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Identification of C10 biotinylated camptothecin (CPT-10-B) binding peptides using T7 phage display screen on a QCM device

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Abstract—A peptide sequence that can bind to camptothecin (CPT), a natural cytotoxic compound, was screened for using a T7 phage display system combined with a cuvette type quartz crystal microbalance (QCM) device. In this screen, after only 10 min of monitoring of the interaction between injected T7 phage pool with immobilized C10 biotinylated CPT (CPT-10-B) on a gold electrode surface, six different kinds of phage (A–F) were identified as judged by the size of PCR product on agarose gel electrophoresis. Injection of each single phage (A–E) pool individually caused a frequency decrease, suggesting interaction with the immobilized CPT-10-B. In addition, the peptide sequence displayed on phages A–C is consistent with chemical and biological studies of the interaction of CPTs with topoisomerase I (TopI), human E prostanoid receptor third cytoplasmic polypeptide, and a series of esterases. The efficacy of T7 phage display screening for small molecules on QCM devices, target discovery from primary peptide sequence, and application of this strategy to various drug-like small molecules are discussed.

1. Introduction

Phage display is a molecular biological tool that allows discovery of target-binding peptide sequence.[1,2] In conventional screening, a target of interest is initially immobilized on a carrier, such as a polystyrene microplate, agarose, or sepharose beads or gel.[3–6] An aliquot of phage pool is then added and incubated for several hours. After washing more than 10 times (each wash is 5 min in length and thus the total wash time is 50 min) with adequate saline solution which contains detergent, salt, or denaturant to remove the unbound or non-specifically bound phage, the remaining phage is dissociated using a saline solution containing higher concentrations of detergent, salt, denaturants, or a mixture thereof. For use in the next round of screening, the recovered phage particles are amplified by infection of a log-phase culture of *Escherichia coli*. Following several rounds of selection, a portion of the recovered phage clones are arbitrarily isolated from the whole phage pool and disrupted to allow extraction of phage DNA. The gene coding for the region of capsid protein is cloned by PCR and then separated by electrophoresis on agarose gel to characterize the population of recovered phage with respect to size of PCR product. Finally, the base sequence is determined and translated into peptide sequence and thus the peptide sequence displayed on the phage capsid that potentially interacts with the target molecule is ascertained.[7–9]

Previously, using conventional T7 phage display methods with a 96-well streptavidin-coated polystyrene microplate, we identified a CPT-20-B-binding phage displaying NSSQSARR on the phage capsid (Fig. 1b).[9,10] In this screen, no adequate elution conditions were found for the second and third round. We therefore had to directly recover binding phages using host cell solution in order to obtain CPT-20-B-binding phage. In T7 phage display screens that use a series of these end-point methods, determination of the wash and elution condition can be quite difficult and is the rate-limiting step because these conditions vary depending on the physico-chemical properties of each target. In addi-

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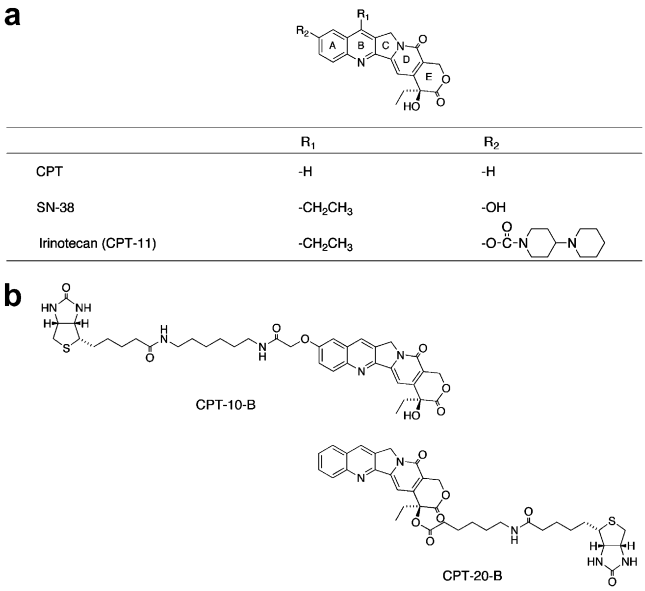

Figure 1. Structure of CPTs (a) and biotinylated derivatives (b).

tion, when evaluating a screen, more than 10^9 plaque forming units (pfu) of phage pool are needed in order to detect potential target-binding phage. This causes a concurrent increase in the non-specific carrier binding phage and produces false positives. These background phages are particularly problematic in T7 phage display screening of small molecules and make the screening data ambiguous.

In this study, we performed a CPT-10-B-binding peptide screen using a T7 phage display system combined with a cuvette type QCM device, AffinixQ (Initium Inc., Tokyo, Japan). QCM is a very sensitive and accurate mass measuring signal-converting chip whose signal is derived from the distortion of a crystal (SiO_2, 0.06 mm thick, 9 mm in diameter, 64 mm^2) produced by an alternating current and converted to a resonant frequency that proportionally decreases as the mass increases on the gold electrode (Au, 0.1 mm thick, 2.5 mm in diameter, 4.9 mm^2).[11,12] If phage particle injected into a cuvette which contains a saline solution stirred at a rate of 1000 rpm binds to the immobilized target on the gold electrode surface, the binding can be detected as a frequency decrease in real time. Using this screening environment with a smaller amount of phage pool and without troublesome exploration of washing or elution conditions, we attempted to capture CPT-10-B-binding phage from a phage pool and identify the potential CPT-binding peptide sequence on phage capsid. We have also related the resulting peptide sequences to the results of other work in relation to the bioactivities of CPTs.

2. Results

2.1. Screening of CPT-10-B-binding phage on a QCM device

Using a T7 phage display system with a QCM device, AffinixQ (Initium), we screened for CPT-10-B-binding phage (Fig. 1b).[11,12] The strategy is summarized in Figure 2. A ceramic QCM sensor chip whose gold electrode surface was coated with avidin bound to approximately 3 ng of CPT-10-B (Fig. 1b) was attached to the device. After stabilizing the QCM sensor, 80 µl of a T7 phage library was injected into a cuvette containing saline solution. As shown in Figure 3, a decrease in frequency was observed after injection of the T7 phage library, indicating that phage clones may interact with the immobilized CPT-10-B. After monitoring the frequency decrease for 10 min,

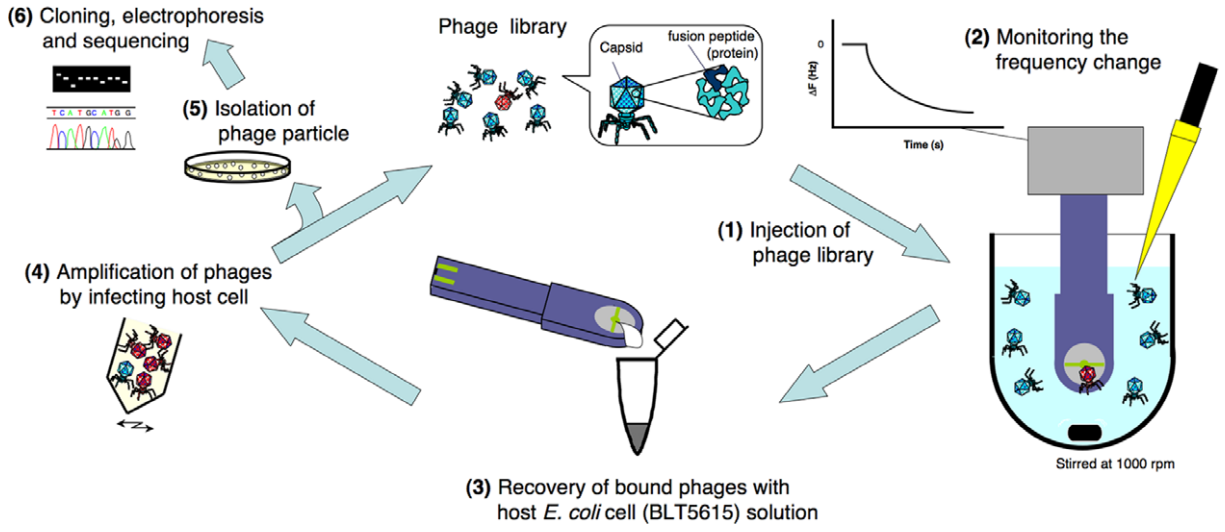

Figure 2. Schematic diagram of T7 phage display screen using cuvette type QCM device (AffinixQ, Initium Inc.). (1) An aliquot of T7 phage library is injected into a cuvette containing saline solution stirred at 1000 rpm. (2) The frequency change that occurs upon binding of the phages to a target of interest immobilized on the gold electrode is monitored in real time. (3) The bound phages are recovered using host cell (*E. coli* BLT5615, Novagen) solution. (4) The resulting solution is added to another host cell solution and amplified. (5) A part of the recovered phage is arbitrarily isolated. (6) The capsid gene-coding region of each phage is cloned and analyzed by agarose gel electrophoresis followed by DNA sequencing.

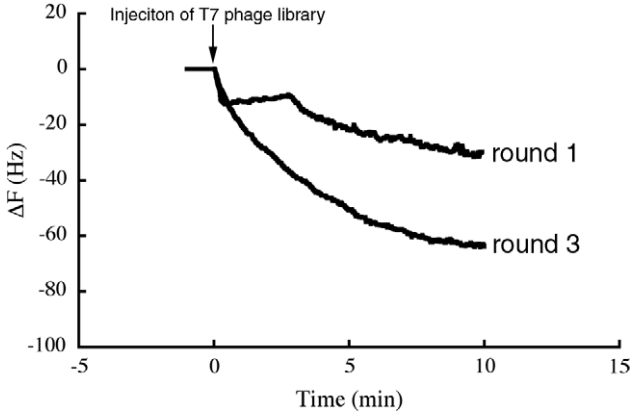

Figure 3. Sensorgram of T7 phage display screen with QCM device. After injecting an aliquot of 10^8 pfu (final) of T7 phage library, the frequency change was monitored for 10 min.

the sensor chip was dislodged from the device. The remaining phages on the gold electrode surface of the sensor chip were recovered using host cell (*E. coli* BLT5615) infection and then amplified for use in the next round of screening. It is noteworthy that the technique did not require troublesome rounds of washing and elution as needed in conventional methods. Thus, the length of the screening process was dramatically shortened and most of the operational errors reduced. After performing three rounds of selection, a portion of the recovered phages was arbitrarily isolated and part of the capsid-coding gene amplified by PCR and then analyzed using agarose gel electrophoresis. As shown in Figure 4a and b, a clear enrichment of several kinds of phage clone was observed in the first round of selection in the QCM screen. These were classified into six groups (A–F) based on the size of PCR product. Such remarkable enrichment was not observed even after four rounds of selection using a 96-well streptavidin microplate (Fig. 4c). The peptide sequence displayed on each phage capsid and which potentially binds to CPT-10-B was then determined. The phage clone groups were found to have different peptides on their capsid with length and sequence being quite different (Table 1).

Table 1. Summary of screening results and (poly)peptide sequences

Phage	Ratio	PCR product (bp)	Peptide (mer)	Sequence
(A)				
A	11/41	520	5	NSLKD
B	8/41	290	53	NSEDQIPAHGPERETQAL GGHVPLALQ PRCKVGDG LEEGGREAVQSLRPHSSN
C	5/41	220	11	GDPNSVILFKQ
D	2/41	380	14	NSTRDTRRNWYHSF
E	1/41	560	9	NSNATDGGP
F	14/41	190	19	NSPAGISRELVDKLAAALE
(B)				
A	29/47	520	5	NSLKD
B	3/47	290	53	NSEDQIPAHGPERETQAL GGHVPLALQ PRCKVGDG LEEGGREAVQSLRPHSSN
C	5/47	220	11	GDPNSVILFKQ
E	1/47	560	9	NSNATDGGP
F	9/47	190	19	NSPAGISRELVDKLAAALE

(A) The results of the first round. (B) The result of the third round. Phage F (NSPAGISRELVDKLAAALE) is a control phage that is naturally generated with no insertion of cDNA.

2.2. Characterization of the interaction between CPT-10-B and single phage

In order to characterize the interaction with CPT-10-B, each phage was amplified by infection of host cells and used for binding experiments. For the screening procedure, an aliquot of 10^8 pfu of each single phage pool

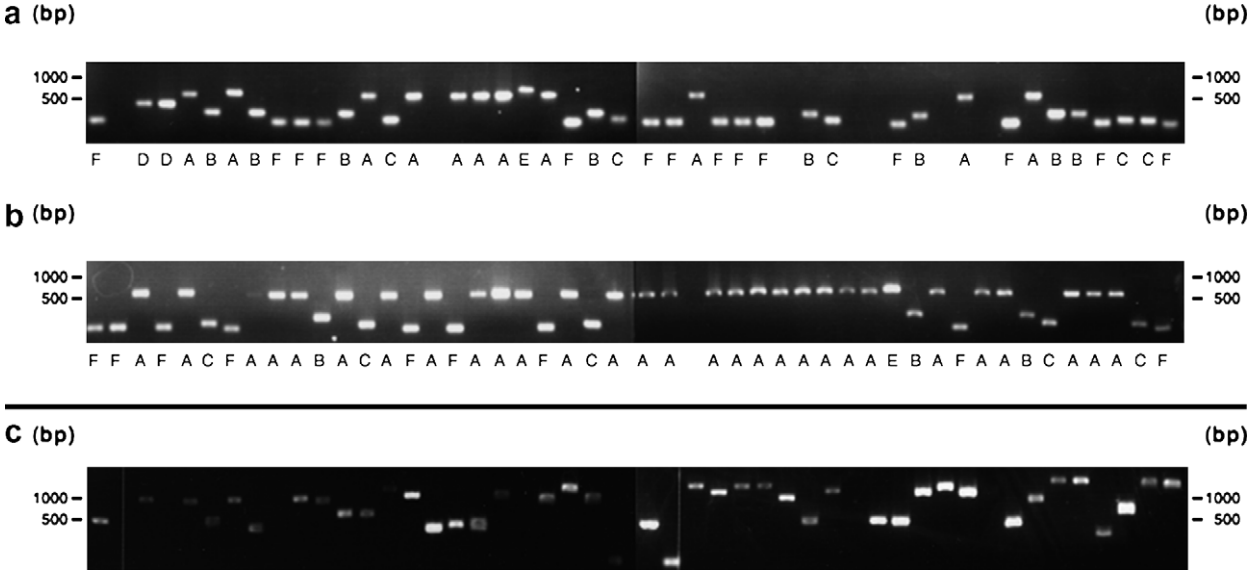

Figure 4. 1% Agarose gel electrophoresis. (a) Population of phage particles arbitrarily selected after first round of selection. (b) After third round of selection. (c) After fourth round using 96-well streptavidin-coated microplate.

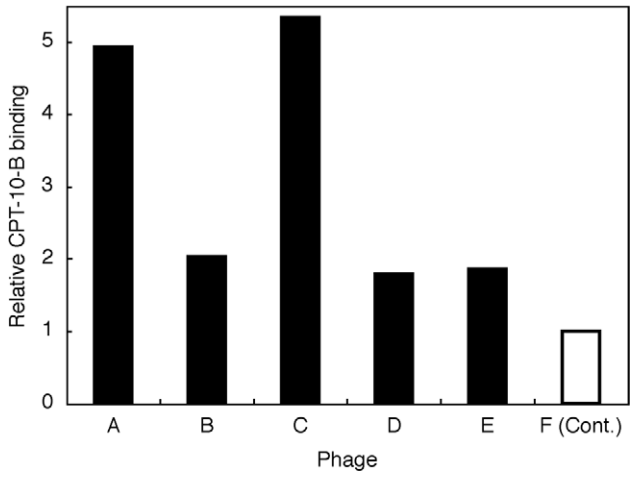

Figure 5. Relative binding of phage clones to CPT-10-B. An aliquot of 10^8 pfu (final) of phage was injected and the frequency changes monitored for 10 min. The size of each frequency change is compared with that seen with control phage F (white bar) and shown as a black bar.

was individually injected into the cuvette and frequency change monitored for 10 min. Figure 5 shows the relative binding of each phage to CPT-10-B as calculated from the frequency decrease 10 min after injection. The frequency change obtained with control phage F is also shown as a reference. Phages A and C, which were both enriched in the first and third round of enrichment, showed the strongest interaction with CPT-10-B. Injection of phages B–E also yielded an approximately twofold higher frequency change than that of control phage F, signifying interaction with CPT-10-B.

2.3. Biological significance of CPT-10-B-binding peptide sequences

Since the binding experiment clearly demonstrated interaction between CPT-10-B and the obtained phages with the exception of phage F (Fig. 4), an amino acid residue or a sequence included within these peptides should chemically recognize the immobilized CPT molecule. Based on the chemical and biological information available about CPTs reported, we have analyzed the significance of the obtained peptide sequence.

2.3.1. 5-mer peptide, NSLKD on phage A. The manner of docking between CPT and human topoisomeraseI (hTopI), reported as a molecular target of CPT,[13] has already been precisely evaluated.[14–16] In the proposed docking model, side chains of Arg364, Lys532, Asp533, and Asn722 of hTopI and a base of DNA surround the CPT, forming a ternary complex.[14–16] Interestingly, the sequence NSLKD of phage A (the most enriched phage in the first and third rounds) includes Asn, Lys, and Asp residues within the sequence. Thus, it is possible that this peptide may be able to mimic Asn722, Lys532, and Asp533 of hTopI, which form hydrogen bonds with the 16a carbonyl, 21 carbonyl and 20 hydroxyl group of CPT (Fig. 6a). In addition, because the biotin linker was introduced at the C10 position of CPT, the manner of interaction between immobilized CPT-10-B and NSLKD on the phage capsid should be similar even though the ligand is attached to the gold electrode surface. Such information cannot be obtained from a simple homology search. Moreover, without the phage display technique and the highly sensitive QCM biosensor, the interaction between such a short peptide and a small molecule would be quite difficult to detect.

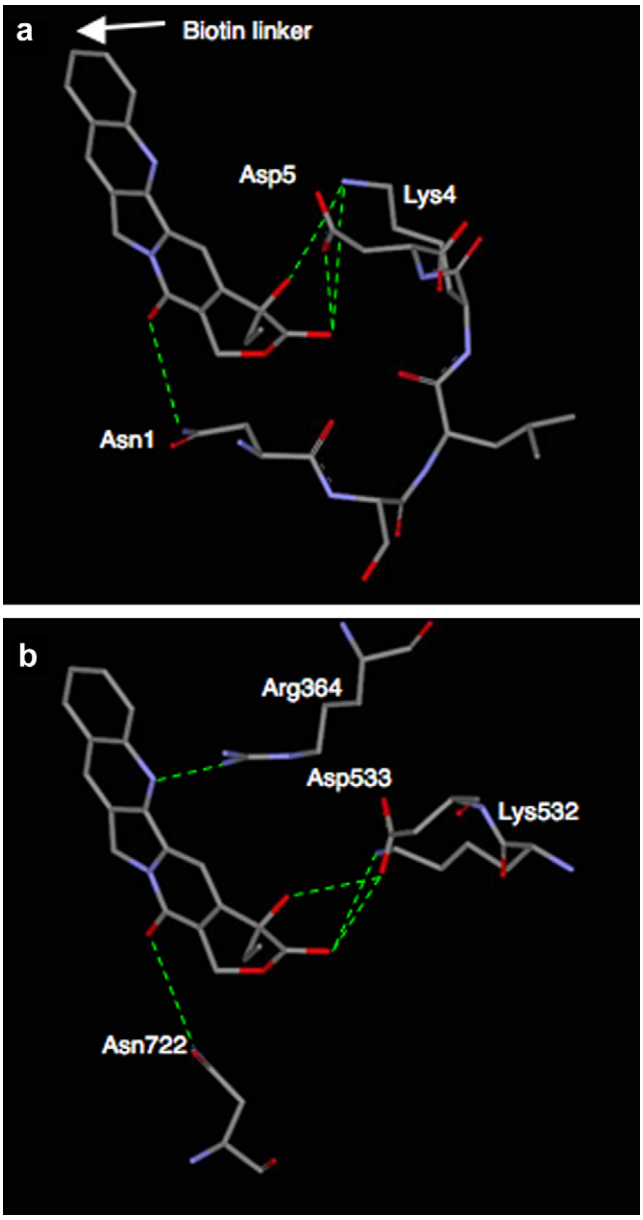

Figure 6. Binding mechanism of CPT to 5-mer peptide, NSLKD, on phage A or hTopI. (a) A possible binding mechanism of CPT to NSLKD. The conformation of NSLKD was refined by energy minimization using Insight II/Biopolymer (Accelrys Inc.) and then located nearby the E ring of CPT according to the hTopI-CPT docking model. (b) Binding model of CPT–hTopI–DNA ternary complex (PDB: 1T8I). For convenience, most amino acid residues of TopI and DNA chains are not shown. The atoms comprising the structure of CPT and NSLKD are color coded: carbon in gray; oxygen in red; nitrogen in blue. The green lines indicate the distance between two atoms connected.

2.3.2. 53-mer polypeptide on phage B. Although the strength of phage B binding toward CPT-10-B was considerably weaker than that of phages A and C (Fig. 5), the 53-mer polypeptide was found to include a partial sequence similar to 237 R-258P of the third cytoplasmic polypeptide of the hEP_1 receptor whose interaction with CPT has previously been demonstrated (Fig. 7a).[10] In our previous study, a CPT-20-B-binding peptide, NSSQSARR, was identified and found to have homology to 280R-287R in the third cytoplasmic polypeptide. This is a different position to that identified in the CPT-10-B screen. The different position of the introduced biotin linker (Fig. 1b) may be responsible for recognition by a different region within the third cytoplasmic polypeptide. Thus, the results with the 53-mer polypeptide reinforce previous data which demonstrated CPT binding to a non-structural hEP_1 receptor cytoplasmic loop.

2.3.3. 11-mer peptide, GDPNSVILFKQ on phage C. It is known that irinotecan (CPT-11), a water-soluble CPT derivative, is converted to SN-38, an active TopI poison by carboxylesterase (CE) (Fig. 1a).[17–20] From a BLAST search, the 11-mer CPT-10-B-binding peptide was identified in part of human carboxylesterase (hCE). The homologous region, 210G–220E (hCE1), is highly conserved within a series of esterases and is a potentially flexible portion because this portion is adjacent to Ser221 that forms the catalytic triad with Glu354 and His468 (Fig. 7b).[21–23] The hydroxyl group of Ser221 is part of the catalytic core, forming a covalent bond with a dipiperadino moiety at the C10 position of CPT-11 and producing SN-38.[17–20]

The cholinergic effect of CPTs has been demonstrated previously.[24–26] In particular, CPT-11 shows the strongest inhibitory activity against human acetylcholinesterase (hAChE) by binding directly.[24] Furthermore, a co-crystal structure of CPT-11 and torca AChE (*Torpedo californica* AChE, *Tc*AChE) has recently been reported.[27,28] The highly conserved region mentioned above is also found within a series of ChE as well as CE. A BLAST search using the 11-mer CPT-10-B-binding sequence also succeeded in identifying hAChE and butyrylcholinesterase (hBChE) (Fig. 7b).

2.3.4. Others. The interaction between fusion peptides of phages D and E and CPT-10-B was also observed in binding experiments (Fig. 5). Although BLAST searches gave a large number of protein hits having homology with these two peptide sequences, no target protein was found that had biological relevance given current knowledge of the biology of CPTs. However, from the

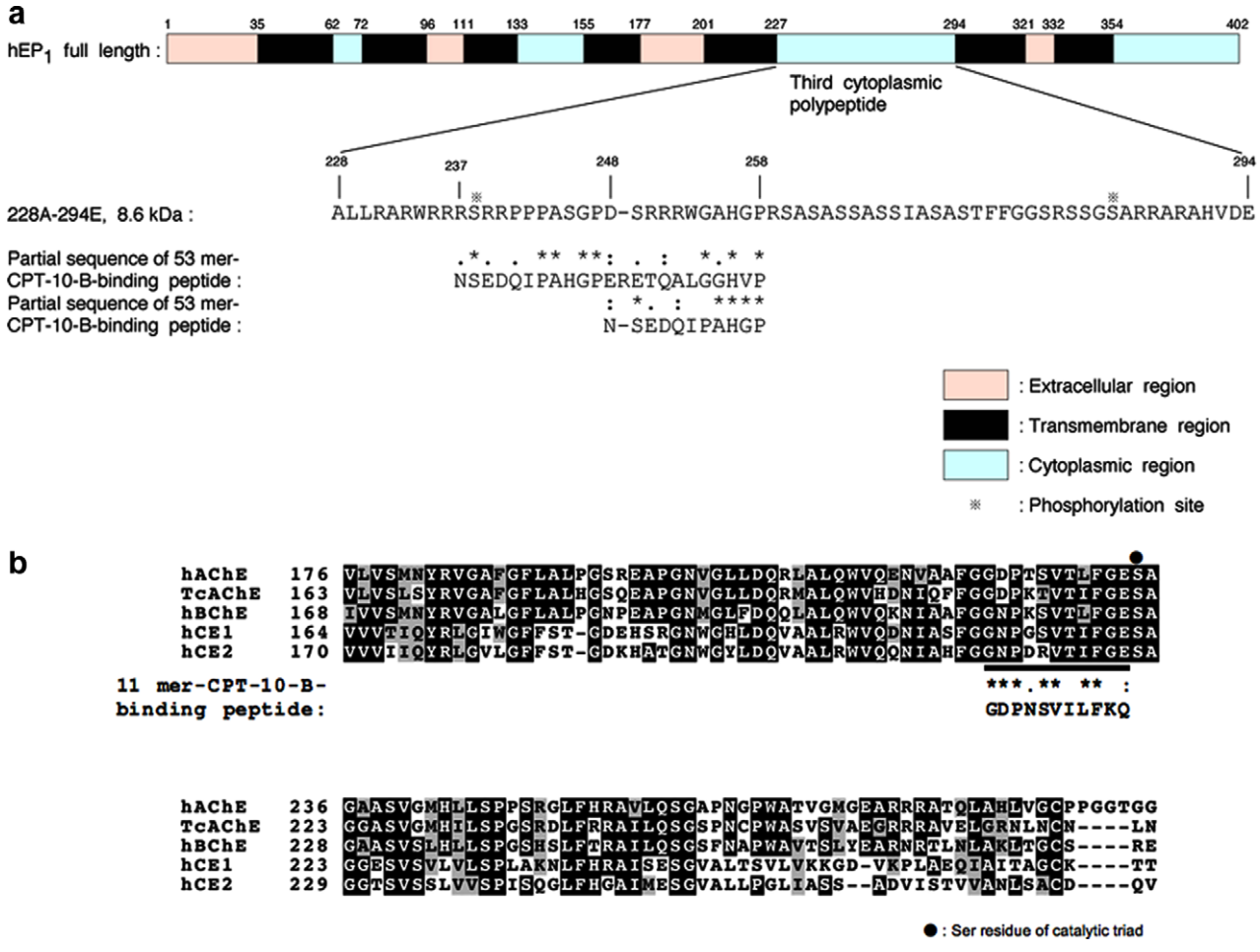

Figure 7. Similarity between CPT-10-B-binding (poly)peptide and protein. (a) Partial peptide sequence of 53-mer CPT-10-B-binding peptide (on phage B) and hEP_1 receptor third cytoplasmic polypeptide. (b) 11-mer CPT-10-B-binding peptide, GDPNSVILFKQ (on phage C) and esterase.

list of these proteins, new findings as well as new biological targets of CPT may be discovered.

3. Discussion

3.1. QCM device as a platform for T7 phage display screen

In this study, we screened CPT-10-B-binding phage using T7 phage display combined with a QCM device, AffinixQ. Although the platform itself combined with QCM is not new for phage display,[29–37] this study seems to be the first to report the combination of T7 phage display screen with QCM biosensor for target discovery of drug-like organic compounds. Compared with conventional end-point style phage display, this strategy needs no wash and elution conditions exploration or troublesome handling. In addition, the target-binding phage could be captured within 10 min, monitoring of binding was in real time (Fig. 3), and there was no need for repeated selection (Fig. 4a and Table 1A). Moreover, the background phage detection seems to be decreased in this system, at least, in the CPT-10-B screen (Fig. 4). Thus, this platform may be a means of phage display more effective than the conventional method.[10] To demonstrate the efficacy and generality of this platform, further peptide screening against several bioactive compounds is currently underway.

3.2. Distinctive aspects of target discovery using primary binding-peptide sequence

At least from our screening results using a QCM device, it may be considered that a search for binding proteins using the primary peptide sequence can demonstrate the importance not only of direct-binding amino acid side chains with the compound (NSLKD on phage A, Fig. 6), but also of the flexible portion near the small molecule-binding site (Fig. 7).[3,38] In the case of the 11-mer GDPNSVILFKQ on phage C, this CPT-10-B-binding peptide was used to identify a series of esterases whose interaction with CPT or its derivatives has previously been demonstrated. Although the homologous region, 210G–220E, within hCE1 does not include the directly-binding residues as proposed from the crystal structure with CPT-11,[17,18] such a flexible portion was clearly identified, indicating that amino acid residues close to Ser221 (e.g., 210G–220E), which form part of the catalytic core, may be important for recognition of the CPT skeleton by hCE.

Another T7 phage display screen with CPT-20-B using a QCM device and an identical lot of T7 phage library has also demonstrated these observations. In this screen, a 15-mer CPT-20-B-binding peptide, NSASRGGSQR GRGEH, was found among the resulting peptides. Surprisingly, part of this peptide was found to have homology to 359G–367H of hTopI, a flexible portion involved in DNA recognition and includes Arg364 that forms hydrogen bonds with CPT (Fig. 8).[14–16] Although direct interaction of CPT is only with the Arg364 side chain within this region in the proposed docking model (Fig. 6b), such a homologous fragment was specifically identified as one of the CPT-20-B-binding peptides, indicating that amino acid residues close to Arg364 may also be important when CPT forms a ternary complex with TopI and DNA.

```
                                        364
                                         |
htopI        346   HKERIANFKIEPPGLFRGRGNHPKMGMLK   374
CPT-20-B-                        *  ****.*
binding peptide:        NSASRGGSQRGRGEH
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Figure 8. Similarity between partial sequence of hTopI (359G–367H) and a 15-mer CPT-20-B-binding peptide obtained from T7 phage display screen with QCM device. The homologous region within hTopI includes 364R, which forms hydrogen bonds with the oxygen of carbonyl 21 of CPT.

The hEP_1 receptor is indicated to interact with CPT by identification of a 53-mer CPT-10-B-binding polypeptide on phage B (Fig. 7a).[10] The region implicated is the third cytoplasmic loop which is predicted to be a nonstructured and flexible portion, the three-dimensional analysis of which is likely to be quite difficult. The binding of CPT may produce structural change in the GPCR following regulation by PGE_2 binding to the extracellular domain of this receptor.[39]

Using the findings of sensitive and accurate T7 phage display screens using the QCM platform, target discovery using a primary structure approach could provide us with not only the identification of the target protein but also the specific aspects of the interaction i.e. the importance of the flexible portion near the small molecule-binding site.[3,38] Such information is hard to ascertain using the conventional three-dimensional analyses.

3.3. Interpretation of the resulting peptide length and sequence from library construction

To our knowledge, the construction of phage libraries is generally performed by one of two methods depending on the method of preparation of DNA library. The first method is a phage library construction using a shorter random DNA library produced by chemical synthesis.[3,31–36,38,40] In this approach, phage particles display shorter peptides having the same length with the length being totally flexible and dependent on the length of the DNA library prepared. Generally, from 7- to 15-mer peptides are generated and used. In this case, in order to identify the potential binding target, we search for candidates from the resulting peptides taking advantage of a homology search or a conserved peptide motif. In contrast, genomic DNA or mRNA derived from biomaterials can be used for library construction.[4–10] In such a preparation, with the exception of the two kinds of shifted reading frames that can be generated,[10] most of the phage obtained from a library contain primary peptide sequence 100% identical to that translated in the proto biomaterial. Thus, the screened target should be more obvious than in the previous method. Furthermore, in the T7 phage system of Novagen, since proteins of a large size (<1200 aa) can be displayed on the phage capsid, a wide variety of protein can be covered by a library.

Even though we used a T7 phage library constructed from mRNA from 10 kinds of human cancer cell for

the CPT-10-B screen, no phage fully matching with hTopI, hCE, or hAChE was identified. This may be because the corresponding phage particles may not be included in this library because of low abundance of corresponding mRNAs or perhaps more importantly, it is possible that CPT immobilized via a linker shorter than 20 Å could not reach its binding site within these proteins (Fig. 1b). According to the crystal structures of CPT bound to hTopI or hCE[14–18] and CPT-11 with *Tc*AChE,[27,28] the CPT-binding region or the region homologous to the CPT-binding peptides is more than 20 Å distant from the protein surface. Therefore, a longer linker of more than 20 Å that can reach the bottom of the gorge would be needed to identify the corresponding phage particle. The need for DNA must also be taken into account when considering CPT binding to TopI. With regard to hEP_1 receptor, display of full-length, insoluble GPCR on the phage capsid is impossible. Thus, hEP_1 receptor itself cannot be identified.[10]

Nevertheless, based on the primary peptide sequence of the phage obtained from an effective T7 phage display screen using a QCM device, we have attained chemical and biological insights into CPTs, with direct information about the binding regions of protein ligands and without dependence on the physico-chemical characteristics of the proteins themselves. These advantages are considered to be one of the most powerful aspects of the phage display technique. From such a point of view, target hunting with homology searching or motif detection using random shorter peptide library is superior.[3,38] In addition, the development of bioinformatics tools is helpful for understanding results from random peptide library screens. Rodi and Makowski et al. have already created a series of systems with 7- or 12-mer random peptide phage libraries, which can detect small molecule-binding regions of a target protein.[41,42] Given the success described here in obtaining results from an effective platform, we now aim to develop the technology further such that it can be used to systematically detect binding targets using peptide sequences of various lengths and in particular ones which spatially mimic the binding site of the compound as reported.

4. Experimental

4.1. Instrumentals

A 27-MHz QCM device, AffinixQ, and ceramic sensor chip was purchased from Initium Inc. (Tokyo, Japan). PCR was performed using a PTC-200 (Peltier Thermal Cycler) (Bio-Rad). Sequencing analysis was carried out using an ABI PRISM 3100 Genetic Analyzer (ABI).

4.2. Bioinformatics Tool

We searched for the homologous proteins to CPT-10-B-binding peptide using Basic Local Alignment Search Tool (BLAST, NCBI) (http://www.ncbi.nlm.nih.gov/BLAST/). We also elucidated the similarity between CPT-10-B-binding peptide and protein using **CLASTALW** (Kyoto University Bioinformatics center) (http://clustalw.genome.jp/). The multiple alignment of esterase was generated using BOXSHADE (ch. EMB net. Org.) (http://www.ch.embnet.org/software/BOX_form.html).

4.3. Synthesis of biotinylated CPT derivatives

CPT-10-B (**3**) was synthesized by the condensation reaction of commercial 10-hydroxy CPT (**1**) and iodoacetyl-LC-biotin (**2**) with K_2CO_3 in DMF at 50 °C (Scheme 1). The reaction products were analyzed by NMR and ESIMS. NMR data were obtained from JEOL JNM-LA400 (400 MHz for 1 h, JEOL, Tokyo, Japan) in a mixture of CD_3OD and $CDCl_3$. Chemical shifts were expressed by δ ppm using TMS as an internal standard. Mass spectrometry data were collected on an ABI QSTAR (Applied Biosystems Japan (ABI), Tokyo, Japan) using ESI in positive ion mode.

CPT-10-B; $C_{38}H_{46}O_8N_6S$. 1H NMR (1:1, CD_3OD—$CDCl_3$); d 1.04 (3H, t, $J = 7.4$ Hz), 1.25–1.75 (14H, m), 1.97 (2H, m), 2.19 (2H, t, $J = 7.1$ Hz), 2.73 (1H, d, $J = 12.7$ Hz), 2.93 (1H, dd, $J = 4.6$, 12.7 Hz), 3.16 (4H, m), 3.63 (1H, m), 4.32 (1H, dd, $J = 3.4$, 4.4 Hz), 4.51 (1H, dd, $J = 3.2$, 4.6 Hz), 4.70 (2H, s), 5.32 (2H, s), 5.34 (1H, d, $J = 16.4$ Hz), 5.65 (1H, d, $J = 16.4$ Hz), 7.35 (1H, s), 7.63 (1H, d, $J = 9.3$ Hz), 7.70 (1H, s), 8.14 (1H, d, $J = 9.3$ Hz), 8.47 (1H, s); HRMS calcd for $C_{38}H_{47}O_8N_6S$ $(M+H)^+$ *m/z* 747.3176, found 747.3190.

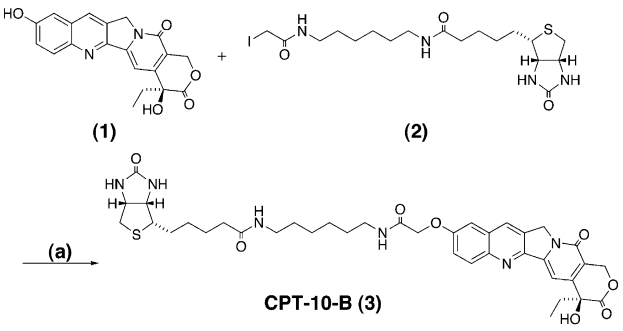
Synthesis of CPT-20-B (Fig. 1b) was described previously.[9]

4.4. T7 phage display screen using 96-well streptavidin-coated microplate

The procedure for T7 phage display screen using 96-well streptavidin-coated microplates has been described previously.[9]

4.5. T7 phage display screen on QCM device

Screening of CPT-10-B-binding peptide was performed with a 27-MHz QCM (AffinixQ, Initium). Two microliters of 100 μg/ml avidin was applied to the gold electrode surface of the ceramic sensor chip and left for 30 min to immobilize. After washing the surface, the



Scheme 1. Synthesis of C10 Biotinylated CPT Derivative (CPT-10-B). Reagents and condition. (a) K_2CO_3 in DMF at 50 °C.

sensor chip was immersed into the cuvette containing 8 ml of saline solution (150 mM NaCl in 50 mM phosphate buffer, pH 7.0, 10% DMSO) stirred at a rate of 1000 rpm. CPT-10-B (100 μM in DMSO) was then injected into the cuvette and the immobilization was followed by monitoring alterations in frequency (ΔF) resulting from changes in mass at the electrode surface. Eighty microliters of phage library (final 1.0×10^8 pfu) was then injected into the cuvette containing saline solution (150 mM NaCl, 0.5% Tween 20 in 50 mM phosphate buffer, pH 7.0) at 25 °C. After monitoring the alterations in frequency (ΔF) for 10 min, the sensor chip was desorbed from the equipment. The bound phage on the gold electrode surface was then recovered by adding 10 μl of host *E. coli* cell (BLT 5615) solution followed by incubation for 30 min with shaking. The resulting solution was mixed with another 1 ml of host cell solution and then incubated for 3 h at 37 °C to amplify the recovered phages.

4.6. Characterization of binding phage

Forty-eight clones were isolated from the phage solution. Each clone was dissolved in phage extraction buffer (100 mM NaCl, 6 mM $MgSO_4$ in 20 mM Tris–HCl, pH 8.0). These clones were amplified and used for binding experiments with CPT-10-B according to the screening procedure described above. Phage particles were disrupted by heating the extract to 65 °C for 10 min and DNA extracted from each phage. The capsid-coding region (gene10B) was subject to PCR using the forward primer 5′-TGCTAACTTCCAAGCGGACC-3′ and the reverse primer 5′-AAAAACCCCTCAAGACCCGTT TA-3′. The PCR product was then analyzed by 1% agarose gel electrophoresis or purified with ExoSAP-IT (USB, Ohio, USA) and EtOH precipitated. The purified fragments were sequenced on an ABI PRISM 3100 Genetic Analyzer (ABI) to determine the corresponding amino acid sequence displayed on the T7 phage capsid.

4.7. Construction of docking model between CPT and NSLKD

An initial three-dimensional structure of CPT and the conformation of NSLKD was generated using Insight II software (Accerlys Inc.) as described previously. The possible docking manner (Fig. 6) was produced using PD viewer 5.0 (Accerlys Inc.).

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Supplementary data

An electropherogram obtained from the sequencing analysis of the region coding for the C-terminal part of the capsid (Table S1). Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2007.09.002.

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