Peptide library screening based on the one-bead one-peptide concept

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Introduction

Combinatorial library methods are valuable tools for drug discovery and basic research. There are three general methods to generate and screen huge peptide libraries (over 10^6 peptides): (i) the biologic peptide libraries using filamentous phage [1], plasmids [2], and polysomes [3], (ii) combinatorial peptide libraries with iterative process [4, 5] and positional scanning [6], or (iii) combinatorial peptide library based on the one-bead one-peptide concept or the Selectide process [7].

In the Selectide proces, we use a "split synthesis method" [7,8] to generate random peptide libraries such that each resin-bead expresses only one peptide entity [7]. These huge libraries are then mixed with an enzyme-receptor complex. The beads that interact with the enzyme-receptor complex turn color in the presence of substrate. The color beads are then physically isolated for microsequencing [7,9]. More recently, with a modified functional screening method, peptide substrate motifs for cAMP-dependent protein kinases and c-src protein tyrosine kinases ($p60^{c\text{-}src}$) were also determined. In this article, the general method as well as examples of some of the applications of the Selectide process will be discussed.

Peptide Library Synthesis

Peptide libraries were synthesized with a split synthesis approch using standard Fmoc chemistry [7,9]. Polystyrene beads grafted with polyoxyethylene (TentaGel S, Rapp Polymere, Germany) were used as solid-phase for the peptide synthesis. Details of the library synthesis method were described previously [7,9]. In our standard peptide library synthesis, all 19 eukaryotic amino acids except for cysteine were used in each randomization step. Cysteine was excluded to eliminate undesirable crosslinking by disulfide bond. Linear peptide libraries as long as 15-mer had been synthesized and screened. After the desired length of the library was achieved, the N^{α}-Fmoc and side chain protecting groups were removed, and the beads were thoroughly washed with an aqueous buffer prior to screening [7,9].

Peptide Library Screening for Binding

In this method, the positive beads were identified by their ability to interact with a tagged-acceptor molecule. The acceptor molecule (e.g. receptor, enzyme, virus, or even

small molecules) can be tagged directly by an enzyme [7,9], a fluorescent probe [10], a radionuclide [11], or a chromophore [12]. Alternatively, a secondary reagent with the above reporter molecule can also be used. For example, biotinylated-acceptor molecule can be the primary reagent, and streptavidin-alkaline phosphatase conjugate the secondary reagent. In the enzyme-linked detection scheme, the beads that interacted with the acceptor-enzyme conjugate turned color upon addition of substrate. The color beads were then physically isolated under a dissecting microscope and subjected to microsequencing as described [7,9].

We have applied this library screening method to various biological systems such as monoclonal antibodies (specific for continuous [7], and discontinuous [13] epitopes), streptavidin [7,14], avidin [14], MHC-class I molecules (B2 and A2) [15], and thrombin.

Peptide Library Screen for Post-translational Modification

In this method, the positive beads were identified by their ability to be enzymatically modified (e.g. phosphorylation) by an enzyme (e.g. protein kinase). To identify peptide substrate motif for cAMP-dependent protein kinase, we incubated a random heptapeptide library with cAMP-dependent protein kinase and [γ-^{32}P]ATP [16]. The bead-library was then washed thoroughly and immobilized on a glass plate with 1.5% agarose (w/v). The [^{32}P]-labelled beads were then localized by autoradiography. Using this method we identified peptide substrates for both cAMP-dependent protein kinase wd p60$^{c\text{-}src}$ protein tyrosine kinase (Table 1). Potentially, this can be used as a general method for identification of linear substrate motifs for other post-translational modifications of proteins provided that modifying enzymes and radiolabelled donors are available. If radiolablelled donors are not available, one may still be able to detect the covalently modified sites by antibodies that recognize these modified sites.

Table 1 *Identification of peptide substrate for protein kinase*

(I) cAMP-dependent protein kinase	RRYSV IIRRKSE SQRRFST YRRTSLV
(II) p60$^{c\text{-}src}$ protein tyrosine kinase	YIYGSFK

Identification of Peptides that Interact with Small Molecules

To test whether the combinatorial library method can be used to identify binding motif for small molecules, we used indigo carmine as a small molecule target [17]. Indigo carmine was chosen because it is a small water soluble organic dye molecule (M.W. = 466.56, Fig. 1). Since it is intrinsically colored, no additional reporter

molecule is needed. The peptide libraies were incubated with 10 μM indigo carmine in the presence of 0.26 M NaCl (to reduce non-specific ionic interaction) and 0.1% Triton X-100 (to reduce non-specific hydrophobic interaction). After an hour, the color of a few beads became intensively blue. Some blue beads were isolated for microsequencing. The amino acid sequence of these beads is shown in Table 2. All ligands isolated had a genaral motif of "+OOO+" where '+' represents either Lys or Arg and 'O' represents relatively hydrophobic or aromatic amino acids. The two flanking basic amino acids are highlighted in boldface in Table 2.

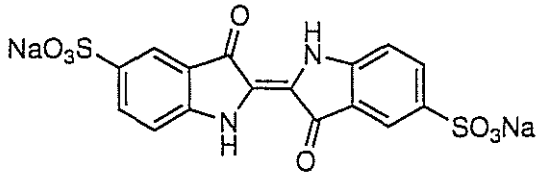

Fig. 1. Chemical structure of indigo carmine.

Table 2 *Identification peptides that interact with indigo carmine*

Libraries	Peptide
xxxxxxxx	akwkwvyr, ikivyrfr ykvvyris, vkkmvikf
XXXXXXX	LTKLVLK, YKVVYKL, VTKIIFK

Perspectives

This article summarizes principles and methods of combinatorial peptide library screening based on the one-bead one-peptide concept (Selectide process). Only a few examples are given. Over the last few years, there has been enormous progress in various aspects of the Selectide process. Using double-releasable linkers, peptides can be released from the beads for solution-phase assay [18]. Libraries with cyclic structures, unnatural amino acids and non-peptide moieties were synthesized and tested. Structural determination of small organic non-peptide ligand \using mass spectroscopic or peptide-encoding methods was accomplished [19]. Non-peptide libraries with rigid or flexible scaffolds (e.g. steroid, Kemp-triacid) were also developed. New coupling chemistries for combinatorial are currently under intensive investigation from many laboratories [20].

As an alternative to the synthetic approach described in this paper, the Selectide bead-library can be synthesized biochemically using enzymes. With the appropriate glycosyl-tranferases and precursors, oligosaccharide libraries can be synthesized. A similar approach can also be applied to polyketides.

Although our major focus has been on biological problems, we believe the Selectide

process can also be applied to other disciplines such as materials science. For example, one could develop new material with desired photoelectric, physical, electronic, or electromagnetic propertis provided that an appropriate detection scheme can be developed. Combinatorial library method is an emerging field and will likely be useful for investigators from many disciplines. Table 3 summarizes some potential applications of the Selectide Process.

Table 3 *Some potential applications of the Selectide process*

1.	Basic research (identification of binding ligands, protein-peptide and protein-protein interaction, molecular recognition)
2.	Drug-discovery (lead discovery and optimization of leads, receptor binding, biological activity)
3.	Materials science (optical, photochemical, photoelectrical, electronic and electromagnetic properties, molecular switch)
4.	Molecular assembly (binding pairs with high affinity)
5.	Artificial enzyme (e.g. cyclized peptides with/without co-factors as side chains)

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