Application of Selectide Technology in identifying (i) a mimotope for a discontinuous epitope, and (ii) D-amino acid ligands

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Introduction

Selectide Technology is a synthetic peptide library method that is based on the "one-bead one-peptide" concept [1]. The peptides are synthesized on solid-phase beads using a "split synthesis" method [1, 2] resulting in a huge library of peptide beads where each solid-phase bead expresses only one peptide entity [1]. The entire library of peptides (10^6-10^8) is then screened and the reactive bead identified, physically isolated, and microsequenced. Since the Selectide Technology is based entirely on synthetic chemistry, unnatural amino acids and even non-peptide chemical subunits can be used in the construction of the chemical library [3]. In this paper, we report on the application of Selectide Technology in identification of ligands for an anti-insulin monoclonal antibody (MoAb). This murine Ab (Clone AE9D6) recognizes a discontinuous epitope of insulin with a binding constant of 0.01 μM.

Results and Discussion

Table 1 shows the various motifs identified when a linear or cyclic peptide library was screened with the anti-insulin MoAb. Immediately apparent is that several distinct peptide motifs were identified using this parallel approach of peptide library screening. This is in contrast to the convergent approach of screening where an iterative process was used [4], and only one motif was identified. Table 1 also shows that the motif identified is dependent on the length as well as the secondary structure of the library. The binding affinities of most of these ligands identified during the primary screen are relatively low (>10 μM). A sequential screening approach was then applied to one of these motifs, _ W _ _ GF, identified from the primary screen (Table 2). Based on the motif of the primary screen, longer secondary libraries were synthesized and screened under higher stringency. This process was repeated several times until ligands of higher affinity were finally isolated. The binding affinity (IC_{50}) of the best ligand identified (SKQDIWGRGF) thus far was 0.05 μM, 5 fold weaker than that of insulin, the native ligand. The subsequent libraries synthesized, however, need not be linear. We are in fact currently

developing libraries where the randomization steps are carried out at a branching arm from the middle of the peptide motif identified from a previous screen. It is hoped that this will generate additional contact points resulting in ligands with affinities higher than that of insulin. In addition, we have also used the anti-insulin MoAb to screen all D-amino acid linear hexa- and octa-libraries. The common motif identified was _ _ _ q _ Gs_G. This motif does not resemble ligands identified from the L-amino acid libraries.

Table 1 *Peptide motifs identified on the primary screen of an anti-insulin MoAb*

Tetra	QNPR	Deca	____W__GF
Penta	FNW__	Quindeca	_________W__GF
	FDW__		_FDW___________
	_QDPR		______W__GF____
Hexa	_W__GF		
	FDW___	Cyclic 7-mer	CW__GF__C
	FNW___		
	__QDPR	Cyclic 8-mer	C_F_W__GGC
Octa	___W__GF		C____HGVQC
	______GF		
Nona	___GF__GF	Cyclic 9-mer	CQDI_Y____C

Table 2 *Optimization by sequential screening*

Library		Motif
1°	XXXXXX	_W__GF
2°	XXXWXXGF	___WKYGF, Q_IWG_GF
3°	XXXXWKYGF	NH_(G)WKYGF
	XXQXIWGXGF	S(R/K)Q(D/A)IWG_GF

The V_H and V_L genes of this anti-insulin MoAb were cloned and a single chain Ab has been constructed. This single chain Ab, when compared to the whole native antibody, showed a similar but not identical binding profile to insulin and the various peptide ligands. Work is currently underway to use this single chain Ab and its CDR loop variants to explore antibody-peptide interaction at the molecular level.

References

1. Lam, K.S., Salmon, S.E., Hersh, E.M., Hruby, V.J., Kazmierski, W.M. and Knapp, R.J., Nature (London), 354 (1991) 82.
2. Furka, A., Sebestyen, F., Asgedom, M. and Dib, G., Int. J. Peptide Protein Res., 37 (1991) 487.
3. Nikolaiev, V., Stierandova, A., Krchnak, V., Seligmann, B., Lam, K.S., Salmon, S.E. and Lebl, M., Peptide Research, 6 (1993) 161.
4. Houghten, R.A., Pinella, C., Blondelle, S.E., Appel, J.R., Dooley, C.T. and Cuervo, J.H., Nature (London), 354 (1991) 84.