a competitive ELISA assay was used for screening. Two positive beads were identified both with the following sequence: CRGDC. The RGD sequence is well known to be the native binding motif for gpIIb/IIIa.

These results demonstrate that our two-stage releasable peptide library method can be used to identify binding ligands in solution-phase assays, enabling the easy adaptation of many in vitro bioassays to this methodology. Furthermore, we may now screen for biological endpoint with a functional assay where the target molecule is either not yet purified or even totally unknown. As our libraries are produced synthetically, we have adapted this method to non-peptide chemical libraries which include a coding peptide sequence [4]. The non-peptide chemical structures are first released into solution and screened in a two stage process as described above. The remaining arm on the bead is the peptide coding sequence from which the non-peptide chemical structure can be determined.

References

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