Streptavidin-peptide interaction as a model system for molecular recognition

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Introduction

Peptide library screening has been proven to be a valuable tool in identifying peptide ligands for various macromolecular targets [1]. Our peptide library method (Selectide Technology) is based on the "one-bead one-peptide" concept where each individual bead expresses only one peptide entity [2, 3]. Using this technology, we have identified several peptides with HPQ and HPM motifs that interact specifically with streptavidin [2]. When a histidine depleted library was used, several non-HPQ motifs were identified [3]. When avidin was used as a target macromolecule, four new peptide motifs were identified. One of these motifs, HPYP_, binds to avidin but not to streptavidin. On the other hand HPQ binds to streptavidin but not to avidin [3]. Weber et al. [4] have reported the high resolution X-ray crystallographic structure of streptavidin: FSHPQNT peptide complex. In this paper, we report on our continuing work on using streptavidin as a model system to screen our D-amino acid containing and cyclic peptide libraries.

Results and Discussion

Table 1a shows the structure of the D-amino acid containing peptide ligands isolated when penta-peptide libraries were screened with streptavidin. When an XxXxX penta-peptide library (wherein 'X' represents L amino acids and 'x' represents D amino acids) was screened, three distinct motifs were identified: _ _WpH, _w(F/Y)pH and Y_ _fP. Interestingly, a D-Pro-L-His (_ _ _pH) is present in two of these three motifs. This sequence has some resemblance to that of HPQ or HPM when an all L-library was screened. In contrast, a totally different motif was identified when an all D-amino acid penta-peptide library was screened: wy_ _a. Biotin at 0.1 μM, completely abolishes the binding of streptavidin to these peptide-beads. Furthermore, similar to HPQ and HPM, many of these ligands interact with streptavidin but not with avidin.

Table 1 *Ligands that interact specifically with streptavidin*

	XXXXX	XxXxXx		xxxxx	
a.	HPQ	WkWpH	RwYpH	wyqea (3)	wfrya
	HPM	YgWpH	DwFpH	wyhea	wymel
		QrWpH		wydya	
		LqWpH	YvIfP	wyefa	
		AfWpH	YpFfP	wyfya	
		SyWpH			
b.	**CXXXXC**	**CXXXXXC**		**CXXXXXXC**	
	HHPM	HPQNV	SHPQF	HPQAPK	HPQGPG
	NHPM	HPQNN	LHPQN	HPQAPY	HPQNGG
	QHPM	HPQQV	DHPQN	HPQFAS	HPQNAQ
	LHPM		WHPQN	HPQFAR	HPQVGI
		HPMNA		HPQFPA	HPQSGM
	RHPQ	HPMNP		HPQFPQ	
				HPQGPA	HHPQFP

X = all 18 L amino acids plus glycine, excluding cysteine.
x = all 18 D amino acids plus glycine, excluding cysteine.

We have also screened some cyclic all L-amino acid containing libraries. The results are shown in Table 1b. Disulfide cyclization was accomplished by incubating the library overnight with 20% dimethylsulfoxide. Similar to the linear libraries, HPQ and HPM motifs were both identified. However, HPM was preferred in the cyclic tetrapeptide libraries whereas HPQ was preferred in both cyclic penta- and hexa-peptide libraries. Furthermore, the location of this triplet motif with respect to the flanking cysteine residues depends greatly on the ring size.

Work is currently underway to determine the binding affinities of all these ligands. When combining these data with X-ray crystallography and computer modeling, we hope to gain insights into the understanding of peptide-protein and ligand-protein interactions at the molecular level.

References

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