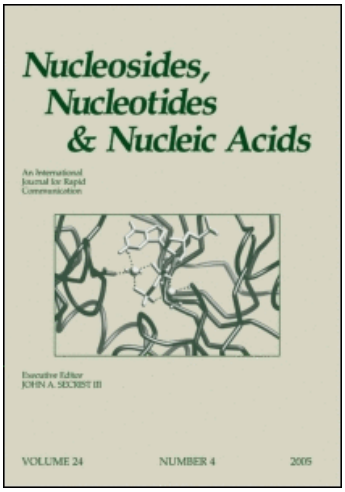

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A DNA-BINDING PEPTIDE FROM A PHAGE DISPLAY LIBRARY

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A DNA-BINDING PEPTIDE FROM A PHAGE DISPLAY LIBRARY

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ABSTRACT

A DNA-binding peptide was selected from a random peptide phage display library. For competitive elution using the DNA methyltransferase M · *Taq*I in the selection step, a biotin-labeled duplex oligodeoxyribonucleotide containing the 5′-TCGA-3′ recognition sequence of M · *Taq*I was employed. Nine of ten phages selected were found to have the same deduced amino acid sequence SVSVGMKPSPRP. The selected phage binds to DNA, as demonstrated in an ELISA.

In contrast to DNA-binding proteins with a built-in secondary and tertiary structure only very few short DNA-binding peptides have been identified by use of phage display libraries, in which random peptide sequences are displayed fused to coat proteins of filamentous phages, allowing the simultaneous screening of a vast number of peptides (1,2). Krook *et al*. used phage display to select 6-mer peptides binding to single-stranded oligo-dC (3). However, no consensus sequence in the selected peptides was found, and only a moderate discrimination between oligo-dC, -dG, -dA and -dT was observed. In contrast to the studies so far, which selected DNA-binding phages by elution with acid, base or salts, we employed a novel way of selection using a DNA-binding protein in the elution step and describe the successful selection of a new DNA-binding peptide.

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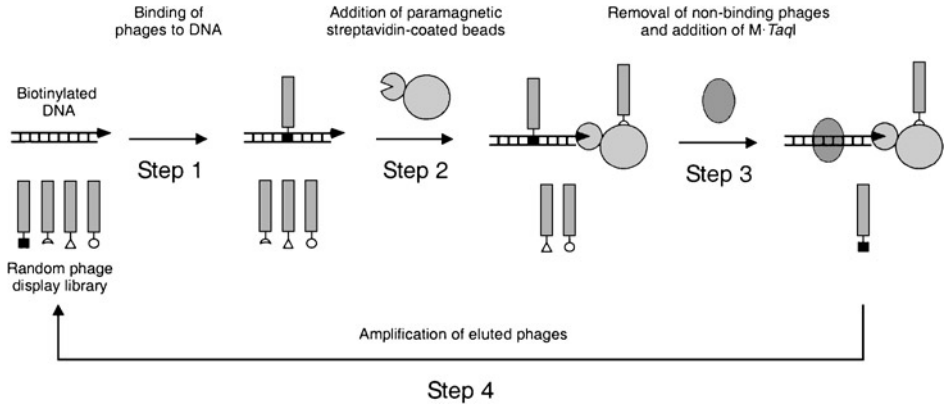
For the selection of a DNA-binding peptide we used a randomized phage display 12-mer peptide library (Ph.D.-12™ Phage Display Peptide Library Kit, New England Biolabs) consisting of 1.9 ### 10 (9) recombinants. The peptides were expressed at the N-terminus of the gene 3 coat protein (g3p) of the filamentous phage M13. The preparation of the DNA methyltransferase M · *Taq*I was described previously (4). We had observed that the N6-adenine DNA methyltransferase M · *Taq*I binds with high affinity to double-stranded oligodeoxyribonucleotides (duplex ODNs) containing the 5′-TCGA-3′ recognition sequence. The binding affinity is even increased, if the recognition sequence is flanked by a stable abasic site (1,2-dideoxy-D-ribose) (Δ) at the 3′-end (5). We, therefore, synthesized a biotinylated duplex ODN of 36 base pairs, containing the sequence 5′-TCGA###Bio-3′, which could lead to the isolation of DNA-binding peptides after elution with M · *Taq*I. Recently, we found that the dissociation constant of the complex between M · *Taq*I and the duplex ODN TCGA### is in the picomolar range (5). Consequently, competitive displacement with M · *Taq*I could lead to the isolation of DNA-binding phages with very high affinities. For the immobilization of the target duplex ODN TCGA###Bio on streptavidin-coated paramagnetic beads the 3′-end of the ODN strand containing the abasic site was labeled with biotin via a PEG-linker.

The selection procedure is described in Scheme 1. The N-terminal amino acid sequence SVSVGMKPSPRP was identified in nine out of ten phages sequenced after eight rounds of selection. DNA-binding of the phage containing the SVSVGMKPSPRP peptide (selected phage) and of a sequenced phage of the primary library (control phage), containing the amino acid sequence FSIVQLSPSSMS



Scheme 1. Selection of DNA-binding phages from a random peptide phage display library using a DNA-binding protein for competitive elution in the selection step. The instruction manual for the Ph.D.-12™ phage display peptide library kit by New England Biolabs (1997) was adapted. A random phage display library and biotinylated DNA are incubated (Step 1) and paramagnetic streptavidin-coated beads are added to this solution (Step 2). After incubation, biotin is added to reduce binding of phages to streptavidin (not shown). Then, non-binding phages are removed and the DNA methyltransferase M · *Taq*I is added to the beads (Step 3). The eluted phages are amplified (Step 4) and used in the next selection round.

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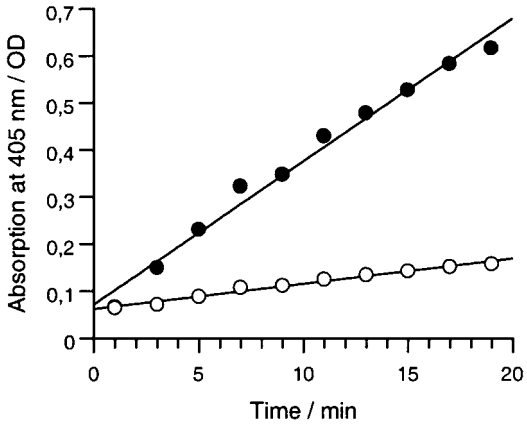

Figure 1. DNA-binding of the selected phage and of the control phage from the primary library by enzyme-linked immunosorbent assay (ELISA). The duplex ODN TCGA###Bio was immobilized in the wells of a microtiter plate coated with streptavidin and the selected phage containing the SVSVGMKPSPRP peptide (closed circles) or a control phage from the primary library (open circles) were added. After addition of horseradish peroxidase / Anti-M13 conjugate, hydrogen peroxide and ABTS (2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid)) the reaction was followed by measuring the increase of UV absorption at 405 nm in a microtiter plate reader.

was tested in an enzyme-linked immunosorbent assay (ELISA) (Fig. 1). The selected phage led to an UV absorption increase of 30 mOD/min, while the control phage from the primary library yielded only an increase of 5 mOD/min. An UV absorption increase of 5 mOD/min was also observed for both phages in the absence of TCGA###Bio (data not shown) and can be regarded as the background of this assay. Thus, the phage containing the SVSVGMKPSPRP peptide in fact binds to DNA.

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