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Minireview

Peptide display on filamentous phage capsids

A new powerful tool to study protein–ligand interaction

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Peptides can be displayed on the surface of filamentous bacteriophages by fusion to phage coat proteins. It was recently shown that vast (10^8) collections of phages, each exposing a variant of the original peptide, can be constructed and utilized as a general source of peptide ligands. By panning these libraries on a target molecule linked to a solid support it is possible to select, out of the hundreds of millions of clones, those few phages that display a peptide that binds the target molecule. Searching these libraries is a powerful tool to be applied in many areas of fundamental and applied biology.

Peptide library; Filamentous phage; Ligand binding

1. INTRODUCTION

The combination of protein over-expression technology and site-directed mutagenesis has allowed considerable progress in the understanding of the relationship between amino acid sequence and protein conformation and function. Progress is limited, however, when the scarce understanding of the process of interest prompts researchers to formulate poor models that are not easily tested with the construction and characterization of a workable number of site-directed mutants. These problems are better approached by selective methods involving the simultaneous construction of a large collection of random mutants and the selection of those that are compatible with a given property.

Recently a new general technology has been developed that permits the application of a selective approach to the study of molecular recognition in proteins. Most importantly this technology does not rely on the recognition mechanism under investigation having a phenotype associated in common prokaryotic expression organisms and can, in principle, be applied to eukaryotic or artificial peptides.

2. PHAGE EXPOSITION TECHNOLOGY (PET)

The method, which we call PET (Phage Exposition Technology) stems from the pioneering work of G. Smith [1] and relies on the ability to display a peptide of interest on the surface of a bacteriophage capsid (Fig. 1). The method begins with the fusing of a peptide (X), whose function under study is that of being able to bind a second molecule (Y), to a phage capsid protein. The fusion is engineered so that the foreign peptide does not affect phage capsid assembly and remains exposed to the solvent available for interaction with the target molecule (Y). Usually the second step consists of the construction of a large collection of phage clones each synthesizing a different sequence variant of the fused peptide. The target molecule (Y) attached to a solid phase can then be used to separate, by standard chromatographic means, those phages presenting a protein X proficient in binding. The important point is that the physical linkage between protein X and its gene, mediated by the fusion to the coat protein, permits cloning and sequencing of the nucleotide sequence encoding the peptide X variants that bind the ligate Y. The comparison of the predicted amino acid sequences of the peptide X displayed on the selected clones is expected to reveal details of the recognition mechanism. Furthermore PET can be used to select from such variant libraries novel binding properties that were not present in the original peptide.

In principle other vectors can be used for studying protein–ligand interaction in a manner similar to what we have just described. For instance a variety of outer membrane proteins [2–4], or proteins that form the building block of bacterial thread-like organelles [5,6] are capable of presenting oligopeptides inserted into

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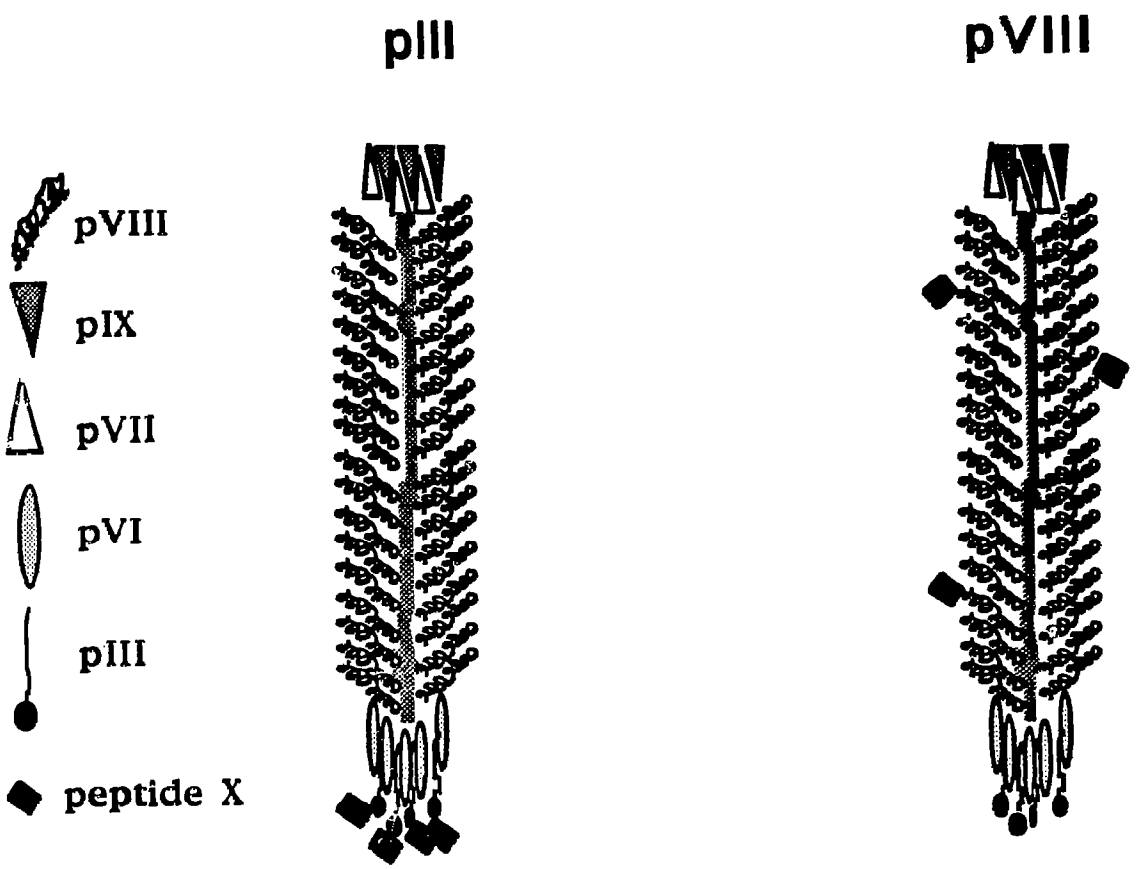

Fig. 1. Schematic representation of hybrid filamentous phages displaying the X peptide fused either to pIII (left) or to pVIII (right). As described in more detail in the text, the display by fusion to pVIII is achieved by phenotypic mixing of a hybrid peptide X–pVIII fusion protein dispersed in a wild-type phage coat. The exact number of minor and major coat proteins is not known.

specific regions of their structures in a form available for interaction with ligands present in the medium. Utilizing phages instead of bacteria, however, raises the number of clones that can be handled in a single experiment by a factor of 10^3 and facilitates the construction and storage of large libraries. Furthermore bacteriophages are simpler and more compact structures, and are therefore likely to have a smaller number of aspecific interactions in the selective procedure.

3. FILAMENTOUS PHAGE CAPSID

In contrast to virulent bacteriophages that complete their life cycle by lysing the bacterial cell, filamentous phages do not kill their host. New phage particles are secreted without breaking the integrity of the cell envelope. The secreted particles consist of a single molecule of circular single-stranded DNA covered in a tubular array by approximately 2,700 copies of a small helical protein, the product of gene VIII (for a review see [7]). The virion structure has been studied in some detail by physical methods, and a model of the helical arrangement of the major coat proteins has been proposed [8]. The two ends of the viral filament are capped by two structures formed by four minor coat proteins that are present in 4–5 copies per phage particle; the products of gene VII and IX, on one side, and VI and III, on the other side (Fig. 1). pIII, which emerges last from the infected bacterium, can be proteolytically split into two domains of similar dimensions [9]; the C-terminal domain is necessary to stabilize the viral particle and to prevent the formation of polyheads while the N-terminal domain binds to the phage receptor, the tip of F pilus in male bacteria.

Smith and his collaborators have first shown that short peptides can be inserted close to the N-terminus of the pIII protein without preventing its ability to assemble and to recognize the bacterial receptor [1,10,11]. Furthermore the hybrid protein displays the foreign peptide in a form that can be recognized by a cognate antibody. The same authors have developed an efficient panning technique (Fig. 2) that permits selection of phage particles presenting a peptide that binds an antibody molecule [10,12]. This technique can be easily extended, with minor modifications, to target molecules that are different from antibodies. An important char-

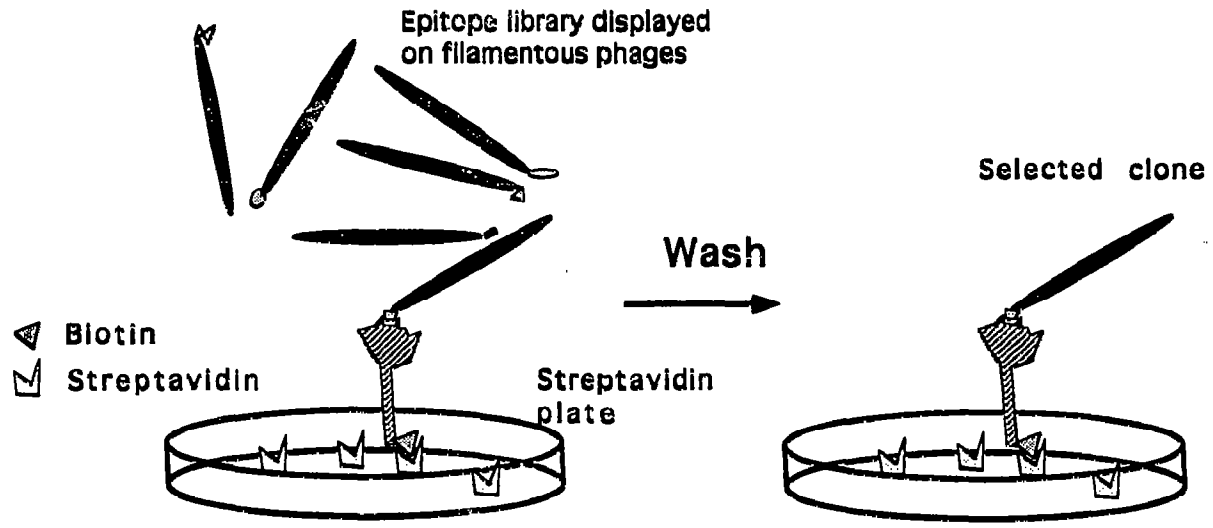

Fig. 2. Selection from an epitope library of a filamentous phage that displays a peptide that binds a Fab fragment of immunoglobulin. The Fab fragment is linked to a plastic Petri dish via a biotin–streptavidin bridge. The selection technique, known as biopanning, is described in detail by Scott and Smith [13]

acteristic of filamentous phages is their ability to survive exposure to extreme conditions (95°C, pH 2) thereby allowing recovery of the bound phages by breakage of the non-covalent bonds between displayed and target molecules without eliminating phage infectivity.

Although only a limited number of experiments have been published, evidence is accumulating that PET is rather general and is subjected to few if any limitations. Most likely any peptide that can be passively translocated across a membrane, once fused to the N-terminus of mature pIII protein, will be displayed on the phage capsid. It is conceivable, however, that some of these N-terminal extensions will decrease or abolish phage infectivity [10]. Even in these cases, however, phage infectivity can be preserved by constructing hybrid phage particles where most pIII copies are wild-type while only one or a few are hybrid pIII carrying the N-terminal extension [13]. This is achieved by phenotypic mixing of the hybrid pIII synthesized by a gene carried on a plasmid and the wild-type gene III product synthesized by a superinfecting phage. If the resident plasmid contains the filamentous phage replication origin (phagemid), the supernatant of the bacterial culture will contain phage and phagemid particles that are both infective and display the peptide fused to pIII, as encoded in the phagemid gene. A further advantage of the phagemid system is the ability to decrease the number of copies of peptides displayed down to fewer than 1 copy per phage particle. This means that each phage is monovalent with respect to binding to the target molecule, thus eliminating possible avidity effects during the enrichment procedure.

A second displayed method, also based on filamentous phages, was developed more recently. It relies on the ability to incorporate short peptides on phage capsids by fusion to the N-terminus of gene VIII protein [14–16]. In contrast to the 4–5 copies that constitute the maximum number of peptides that can be presented on a single phage particle by fusion to pIII, the utilization of pVIII as a carrier permits to cover a phage with up to approximately 2,700 peptides. In practice, however, it has been observed that hybrid pVIII proteins extended at the N-terminus with peptides that are longer than six amino acids do not form viable phage particles [15,16]. This is probably due to some interference between the closely packed N-terminally extended subunits. As a consequence, for most applications, it becomes vital to use a phagemid vector carrying the modified gene VIII. By superinfection with a wild-type helper phage it is then possible to assemble hybrid capsids where the mutated protein VIII is dispersed in an otherwise wild-type capsid. Peptides as small as six amino acids [15] or as long as an entire Fab fragment [17] have been successfully linked to phage capsids by this method. A survey of peptides of 9 amino acids of random sequence has indicated that at least 80% of them can be successfully translocated across the membrane and presented on phage capsids when inserted after the fifth amino acid of the mature pVIII (our unpublished results). The density of the peptide on the phage surface depends somewhat on the peptide sequence and varies between 1,000/phage (approximately 1/3 of the total number major capsid protein subunits) and less than 100/phage. By controlling the activity of the promoter (pLac) in front of gene VIII on the plasmid, it is also possible to modulate, within a factor of ten, the density of a given peptide (our unpublished results).

4. OLIGOPEPTIDE LIBRARIES

The first demonstration of the power of the PET technology came from model experiments that used antibodies as ligate molecule to select ligands from large 'epitope' libraries made of 10^7–10^8 hybrid phages each displaying an oligopeptide of different sequence.

Scott and Smith [11] constructed a library of 200 million different hexapeptides inserted after the first four amino acids at the N-terminus of mature pIII. A subset of this library was used to demonstrate that the biopanning technique is sufficiently sensitive and selective to recover, from this random collection of peptides, those that bind two monoclonal antibodies raised against the hexapeptide, DFLEKI.

In an analogous experiment Dower's group [18] constructed a 300 million hexapeptide library, in which the variable region begins at the N-terminus of mature pIII. A monoclonal antibody that binds tightly to the N-terminus of β-endorphin (YGGF) was used to select phage clones that present peptide ligands on the capsid. As in the previous experiment [11], the majority of the 50 clones that were characterized bore a resemblance to the peptide that was used to raise the antibody.

Our group [16] used a phagemid system to assemble a library of 37 million clones each presenting a nonapeptide of random sequence inserted after the fifth amino acid of mature pVIII. Phagemid clones that bind a monoclonal antibody raised against oligopeptide 163–171 of interleukin 1β were selected and the displayed peptide determined by DNA sequencing. The selected peptides all contain the consensus SND/E that is also present in the IL1β nonapeptide that was used to raise the antibody, thus defining the SND region as a critical region for MAb recognition.

The recovery, in the three different panning experiments, of peptides that resemble the original antigen demonstrates that the biopanning technique preferentially selects peptide clones that bind the 'antigen binding site' with atomic contacts that are similar to those of the natural epitope. Whatever its molecular explanation, this finding renders epitope mapping possible since one can expect to identify an unknown immunogenic epitope by exploiting the consensus, determined from the selected peptides, as a searching template. Stephen and Lane [19] used an hexapeptide library to identify an epitope that is unmasked by a conformational change that occurs in some p53 mutants.

Epitope libraries have also been successfully used to search for peptide ligands that mimic non-proteinous ligands. Devlin's group selected, from a decapentapeptide library on pIII, peptides that mimic biotin binding to streptavidin identifying the consensus tripeptide HPQ. Phages bearing the consensus peptide, YPY, were selected by two different groups from an exapeptide and an octapeptide library using the plant lectin, concanavalin A (Con A), as a ligate [20,21]. This tripeptide (YPY) was found to be highly selective for Con A since phages exposing these peptides do not bind to closely related pea or lentil lectins.

Although we are still in the beginning of the development and exploitation of the PET technology, there is expectation that, with the construction of slightly more complex and sophisticated libraries (larger size, constrained peptides, mixtures of peptide libraries in different structural contexts, etc.), the method could be generally used to find ligands for practically any target molecule. It is not difficult to imagine the possible applications of this type of approach to the development of vaccines or diagnostics and to the discovery of ligands for targets of medical interest. The structural information extracted from the consensus, derived from the selected peptides, could then be used to develop non-peptide drugs of therapeutic interest.

5. DISPLAY OF PROTEINS OR PROTEIN DOMAINS

The applications of PET technology are not limited to the display of libraries of small peptides of random sequence. Although it has been shown that some peptides of larger sizes (approx. 100 amino acids) may prevent phage propagation by interfering with the receptor recognition function of pIII [10], the development of phagemid vectors that permit the assembly of single hybrid pIII protein on otherwise wild-type phage capsids (monovalent display), has removed this limitation. Wells and collaborators have used filamentous phages that display the human growth hormone [13] to select mutants that bind to the cognate receptor with an affinity higher than that of the natural hormone [22]. A similar approach was exploited by Roberts et al. [23] to change the specificity of a protease inhibitor, thereby selecting a molecule that is 50-times more potent in inhibiting human neutrophil elastase (HNE) than any other anti-HNE Kunitz derivative described. Other large peptides that have been successfully displayed in the native conformation are the enzyme, alkaline phosphatase [24], and an immunogenic fragment of the HIV Gag protein [25].

Perhaps the most impressive application of the technology originates from the demonstration that variable fragments of immunoglobulins (either Fab or Fv) can be displayed on phage capsids (phagebodies) [17,26–31]. Combined with the ability to clone, by PCR amplification, large libraries of heavy and light chain immunoglobulin genes, this permits the enrichment of those phagebodies that bind to any ligate molecule, bypassing both hybridoma technology and immunization. Recently, Winter and co-workers have shown that a single large phage library, displaying different combinations of heavy and light chain fragments from a non-immunized individual, can be used to isolate human antibodies against any antigen [32].

6. CONCLUSIONS

A discussion of future developments and applications risks to be superseded by the experiments that are now carried out in various laboratories will probably be published by the time this review goes to press. Applications are only limited by the ingenuity of scientists and are not confined to the study of peptide–ligand interaction. PET technology essentially permits the link between the ability of a peptide to reach the phage capsid and associate and dissociate from a ligand to the phage replication and amplification properties. Any phenomenon that may interfere with the assembly–association–dissociation process can be studied by this technology. Among these are peptide secretion, protein folding and stability, and formation of covalent bonds during an enzymatic reaction.

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