SELF-ASSEMBLED MONOLAYER (SAM) OF SMALL ORGANIC MOLECULE FOR EFFICIENT RANDOM-PEPTIDE PHAGE DISPLAY SELECTION USING A CUVETTE TYPE QUARTZ-CRYSTAL MICROBALANCE (QCM) DEVICE

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Introduction

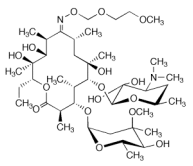
Phage display (PD) technology is a molecular biological tool that allows identification of target-binding peptide sequence displayed on phage capsid [1]. To date, we developed an efficient one-cycle PD protocol using a cuvette type quartz-crystal microbalance (QCM) device for identification of binding peptides of small organic molecule from phage library [2,3]. In this approach we have shown that self-assembled monolayer (SAM) is effective for small-molecule attachment on the gold electrode of QCM sensor chip [4], providing a robust affinity selection of specifically binding phages [3]. Compared with conventional microplate- or bead-based methods, this strategy needs no wash and elution conditions exploration, repeating several cycles of selection, and cumbersome experimental manipulations. In particular, detection of background phages that come from non-specific binding or carryover was dramatically reduced in this strategy [2,3].
Herein we designed an antibiotic roxithromycin (RXM) derivative that forms SAM and performed one-cycle selection of RXM-binding peptides. The resulting RXM-selected peptide sequences were analyzed by making use of receptor ligand contacts (RELIC) suite, a bioinformatics server for analysis of selection from random-peptide phage library [5]. The experimental detail and result of analysis are presented here.

Experimental

Materials
T7Select® System was purchased from Novagen (Madison, WI). PCR reagents were from TaKaRa Bio Inc. (Shiga, Japan). Exo-SAP-IT was obtained from USB Corporation (Cleveland, OH).

Apparatus
A 27-MHz QCM device, AffinixQ, and ceramic sensor chip was purchased from Initium Inc. (Tokyo, Japan). PCR was performed using Thermal Cycler Dice (TaKaRa Bio Inc.). Sequence analysis was carried out using an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA).

Procedures
A 20 µl aliquot of RXM derivative (Fig. 1) (1 mM in 70% EtOH) was dropped onto the gold electrode surface of sensor chip and left for 16 h under a humid and shaded atmosphere at room temperature. The surface of the electrode was washed and setup for the QCM device with the cuvette containing 8 ml of buffer (stirred at 1000 rpm). The QCM sensor was then allowed to fully stabilize. An aliquot of 8 µl of 15-mer random-peptide T7 phage library (1.7×10^{10} pfu/ml) was injected into the cuvette. Frequency changes were then monitored for 10 min. For the recovery of bound phages, 20 µl of host *Escherichia coli* (BLT5615) solution was dropped onto the electrode and then incubated at 37℃ for 30 min. An aliquot of phage was extracted from resulting solution and subjected to PCR following Exo-SAP-IT purification and DNA sequencing. A subset of RXM-selected peptide was obtained and subjected to respective analysis using RELIC suite (http://relic.bio.anl.gov/) [5].


Roxithromycin (RXM)

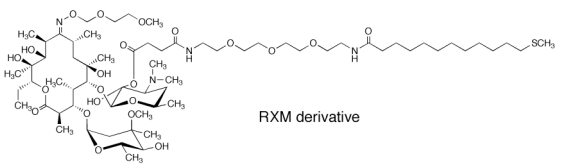

RXM derivative

Fig.1 Structure of RXM and its derivative

Results and discussion

The structure of RXM derivative we designed was shown in Fig. 1. This derivative comprises four units: (i) methylthio terminal for chemisorption on the gold electrode surface of the sensor chip via thiol-Au interactions, (ii) a C11 alkyl chain for accumulation of the RXM molecule on the gold, (iii) diethylene glycol (DEG) for reducing the non-specific binding (mainly via hydrophobic interaction) with the gold surface or linkers, and (iv) RXM to act as bait during screening.

Fig. 2A shows representative QCM sensor gram that was obtained by the monitoring the binding of T7 phages to RXM-SAM. After 10 min of interaction, binding phages were recovered and sequenced the peptides that were displayed on the capsid. After 12 sets of one-cycle selection, we obtained over 100 of 15-mer peptide sequences that are considered to selectively recognize RXM.

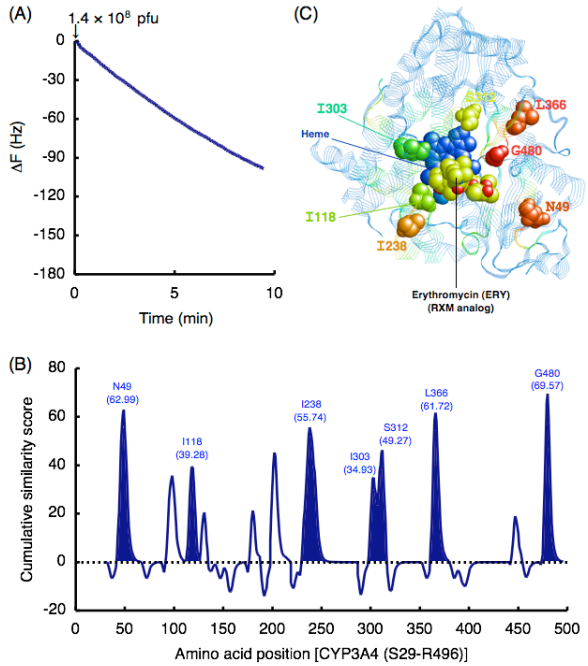


Fig.2 Detection of phage binding to RXM-SAM on the gold electrode after injection of a random-peptide T7 phage library. (A) A representative QCM sensorgram. (B) Similarity scores for the sequence of CYP3A4 (S29-R496) against the sequences of 25 peptides selected for affinity to RXM-SAM. (C) Three structural models of ERY-CYP3A4 complex (PDB ID: 2J0D). Similarity is coded by color; red indicates the highest similarity and blue the lowest.

Figs. 2B and C show a result of validation of known interaction between RXM and cytochrome P450 3A4 (CYP3A4) using RELIC/HETEROalign [5]. A similarity plot of refined 25 of RXM-selected 15-mer peptide along with the entire sequence of CYP3A4 is shown in Fig. 2B, which was cumulatively calculated using a modified BLOSUM62 score. A similarity plot of 103 peptides (arbitrarily selected from the unscreened parent library) along with the entire sequence of CYP3A4 was subtracted from the data before plotting. The result clearly pinpointed N49, I118, I238, I303, S312, L366 and G480 in CYP3A4 as residues of maximal similarity score. As shown in Fig. 2C, these residues are located to possible contact sites for erythromycin (RXM analog) during the binding process.

Additionally, by using identical subset of RXM-selected peptide, we can predict the unknown protein target(s) of RXM from a database (RELIC/FASTAskan) [5] (data not shown). Thus, with providing the rapid determination of RELIC combinatorial peptide and subsequent rigorous validation of known interactions, our strategy enables comprehensive and high throughput exploration of small-molecule-binding proteins that are responsible for the main or side effects as well as the pharmacokinetic metabolisms or transports.

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