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Methods for building libraries of peptide structures and determination of consensus sequences

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Traditional structure-activity studies are generally unable to discover alternative structures having affinity for a given macromolecular counterpart. The only really rational approach to the discovery of new biologically active motifs is the use of so-called libraries of structures. In the area of peptide molecules, the construction of a library is relatively simple and several groups [1–4] reported the synthesis of libraries of various sizes and complexities. The synthetic approach of Furka et al. [5] permits chemical synthesis of a large number of peptide species. Lam et al. [3] were the first to recognize that this synthetic approach could be used specifically with the objective of synthesizing libraries containing large mixtures of polymeric beads containing a single unique sequence on each particle. This idea allowed for the construction of very large peptide libraries for rapid screening and discovery of an interaction of a peptide bound to the solid support after exposure to its sol-

Sequences found in octapeptide library (5×10^{-10} M MoAb):

DELWGQGF
FHKWESGF
NLVWSMGF

Dedicated library for 'fine tuning':

Library: XXXWXXGF

Found with 100 times lower concentration (5×10^{-12} M) of MoAb:

ADHWKYGF	QHIWGLGF
LNPWKYGF	QQIWGRGF
INYWKYGF	QAIWGYGF

Dedicated libraries for the second generation 'fine tuning':

XXXXWKYGF	XXQXIWGXGF

Found with 10 times lower concentration (5×10^{-13} M) of MoAb:

NHKGWKYGF	SRQDIWGIGF
NHVGWKYGF	SKQDIWGRGF
NHIKWKYGF	SRQAIWGYGF

Consensus structures:

NH_*G*WKYGF	S*R*Q*D*IWG_GF

Fig. 1. Scheme used for finding of consensus sequences of peptides interacting with insulin monoclonal antibody (only three sequences with each motif are given as an example).

uble macromolecular counterpart [3]. We have tested this approach on several model systems (antibodies, enzymes and receptors). As an example, the interaction of a monoclonal antibody against insulin is reported here. Libraries of L-amino acids of various length were tested and structures of peptide ligands were determined by automated Edman degradation.

Two binding motifs were found in an octapeptide library (which was prepared without full representation since this would have required the preparation of 25.6 billion beads, i.e., approximately 25 kg of resin support), and were used as a template for the construction of a dedicated library with only five randomizations (2.6 million possible peptides). Sequences showing binding at 100 times lower concentration of antibody (5×10^{-12} M) were found. The consensus sequences found in these libraries were used in the generation of the next libraries. The two consensus sequences which are responsible for binding of monoclonal antibody to the beads at 5×10^{-13} M concentration and fully competable by insulin are shown in Fig. 1. It is interesting that the sequences found have no sequence similarities to the sequence of native insulin.

Structure determination of positively labeled beads is the slowest part of the Selectide Process. However, if we are able to define the shortest peptide interacting with the acceptor (simply by testing libraries of increasing length until several beads show positive reaction) we can sequence all positive beads in one run. If the interacting peptides contain a consensus sequence, we can immediately analyze the importance of the particular positions in a peptide chain. An example of this approach is given in Fig. 2. Interaction of anti-β-endorphin antibody with a part of an L-pentapeptide library (~1 000 000 beads) gave 99 positive beads, which were sequenced in three parts and the results were accumulated. The importance of positions 1, 2 and 4 and flexibility of positions 3 and 5 is obvious.

The Selectide Process (one bead–one peptide) is not necessarily limited to the screening of soluble acceptors. Equal parts of a peptide can be released repeatably from each bead to a solution and biological tests which are transferable to a microtiter plate format can be performed with the released peptide. The structure of a

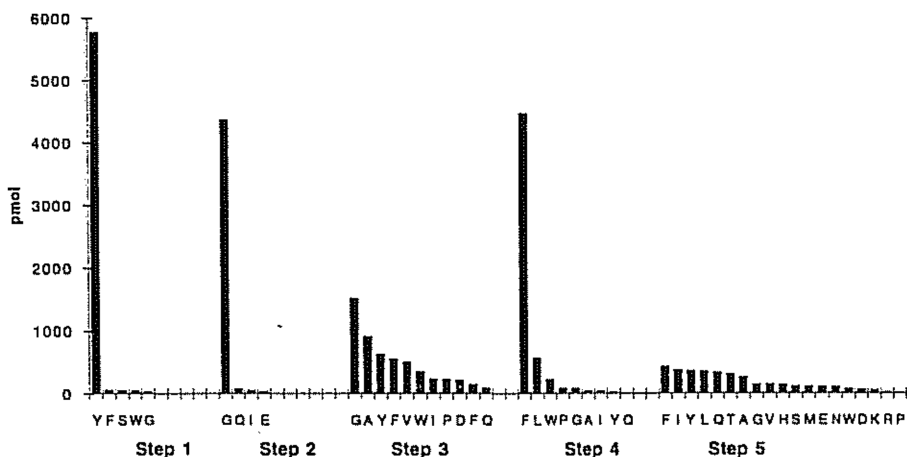
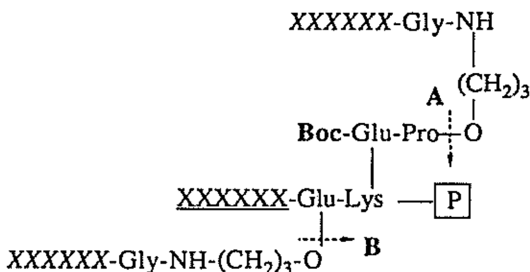


Fig. 2. Results of simultaneous sequencing of 99 beads found positive in anti-β-endorphin antibody screening.



Peptide released in both steps: *XXXXX*-Gly-NH-$(CH_2)_3$-OH

Fig. 3. The structure of a doubly cleavable library releasing the same peptide in both steps.

multiply cleavable library is given in Fig. 3. In the first step a library of millions of peptides is divided into the wells of a microtiter plate by 1000 beads per well. The first part of the peptide from every bead is released by a change of pH to 8.5, when the amino group of glutamic acid is deprotonized and a diketopiperazine structure is formed (cleavage A). Beads from positively reacting wells are redistributed with a single bead per well and a second part of the peptide is released by alkaline hydrolysis or by ammonolysis using gaseous ammonia (cleavage B). The third part of the peptide on the bead is noncleavable and can be used for sequencing. We have shown the applicability of this approach both in antibody and receptor screening projects.

References

1. Parmley, S.F. and Smith, G.P., Gene, 73(1988)305.
2. Fodor, S.P.A., Read, J.L., Pirrung, M.C., Stryer, L., Lu, A.T. and Solas, D., Science, 251(1991)767.
3. Lam, K.S., Salmon, S.E., Hersh, E.M., Hruby, V.J., Kazmierski, W.M. and Knapp, R.J., Nature, 354(1991)82.
4. Houghten, R.A., Pinilla, C., Blondelle, S.E., Appel, J.R., Dooley, C.T. and Cuervo, J.H., Nature, 354(1991)84.
5. Furka, Á., Sebestyén, F., Asgedom, M. and Dibó, G., Int. J. Pept. Protein Res., 37(1991)487.