Peptide library screening based on the one-bead one-peptide concept: An update

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Introduction

Using a 'split synthesis method' [1,2], a random synthetic peptide library can be generated such that each resin-bead expresses only one peptide entity (the one-bead one-peptide concept) [1,3]. This vast library (10^6-10^8 entities) can then be screened with a specific acceptor molecule-enzyme complex. Beads that interact with the acceptor will change color upon incubation with an appropriate substrate. The color beads can then be physically isolated and the bead-bound peptides microsequenced. Over the last three years, we have successfully applied this method (Selectide process) to identify specific peptide ligands for various macromolecular targets such as antibodies (specific against continuous and discontinuous epitopes), streptavidin, avidin, gpIIb/IIIa integrin, thrombin and factor Xa.

Here we report on the application of the Selectide process on several other systems using other approaches for screening. These include the identification of (i) anchor residues for MHC Class I molecules (A2 and B7), (ii) heptapeptides that interact specifically with a small organic molecule, an indigo dye, (iii) peptide substrate motifs for cAMP dependent protein kinase and src tyrosine kinase ($p60^{c\text{-}src}$), and (iv) idiotype-specific peptides for B-cell lymphoma.

Results and Discussion

MHC class I molecules (A2 and B7) were first released from the JY cells by papain and were purified by conventional column chromatography. The class I molecules were then denatured with 5 mol dm^{-3} NaSCN and mixed with a random nonapeptide bead-library and excess β_2-microglobulin. The final mixture was then dialyzed against PBS. The class I molecule reassociated on some of the peptide-beads and these beads were detected with a colorimetric assay using anti-class I molecule monoclonal antibody-alkaline phosphatase conjugate as the probe. The peptide motifs identified from such a random screening were as follows: A2, _(L/M)_ _ _ _ _ _(L/V/I) and B7, _PR_ _ _ _ _(L/V/I). These motifs corroborate very closely with those isolated from the cell-bound peptides. This experiment demonstrates that the peptide anchor residues of class I molecules can be rapidly determined by the Selectide process.

When random all-D amino acid or all-L amino acid heptapeptide libraries were incubated with an anionic small organic dye, indigo carmine, a few beads were stained intensely turquoise/blue. The chemical structure of indigo carmine is shown in Fig. 1. Most of these stained beads had the following motif, where X is any amino acid and O is a relatively hydrophobic residue: X(K/R)OOO(K/R)X. These three relatively hydro-

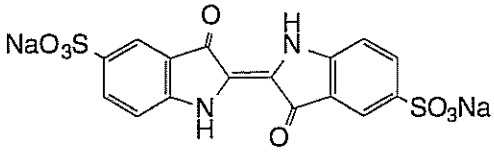

Fig. 1. Structure of indigo carmine.

phobic residues were flanked by two positively charged amino acids, *i.e.* lysine or arginine. This experiment demonstrates that through the use of the Selectide process, one can identify short peptides that interact specifically with a small organic molecule. Applications of this method include design of potent absorbant for toxic waste or design of paired molecules for molecular assembly studies.

We have also applied the Selectide technology for determination of protein kinase substrate motifs. Random peptide libraries were incubated with purified protein kinase and [γ^{32}P]ATP. The libraries were then washed and immobilized on glass plates with agarose. After drying, the immobilized beads were exposed to X-ray films. The radiolabelled beads were then localized, individual beads were isolated and their peptide sequences were determined. We initially applied this method for cAMP-dependent protein kinase. Several beads were positive and four were sequenced: RRYSV, SQRRFST, YRRTSLV and IIRRKSE. The RR-S motif elucidated from this random screening is identical to that described for cAMP-dependent protein kinase, demonstrating that the Selectide technology is generally applicable to the determination of linear motifs for post-translational modification. We have also applied the same methodology to $p60^{c\text{-}src}$, a protein tyrosine kinase (PTK) that is important in signal transduction and tumorigenesis. The peptide that we isolated from random screening of a random heptamer was as follows: YIYGSFK. When compared to the best known peptide substrate for c-src [cdc^2(6-20)] peptide derived from $p34^{cdc2}$, KVEKIGEGTYGVVYK), YIYGSFK was significantly more potent as well as specific for the src-family protein kinase.

Using purified surface immunoglobulins (idiotype) isolated from the murine cell lines (WEHI231 and WEHI279), we were able to screen random peptide libraries and identify peptide ligands (some contained all-D amino acids) that bind specifically to these surface idiotypes. Furthermore, we demonstrated that these peptides were able to bind specifically to the lymphoma cell surface and trigger signal transduction, resulting in an increase in tyrosine protein phosphorylation.

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. Furka, A., Sebestyen, F., Asgedom, M. and Dib, G., Int. J. Pept. Protein Res., 37(1991)487.
3. Lam, K.S., In Meyer, R.A. (Ed.) Encyclopedia of Molecular Biology and Biotechnology, VCH, Weinheim, 1994.