Technical paper

Affinity Ranking of Phage-Displayed Peptides: Enzyme-Linked Immunosorbent Assay versus Surface Plasmon Resonance

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Received: 06-07-2007

Abstract

Peptides with particular affinity and specificity for variety of targets are selected through panning procedure from random peptide phage display libraries. Efficiency and convenience of enzyme linked immunosorbent assay (ELISA) and surface plasmon resonance (SPR) for screening and evaluating peptide-displaying phage clones were compared using streptavidin as a model protein target.

Keywords: ELISA; surface plasmon resonance; Biacore; peptide phage display

1. Introduction

Random peptide phage display libraries are being increasingly used to select peptides with affinity and specificity for variety of targets. The identification of ligands from large biological libraries by phage display has now been used for almost 15 years. In the last few years several improvements have led to numerous high affinity peptide ligands with good biological activity.[1]

In most cases panning procedure is repeated until the percentage of bound phages increases significantly. At this stage a set of 10 to 100 individual clones are analyzed. Confirming the binding properties of selected phage clones is essential to avoid unnecessary sequencing and subsequent peptide synthesis. This is usually carried out in an ELISA like format, using enzyme conjugated antiphage antibodies. Large number of clones can be characterized simultaneously.

Affinity of selected peptide-presenting phages for particular target has also been evaluated using surface plasmon resonance (SPR), although more often analyses of synthesized peptides are performed.[2] Some reports suggest that SPR could be a valuable addition to selecting candidates for sequencing.[2] Other reports describe the use of SPR as an additional method for further evaluation of binding of ELISA-selected phages.[3,4] Moreover, some reports describe the use of SPR for evaluation of the entire eluate[3] or even for performing biopannings on an SPR sensor chip.[5]

Here we compare the two methods for their ability to confirm the binding of phage-displayed peptides to the target molecule and to rank them according to apparent affinity.

2. Experimental

In order to compare the efficiency and convenience of ELISA and SPR assays in screening and evaluating peptide-displaying phage clones, streptavidin was used as a model protein target. Five M13 phage clones from a random cyclic heptapeptide low-avidity type 3 phage display library (Ph.D.-C7C, New England Biolabs) differing in affinity towards streptavidin were used (Fig. 1). Two (K2 and K4), containing the tripeptide sequence His-Pro-Gln, known for its affinity to streptavidin[6], were selected against streptavidin through specific elution with biotin.[7] Three control clones with no anticipated affinity for strep-

tavidin, selected against pancreatic lipase in a previously reported study[8], were used as negative controls. However two of them contained a similar motif.

2. 1. ELISA Assay

Microtitre plate wells (Maxisorp, Nalge Nunc International, Roskilde, Denmark) were coated with 100 μl of streptavidin solution (50 μg/ml) in 50 mM $NaHCO_3$, pH 8.5 overnight at 4 °C and blocked with 200 μl blocking buffer (1% non fat dried milk in phosphate buffered saline (PBS) buffer; 135 mM NaCl, 3 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4; pH 7.4 for 2 hours. As a negative control a separate set of wells was treated with blocking buffer without previous streptavidin immobilization. Equal titers of phages ($1 \cdot 10^8$ plaque forming units (pfu)) were diluted to 200 μl with blocking buffer and transferred to the coated wells. Next ELISA assay was performed as previously described.[8]

2. 2. Surface Plasmon Resonance Assay

Interaction of phages with streptavidin was monitored using Biacore X system (BIAcore, Uppsala, Sweden). To rank the peptide presenting phages in order of binding affinity, phage stocks were prepared in PBST buffer (PBS with 0.1% Tween 20; $1 \cdot 10^9$ pfu/μl). Assays were performed on a SA sensor chip pre-immobilized with streptavidin (BR-1003-98 BIAcore). Reference cell was blocked with biotin (10 μM, 10 min). The chip was then washed with 5 μl of 0.5% SDS at a flow of rate 40 μl/min. Identical wash cycles were used to regenerate the chip between assays. All the steps were performed at 25 °C with a flow rate of 5 μl/min in 0.1% PBST running buffer. Twenty-five micro liters of phage suspension was injected for each assay.

3. Results and Discussion

Both methods require amplification, isolation of phage clones and determination of phage titer. However, ELISA screening can be simplified by using amplified clones in a growth media separated from bacteria by centrifugation, omitting subsequent steps. This enables screening of large number of different clones without time consuming laboratory work. But when a potential strong binder is present in low concentration and thus giving low ELISA signal, there is a risk of overlooking it.

Both methods, SPR and ELISA, clearly confirm or refute the binding affinity of different clones. The ELISA results in Fig. 1 show very strong affinity of K2 and K4 for streptavidin. K4-signal is 0.5 absorbance unit higher than K2-signal, suggesting improved binding properties when Asp is replaced with Ala in position 4. The same results are obtained with SPR. The difference in binding properties of high affinity/avidity clones in ELISA assay is more obvious at lower titers of phages ($1 \cdot 10^8$) (Fig. 2). With high phage titers ($5 \cdot 10^9$) smaller difference can be attributed to the nonlinearity of absorbance measurement at high absorbencies. The results of SPR assay are not influenced by phage titer.

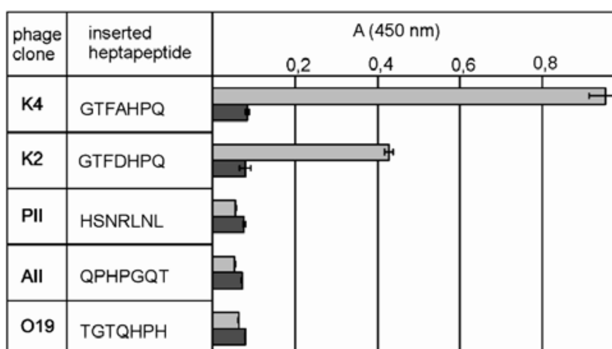

Figure 1: Inserted amino acid sequences of phage clones and their affinity determined in ELISA assay. Displayed heptapeptides are flanked by two cysteine residues (not shown) and therefore partially constrained through cyclization. Light gray bars represent phage clones binding to streptavidin immobilized on microtitre plates. Dark gray bars represent negative controls (phage clones binding to non-fat dried milk blocked microtitre plates). Black bars represent standard deviation of three measurements.

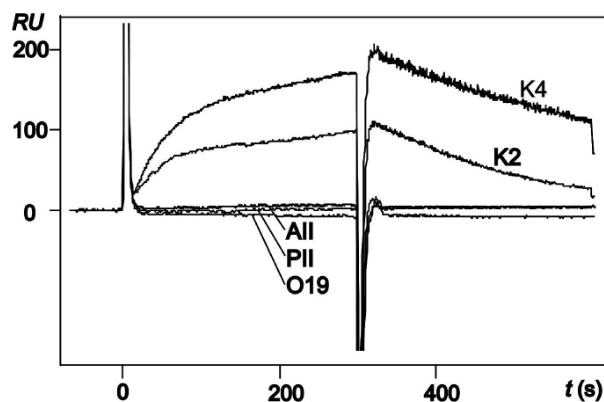

Figure 2: Overlay plot of sensograms of phage clones displaying different peptides. Interaction between streptavidin and phage displayed peptides is indicated by an increase in RU (resonance unit) value.

Detection limit in SPR depends on the amount of immobilized ligand and molecular weight of analyte and ligand, and of course on the affinity of the analyte towards immobilized ligand.

As stated in the literature the binding of phages can be monitored at titers as low as $5 \cdot 10^8$ pfu/ml.[4] However different concentrations of phages up to $10 \cdot 10^{13}$ (Tamm I 2003) were used in SPR experiments. In our case limit of

detection for the phage clone K4 was below $7 \cdot 10^{11}$ pfu/ml. Titer used in affinity ranking experiments was $1 \cdot 10^{13}$ pfu/ml.

Detection limit in ELISA experiment depends on the amount of microtitre plate bound target and affinity or avidity of the phage displayed peptide towards the target. The detection limit for K2 was below $1 \cdot 10^{8}$ pfu, considering criteria that the ELISA test is positive when absorbance is above 0.3 and exceeds negative control by at least 2 fold. The amount used in ELISA test was $1 \cdot 10^{8}$ pfu.

Affinity ranking of various phage clones by determination of kinetic parameters (k_a and k_d) proves to be difficult.[2] Because multiple copies of peptide are displayed per phage, the actual binding mechanism may be too complicated to model.[3] With a simplification to treat a virion displaying up to five copies of the peptide as a single particle, sensograms can be fitted to a single site binding model (1:1 Langmuir binding) or other models with BIA evaluation software.[3,9] It is possible to obtain the information on the apparent affinity and on apparent association and dissociation rates from a single experimental run, while ELISA assay provides only information on apparent affinity.

However, SPR demands more optimization. Various experimental conditions were reported, significantly differing in phage titer and volume of samples, flow and regeneration procedure. But once the optimal conditions are established, the analysis is fast and robust. ELISA on the other hand requires less preparation. 48 clones may be screened in a single microtitre plate in as little as 4 hours.[10]

The most important drawback of ELISA and advantage of SPR is the amount of target protein needed for the assay. Performing ELISA, a separate microtitre plate well must be coated with target protein for each clone and can be used only once. A range of SPR sensor chips ensures that the most suitable sensor surface is chosen according to the nature of the molecule to be coupled and the requirements of the analysis. Standard covalent amino-coupling procedure is frequently used for immobilization of proteins. Once the target molecule is firmly coupled to the surface, the chip can be regenerated many times without the loss of ligand. Therefore much smaller amount of target molecule is required.

Using SPR, the target protein can also be incorporated in a model membrane system, such as a monolayer or bilayer, thus achieving the correct conformation.

4. Conclusion

Efficiency and convenience of enzyme-linked immunosorbent assay (ELISA) and surface plasmon resonance (SPR) for determining the binding properties of phage display selected clones were compared. Both methods can be successfully used for screening; however the choice depends on the nature of target protein, the amount of target protein and equipment available. Versatile SPR sensor chips enable immobilization of almost any target molecule, regardless of their physiochemical properties and when there is only a small amount of target molecule available, SPR would be a method of choice. However, the equipment needed for SPR assays is far more costly than the one needed to perform ELISA test, where the higher amount of target protein is needed.

6. References

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Povzetek

Pri rešetanju in ovrednotenju bakteriofagnih klonov, ki izražajo izbrane peptide, se uporabljata encimskoimunski test na trdnem nosilcu (ELSIA) in površinska plazmonska resonanca (SPR). Z uporabo streptavidina kot modelne ciljne molekule smo primerjali učinkovitost in primernost obeh metod.