Cellulose-binding Heptapeptides Identified by Phage Display Methods

Takeshi Serizawa,*[1,2] Kyoko Iida,[1,3] Hisao Matsuno,[4] and Kimio Kurita[3]
[1]*RCAST, The University of Tokyo, 4-6-1 Komaba, Meguro-ku, Tokyo 153-8904*
[2]*PRESTO, Japan Science and Technology Agency, 4-1-8 Honcho, Kawaguchi 332-0012*
[3]*Graduate School of Science and Technology, Nihon University, 1-8-14 Surugadai, Kanda, Chiyoda-ku, Tokyo 101-8308*
[4]*KOL, The University of Tokyo, 4-6-1 Komaba, Meguro-ku, Tokyo 153-8904*

(Received May 18, 2007; CL-070543; E-mail: t-serizawa@bionano.rcast.u-tokyo.ac.jp)

Phage libraries displaying random and linear heptapeptides were applied to crystalline cellulose. Binding affinities of obtained phage clones were quantitatively analyzed by enzyme-linked immunosorbent assays (ELISAs). Clones were obtained with greater apparent affinity constants (K_{app}s) than those of the library, suggesting the isolation of cellulose-binding peptides with characteristic sequences.

Due to recent energy issues, efficient methods for producing "bio-" ethanol have gained increasing interest. A possible raw material to produce bio-ethanol is naturally abundant cellulose that originates from wood and grass. Unfortunately, cellulose is water-insoluble, and as such, its degradation is commonly inefficient. A significant and environmentally favorable solution is to enhance cellulase activities during fermentation processes.

Cellulase has a tadpole shape composed of cellulose-binding modules (CBMs) with approximately 40 amino acids and catalytic domains.[1] It is known that affinity constants of CBMs against cellulose are relatively small ($<10^6\,M^{-1}$).[2] Increases in CBM affinities are an important target for cellulase modifications. We are interested in cellulose-binding short peptides, because minimized peptides can be used as tandem or clustering motifs for the building up of artificially designed CBMs with higher affinities. However, it is unknown as to whether short peptides with possible irregular conformations potentially recognize cellulose.

Combinatorial biotechnologies such as phage display and cell-surface display methods have enabled to the identification of short peptides that specifically bind to artificial material surfaces.[3] Peptides that recognize synthetic polymers with simple chemical structures similar to cellulose have already been revealed.[4] Accordingly, the aforementioned methods are possible candidates to find novel short cellulose-binding peptides.

In this study, heptapeptides that bind to cellulose were identified from phage display libraries, as schematically shown in Figure 1. K_{app}s of selected phage clones were estimated from dependences of bound phages against phage concentrations by ELISAs, and were compared with those from the primary library. Resulting peptide sequences with greater affinities were classified in terms of amino acid species and positions.

A phage library displaying random heptapeptides (Ph.D.-7 Phage Display Peptide Library Kit, New England Biolabs, Inc.) was applied to cellulose microcrystallines (Avicel® purchased from Merck). Biopanning processes including phage affinities to cellulose, washing of weakly adsorbed phages, elution of bound phages, and phage amplification were repeated for five rounds and under two conditions. Condition 1: Tris-buffered saline (pH 7.4) (TBS) containing 0.05 wt % Tween-20 (TBST) and 0.5 M glycine–HCl (pH 2.2) were used for washing and elution, respectively. Condition 2: TBST containing 1–30 mM cellobiose and TBS containing 0.1 M cellobiose were used, respectively. Tubes and micropipette chips composed of 100% polypropylene were used for biopanning. Conventional methods were used for cloning and DNA sequencing. ELISAs quantified amounts of phages bound onto cellulose. Experimental details are shown in the Supporting Information.[5]

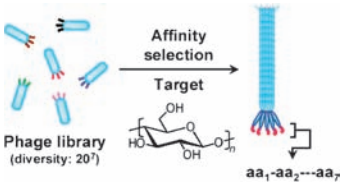

Figure 1. Schematic representation of this study.

Yields, which were estimated by percentages of collected phages relative to applied phages, tended to increase with increasing biopanning rounds (Figure S1),[5] suggesting condensation of the library to the phage pool specific for cellulose. Accordingly, we moved to cloning and sequencing of the phage

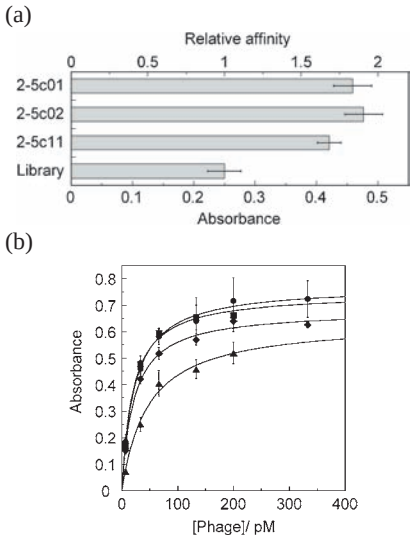

Figure 2. (a) Relative affinities determined by ELISAs at a 33 pM phage concentration. (b) Dependences of absorbance against the phage concentration for 2-5c01 (circle), 2-5c02 (square), 2-5c11 (diamond), and the library (triangle). The curves show the Langmuir fit.

Table 1. Peptide sequences and K_{app}s of identified phage clones and the phage library

Phage[a]	Sequence[b]	$K_{app}/10^{10}$ M^{-1}
2-5c02	HAIYPRH	5.18 ± 0.80
2-5c01	SHTLSAK	4.88 ± 0.69
2-5c11	TQMTSPR	4.86 ± 0.57
1-4c16	YAGPYQH	4.57 ± 0.60
2-5c04	LPSQTAP	4.56 ± 0.21
2-5c09	GQTRAPL	4.49 ± 0.50
1-4c10	QLKTGPA	4.38 ± 0.49
1-4c09	FQVPRSQ	4.28 ± 0.48
2-4c14	LRLPPAP	4.28 ± 0.78
Library	-	2.78 ± 0.48

[a]The first and second numbers for the clone name show the condition and the biopanning round numbers, respectively. [b]Blue: aa containing hydroxy groups; red: aa containing amino groups; green: aliphatic aa; black: others.

pool. Resulting amino acid (aa) sequences of all peptides isolated on two biopanning conditions are shown in Tables S1–S3.[5] Considering clone frequencies, the condensation seemed to proceed successfully on condition 1. However, the frequencies sometimes did not correlate to target specificities, because certain clones seemed to be readily amplified by *Escherichia coli* (*E. coli*). This phenomenon is also outlined in the present case (see below).

Typical ELISA data from a 33 pM phage concentration are shown in Figure 2a. Greater amounts of clones bound onto cellulose compared to the library, suggesting specific affinities of the clones for cellulose. To more quantitatively analyze the affinities, dependences of relative affinities against phage concentrations were estimated, as shown in Figure 2b. Isotherms were saturated against concentrations. Curves were fitted by assuming a Langmuirian adsorption to obtain K_{app}s (coefficients of determination ranged 0.95–0.99), and peptide potentials were analyzed similarly to previous studies.[4d–4f]

The K_{app}s of nine superior clones are shown in Table 1 (for all K_{app}s, see Tables S1–S3).[5] K_{app}s were within the high order of 10^{10} M^{-1}. The K_{app}s may be explained by the following reasons: (1) the non-specific adsorption of phage bodies with 5–6-nm diameters and approximately 1-μm length increased K_{app}s and (2) multiple interactions of 3–5 copy peptides displayed on phage pIII coat proteins increased K_{app}s. Our previous studies similarly observed the high K_{app}s.[4d–4f] Therefore, relative differences of clone K_{app}s from the library K_{app} were discussed.

The K_{app}s were obviously greater than that of the library, indicating that identified peptides show cellulose specificities. The differences are significant if we consider that much smaller peptides are conjugated on much greater filamentous phages. However, the difference of K_{app}s between clones and the library was slightly smaller than in our previous studies.[4d–4f] This study used cellulose particles for ELISAs instead of previous polymer films. Therefore, the present K_{app}s might be underestimating the difference due to inefficiencies of washing out of non-specifically adsorbed phages.

We found significant tendencies for species and positions of aa components in peptide sequences. The sequences were classified into two homogeneous groups, as shown in Figure 3.

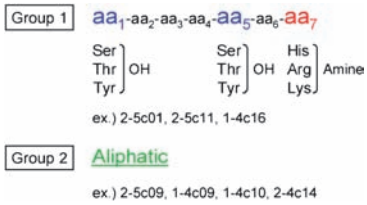

Figure 3. Two patterns of cellulose-binding heptapeptides.

For group 1 corresponding to 2-5c01, 2-5c11, and 1-4c16 with greater affinities, aa_1 and aa_5 were enriched by aa with lateral hydroxy groups, and aa_7 was enriched by aa with amino groups. For group 2 corresponding to 2-5c09, 1-4c09, 1-4c10, and 2-4c14 with smaller affinities, plural aliphatic aa were enriched. The former will interact with cellulose through hydrogen bonds, while the latter will interact through hydrophobic effects. These interactions are similarly utilized by CBMs when CBMs interact with cellulose.[1]

It is necessary to discuss why two groups were obtained. Since the present cellulose microcrystalline contains certain amounts of amorphous components. Therefore, two peptide groups might bind to crystalline and amorphous cellulose. Note that certain CBMs recognize the aforementioned difference.[1] On the other hand, the obtained peptides might recognize the same cellulose component through different interaction modes. Detailed affinity analyses using peptides freed from phages will reveal the mechanism at a molecular level.

This is the first report that quantitatively reveals possible heptapeptides that potentially accommodate cellulose by using phage display methods. Affinity constants of phage clones showed potential affinities of short peptides against cellulose. Peptides with relatively greater affinities were classified into two groups. Peptide affinities depending on different physicochemical states of cellulose are currently being analyzed.

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References and Notes

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5 Supporting Information is available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html.

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