**A multi-substrate fluorometric assay for HIV-1 protease activity**

**and its application in drug resistence detection**

Qinchang Zhu, Tsutomu Kabashima, Takayuki Shibata, Masaaki Kai\*

*Faculty of Pharmaceutical Sciences, Graduate School of Biomedical Sciences, Nagasaki University, 1-14, Bunkyo-Machi, Nagasaki 852-8521, Japan*

***Introduction and Results:***

HIV protease (HIV PR) is one of the major targets of anti-HIV drugs for its critical role in the maturation of HIV. With the emergence and prevalence of drug-resistant strains in recent years, the need for rapid and simple assay for drug resistance detection is increasing. In this study, a three-substrate fluorometric HPLC assay was developed to determine the activity of HIV-1 PR and phenotype its drug resistant mutations, basing on our previous report that the compound catechol could selectively react with peptide containing free N terminus to form fluorescent product[1] [2].

Three HIV-1 PR substrate peptides ([AC]SGIFLETSLE, [AC]SGIFLETSLE and [AC]KSGVFVQNGL) were chosen according to the fluorescence intensity and retention time in chromatography of their cleavage products after reacting with catechol. An acetyl group [AC] was added to these substrates’ N terminus to ensure the catechol only react with the product peptides containing free N terminus. Under an optimized condition, a high sensitivity and good linear relationship between the fluorescence signal and the concentration of synthesized product peptides can be observed (Fig.1).



**Fig.1 HPLC analysis of peptide mixture of LETSLE, FEAM and VQNGL**. (A) HPLC separation and detection of an aliquot of reaction mixture containing 22 pmol of LETSLE, 55 pmol of FEAM and 22 pmol of VQNGL. (B) Standard curve for HPLC separation and detection of product peptide mixture. Peak area is given in arbitrary unit.

In the activity comparison and drug resistance experiment, wild type HIV-1 PR (Wt) and two point-mutated HIV-1 PR (M1:G48V and M4: V32I ) expressed in E.coli were used directly. The result showed that different HIV-1 PR mutants could be distinguished obviously according to their different cleavage activity on the three substrates. And the result of drug resistance assay from three substrates was consistent with previously reported data: M1 shows resistance to saquinavir, while M4 is not. Drug resistance phenotypic assay for HIV-1 PR mutants with this method can be done within 4 days, which is also a relatively simple and economical way so far.

***Keywords:***

HIV-1 protease, drug resistance, phenotypic assay, catechol, HPLC

***Reference:***

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***Introduction to the authors:***

Qinchang Zhu is currently a Ph.D. candidate in Pharmaceutical Sciences at Japan Nagasaki University. Before he started his Ph.D. study, he had worked as a Research Associate from 2006 to 2010 in Guangzhou Institute of Biomedicine and Health, Chinese Academy of Sciences. He received his Master degree and Bachelor degree from Guangzhou Jinan University. His research interests include diagnostic methods and new treatment strategies for infectious diseases, targeted drug delivery vehicle and studies on virus-host interactions.

\***Corresponding author’s E-mail address:** ms-kai@nagasaki-u.ac.jp