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Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 15 (2005) 4850–4853

Camptothecin binds to a synthetic peptide identified by a T7 phage display screen

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Received 1 June 2005; revised 28 June 2005; accepted 7 July 2005
Available online 24 August 2005

Abstract—An analysis of non-biotinylated camptothecin (CPT) binding to the C-20-biotinylated CPT binding peptide NSSQSARR was carried out using two methods, quartz-crystal microbalance (QCM) and surface plasmon resonance (SPR). The peptide was immobilized peptide on a sensor chip and showed a dissociation constant (K_D) of approximately 0.1 μM against CPT in QCM and SPR experiments.
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1. Introduction

Recently, rational molecular design based on docking simulations has emerged as a major approach for generating effective anti-tumor agents. The drugs Gefinitib (ZD1839, Iressa) and Imatinib (STI571, Gleevec), which are used for chemotherapy to treat non-small cell lung cancer and chronic myeloid leukemia respectively, are typical examples developed by this approach.[1–3] Target validation is imperative at the outset of the drug development process to minimize possible side effects. In addition, physiological information, such as the dissociation constant (K_D) value, or binding information deduced from simulations of docking between compounds and their cognate binding domains is useful for molecular drug design.

As discussed in a preceding article, we identified a CPT-20-B-binding peptide sequence, NSSQSARR, by T7 phage display screen.[4] This sensitive method enables the rapid determination of molecular targets from a diverse range of proteins, including those that are membrane-associated or subject to rapid turnover. Furthermore, sets of small molecules, such as biotinylated derivatives, can be used as baits, providing direct information about the binding domains in larger proteins. To date, only a few examples have been reported of target validation using functional small molecules in phage display technology.[5–11]

Here, we carried out a kinetic analysis of QCM and SPR experiments measuring the binding of non-biotinylated CPT to a synthetic CPT-20-B-binding peptide. We also describe a docking simulation carried out using Insight II/Discover program (Accelrys Inc., San Diego, CA, USA).

2. Results and discussion

2.1. Kinetic analysis by QCM

A 27-MHz QCM (AffinixQ, Initium Inc., Tokyo, Japan) was employed to analyze the interaction between CPT and peptide.[12,13] The synthetic peptide was immobilized on a ceramic sensor chip using an amine coupling reaction. Four different concentrations of CPT were added to the peptide immobilized on the ceramic sensor chip with a gold surface. The binding of CPT to this peptide was calculated by monitoring the alterations in frequency (ΔF) resulting from changes in mass on the electrode surface. As shown in Figure 1A, the frequency decreased after injecting each concentration of CPT, confirming that CPT binds to this peptide. Linear-reciprocal plots showed linearity, indicating that CPT showed Langmuir

Keywords: Camptothecin; Binding peptide; QCM; SPR.
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doi:10.1016/j.bmcl.2005.07.017

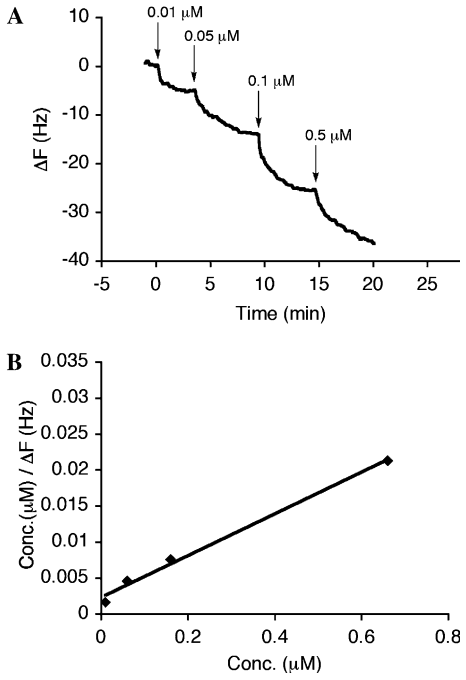

Figure 1. QCM analysis (AffinixQ). Four different concentrations of CPT (0.01, 0.05, 0.1, and 0.5 μM) were added to a cuvette in which they interacted with peptide immobilized on a gold electrode surface of a ceramic sensor chip. (A) Interaction of CPT with NSSQSARR. 1 Hz = 30 pg. (B) Linear-reciprocal plot of concentration (μM)/Δ*F* (Hz) against various concentrations of CPT.

type 1:1 binding to the peptide (Fig. 1B). The K_D value of CPT binding to the peptide, NSSQSARR, was 94 nM.

2.2. Kinetic analysis by SPR

An SPR biosensor, an interaction analysis instrument of the flow injection type, was also employed to analyze the interaction between CPT and peptide. The resulting K_D value was compared to that obtained by QCM, which employs a cuvette-type analytical apparatus. Five different concentrations of CPT were used to measure the binding of CPT to the peptide, which was immobilized on a CM5 sensor chip by amine coupling. CPT bound to the peptide, showing low rates of association and dissociation. The kinetic constants for the interaction were determined by fitting the SPR association and dissociation curves obtained at various concentrations of CPT. K_D values were calculated by global fitting using BIAevaluation 3.2 software. The resulting K_D value was 112 nM (Table 1). Result from the SPR analysis agrees to that obtained by QCM, confirming the binding between CPT and NSSQSARR.

Table 1. K_D values for binding of CPT to the peptide NSSQSARR obtained from QCM and SPR analyses

	QCM[a]	SPR[b]
K_D (nM)	94	112

[a] Value was calculated from linear-reciprocal plot by using AQUA ver1.5 software (Initium Inc.).
[b] Value was calculated from global fitting by using BIAevaluation 3.2 software (BIAcore).

2.3. Docking simulation of CPT binding to the NSSQSARR peptide

A docking simulation using InsightII/Discover (Accelrys Inc.) was carried out to further investigate the interaction between CPT and NSSQSARR. Results indicated that the polycyclic moiety of CPT fits into a complementary groove formed by the peptide and that hydrogen bonding strengthens these interactions, in particular hydrogen bonds that form (1) between the carbonyl oxygen of Arg8 and the hydroxyl group at C-20 of CPT, and (2) between the guanidine moiety of Arg7 and the carbonyl oxygen at C-16a of CPT (Fig. 2).

We are currently validating the candidate protein of CPT from a homology search using NSSQSARR as a query followed by binding analysis with QCM and SPR.

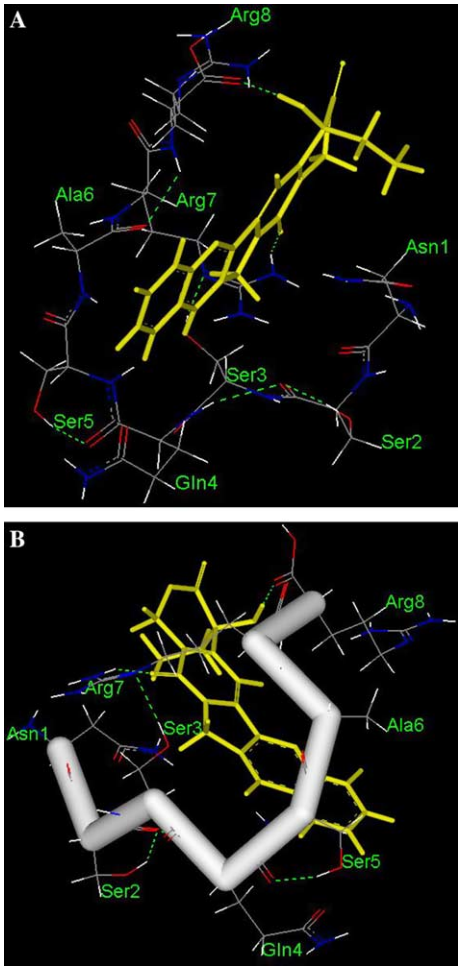

Figure 2. (A, B) Docking simulation of CPT with NSSQSARR, the peptide identified using a phage display method. The structure of CPT is shown in yellow. The atoms comprising the structure of NSSQSARR are color coded: carbon in gray; hydrogen in white; oxygen in red; nitrogen in blue. The green dashed lines indicate hydrogen bonding. This figure was prepared using Insight II/Discover (Accelrys Inc.). (B) Peptide backbone is shown in white.

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