New approach in constructing and screening peptide libraries

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Introduction

It has been recognized from the very early years of peptide research that the structure of a natural peptide can be changed at certain positions without losing biological activity. The crucial structure, or motif, is mainly responsible for binding to the recognition site of an acceptor molecule. Current combinatorial library techniques [for review see *e.g.* 1,2] enable the discovery of individual peptide ligands, but there is no direct technique available to identify critical motifs. In order to identify the critical binding motif in one step, we designed and synthesized a 'library of libraries' in which each resin bead contained a mixture of different peptides sharing a common structural motif.

Results and Discussion

The library of libraries is composed of a number of sublibraries, each one containing all randomized structures within one motif and all sublibraries representing all possible randomized motifs. We synthesized a library of tripeptide motifs in a hexapeptide format. There are 20 different ways to place three positions for randomization inside a hexapeptide. Consequently a hexapeptide library of libraries contains 20 sublibraries. This set of libraries can be synthesized most efficiently using the synthetic scheme presented in Fig. 1. For the synthesis of this library we used 19 proteinogenic amino acids (but not Cys) in the randomized positions and a mixture of the same amino acids in the non-randomized positions.

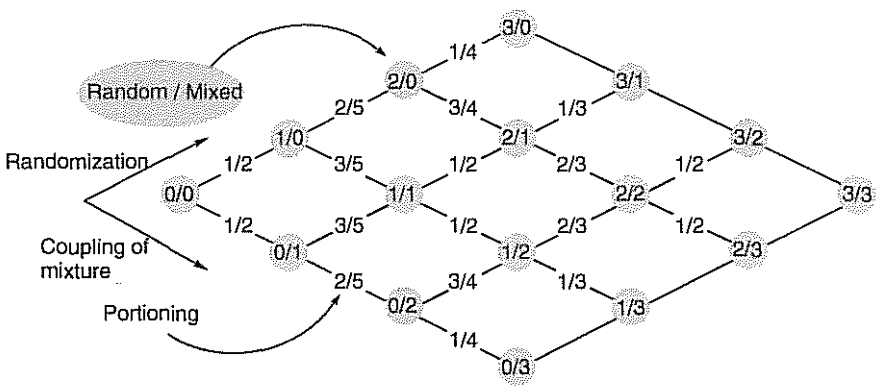

Fig. 1. Synthetic scheme for a library of libraries. Random couplings were performed by split synthesis on the portion of the resin equivalent to the number of remaining randomizations divided by the number of remaining synthetic steps. A mixture of amino acids was coupled to the portion that is equivalent to the number of remaining mixture couplings divided by the number of remaining synthetic steps.

Table 1 *Amino acid sequences of hits from a library of libraries. The number in parentheses shows the frequency of the identified sequence. One letter code was used to abbreviate amino acids and X represents a mixture of amino acids*

Streptavidin		Anti-β-endorphin	Thrombin
XXXHPQ (3)	HPXFXX	YGXFXX (6)	LRYXXX (3)
XXXHPM (2)	XHPXFX	YXGFXX (3)	IRYXXX
XXHPMX		XGAFXX (6)	IRXWXX
XXHPQX	XRXHPX	XGGFXX (2)	LRXWXX
XHPQXX	XXWHPX	YGGXXX	IRWXXX (2)
HPQXXX (2)	XWXHPX	YGXWXX	IXFWXX (2)
WXXHPX		YGAXXX	IXFRXX
FXXXPQ			IFXWXX
WXXXPQ	XXPQFX		
WXXXPM	HXXXPQ		

The library was screened against the anti-β-endorphin monoclonal antibody, streptavidin and thrombin. The results are summarized in Table 1. Known motifs were identified in the streptavidin and anti-β-endorphin assays. A new motif for a thrombin inhibitor was also discovered. The peptide IRFWA (with the mixture of amino acids having been replaced by Ala) was synthesized and tested in a chromogenic assay. The K_i of this composite peptide was found to be 2.3 μmol dm^{-3}.

The library of libraries approach has a number of advantages when compared with other peptide library techniques. It is possible to synthesize complete motif libraries of longer peptides instead of a sample of a complete library. The results from primary screening contain information on SAR, since the critical residues and their relative positions are identified. Non-specific interactions are diminished, since the library does not contain highly hydrophobic or charged species.

References

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