Nonsequenceable and/or nonpeptide libraries

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Introduction

The Selectide process consists of three parts: (i) synthesis of a single structure on each bead in a "library"; (ii) screening the library in a solid phase binding assay or in standard solution-phase assays; and (iii) determination of structure on the "active" bead [1]. The use of amino acids for library construction was the first step toward the generation of increasing structural diversity. Libraries based on amino acids incorporate a limited number of building blocks; however, these compounds are easy to synthesize and sequence. Libraries of non-peptidic compounds offer greater diversity of structures, but may require an alternative means of structure identification, such as coding by amino acid sequence for the nonsequenceable building blocks [2].

Results and Discussion

We have combined the simplicity of peptide structures with the diversity available in alternative building blocks besides regular amino acids. The simplest building blocks used to construct the library are given in Figure 1. We have used trifunctional amino acids and modification of a side chain to achieve the structural multiplicity. Amino acids like diaminobutyric acid, aspartic acid, cysteine and/or iminodiacetic acid are the smallest building blocks onto the side chains of which the universe of carboxylic acids, amines or halides (aliphatic, aromatic, heterocyclic) can be attached. To achieve reasonable binding to a neutral acceptor (receptor, antibody, enzyme, nucleic acid...), the appropriate spatial arrangement of the interacting structures must be realized. Linear presentation of amino acid side chains in peptide libraries may not be the optimal format for selection of the best binding structures.

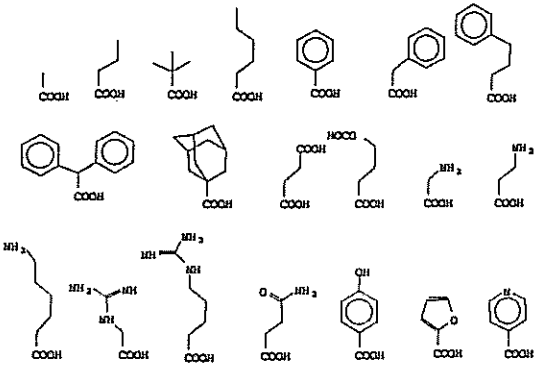

Fig. 1. Blocks used for randomization.

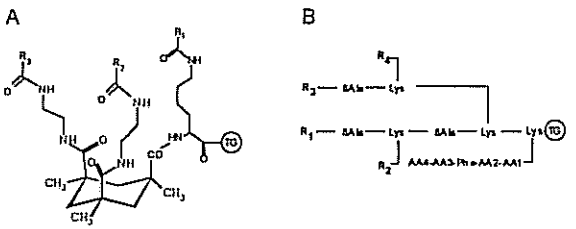

Fig. 2. Structure of nonpeptide library on nonpeptidic (A) and on peptidic (B) scaffolding.

The optimal strategy for displaying the interacting structures might be their placement onto a molecular scaffolding which might map the appropriate segments of space. Inter-relationships of the same set of individual building blocks in the scaffolding arrangement can be varied using different scaffolding.

As an example of a small non-peptidic scaffolding, we have built a conformationally constrained scaffolding based on modified Kemp's triacid and constructed a non-peptide library with 20 different carboxylic acids randomized (Figure 2A). Another scaffolding mapping larger conformational space is a simple branched attachment constructed by consecutive coupling of diamino carboxylic acids (Figure 2B). The synthesis of this scaffold required the use of four independent (orthogonal) protecting groups (Tfa, Npys, Fmoc, Ddz). Unnatural building blocks can be combined in the library with standard amino acids. A small library was synthesized with amino acids randomized in position 1, 2 and/or 3 and (i) a set of aromatic amines coupled to the β-carboxyl group of aspartic acid, (ii) a side chain modified iminodicarboxylic acid, (iii) aromatic acids coupled to the side chain of diaminobutyric acid, or (iv) benzylhalides coupled to the side chain of sulfur containing amino acids (cysteine and penicillamine) in position 4. In this case doublets of 6 amino acids were used to code for 36 different building blocks in position 4 which were not directly sequenceable.

A linear library and both scaffold-based libraries were screened in our model systems to determine ligands to anti-β-endorphin monoclonal antibody and streptavidin. Positively reacting beads were subjected to Edman degradation, and the inter-acting structures, deduced from the obtained data are given in Figure 3. These compounds were resynthesized and have shown specific binding (competable by leucine enkephalin or biotin, respectively).

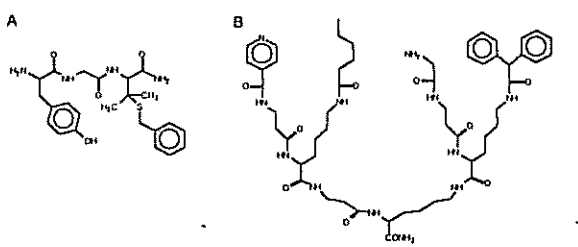

Fig. 3. Structures interacting with anti-β-endorphin (A) and streptavidin (B) found in the test libraries.

References

1. Lam, K.S., Salmon, S.E., Hersh, E.M. Hruby, V.J., Kazmierski, W.M. and Knapp, R.J., Nature, 354 (1991) 82.
2. Nikolaiev, V., Stierandová, A., Krchňák, V., Seligmann, B., Lam, K.S., Salmon, S.E. and Lebl, M., Peptide Res., 6 (1993) 161.