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A novel high-throughput format assay for HIV-1 integrase strand transfer reaction using magnetic beads[1]

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Key words

Abstract

Aim: To develop a novel high-throughput format assay to monitor the integrase (IN) strand transfer (ST) reaction *in vitro* and apply it to a reaction character study and the identification of antiviral drugs. **Methods:** The donor DNA duplex, with a sequence identical to the U5 end of HIV-1 long terminal repeats, is labeled at its 5' end with biotin (BIO). The target DNA duplex is labeled at its 3' end with digoxin (DIG). IN mediates the integration of donor DNA into target DNA and results in a 5' BIO and 3' DIG-labeled duplex DNA product. Streptavidin-coated magnetic beads were used to capture the product, and the amount of DIG was measured as the ST reaction product. The assay was optimized in 96-well microplate format for high-throughput screening purpose. Moreover, the assay was applied in a ST reaction character study, and the efficiency of the assay in the identification of antiviral compounds was tested. **Results:** The end-point values, measured as absorbance at 405 nm was approximately 1.5 for the IN-mediated ST reaction as compared with no more than 0.05 of background readings. The ST reaction character and the half maximal inhibitory concentration (IC_{50}) values of 2 known IN inhibitors obtained in our assay were similar to previously reported results using other assays. The evaluation parameter Z´ factor for this assay ranged from 0.6 to 0.9. **Conclusion:** The assay presented here has been proven to be rapid, sensitive, and specific for the detection of IN ST activity, the reaction character study, as well as for the identification of antiviral drugs targeting IN.

Introduction

HIV-1 integrase (IN) is an important enzyme in the virus replication cycle and an attractive target for antiviral drug design because it catalyzes the integration of the virus genome into the human genome. IN catalyzes a 2-step reaction. The first step is the cleavage of 3' GT dinucleotide from each 3' end of the virus long terminal repeats (LTR) DNA (donor DNA). This reaction is termed 3' processing (3P) and depends on the recognition of the specific sequence at the U5 and U3 ends of LTR. In the subsequent step, strand transfer (ST), the 3'-processed DNA ends acting as nucleophilic agents, attack phosphodiester bonds in the host cellular DNA (target DNA) and splice these 3' ends into the target DNA. This reaction does not absolutely require any specific sequence within the host DNA[1–3].

During the past decade, *in vitro* assays for IN activities have been developed and applied for the screening of inhibitors. These assays include 2 categories in general: (i) low-throughput gel-based assays involving radioactively-labeled oligonucleotides[4,5]; and (ii) high-throughput microtiter assays using biotin (BIO) or digoxin (DIG)-labeled oligonucleotides with enzyme-coupled detection strategies for the quantification of the product[6–8]. Recently, a gel-based assay using microarrayed compound libraries and 1 microtiter assay based on time-resolved fluorescent resonance energy transfer have been developed[9,10]. These assays are established at the aim of high-throughput screening (HTS), high sensitivity, specificity, and reliability. Among these assays, solid-phase microtiter assays in which DNA were immobilized on the microplate surface were most widely

used in the screening of antiviral compounds because they are fast, convenient, and are optimized in HTS format[6–8]. However, the need of plate coating and blocking in these assays is time and labor consuming, and the invalidation of plate blocking causes non-specific binding of DNA or antibodies on the microplate and subsequent higher background readings. Moreover, the immobilization of the DNA substrate on the microplate limits the application of these assays in reaction characters studies.

It is reported that small molecules that inhibit the 3P activity of IN *in vitro* show little antiviral activity *in vivo*, and the inhibition of ST activity is the primary key to antiviral efficiency *in vivo*[11,12]. IN is the only 1 of the 3 enzymes for which clinically useful drugs are not available to date. Therefore, it is important to develop *in vitro* assays for the detection of IN activities, especially ST activity, and apply these assays in the screening of antiviral compounds. In the present study, we describe a rapid and highly robust approach to HIV-1 IN ST activity using HTS technology. This assay was performed in a 96-well microplate. Based on the commercially-available magnetic beads, this assay is rapid, flexible, and specific, and there is no need for plate coating or blocking. Furthermore, 2 kinds of detection strategies, absorbance and fluorescence, have been conducted and 2 antiviral compounds were employed to test the effectiveness of this assay.

Materials and methods

Materials Oligonucleotide sequences corresponding to the U5 terminus of the HIV-1 LTR and the target substrate were synthesized and modified by Shanghai Sangon Bio (Shanghai, China): oligo I BIO-5' ACCCTTTTAGTCA-GTGTGGAAAATCTCTAGCA3', oligo II 5' ACTGCTAGA-GATTTTCCACACTGACTAAAAG3', oligo III 5' ATGTGG-AAAATCTCTAGCGAT3'-DIG, and oligo IV 5' ATCGCTA-GAGATTTTCCACAT3'. Taq DNA polymerase was purchased from Dalian Takara Bio (Dalian, China); restriction endonucleases and T4 DNA ligase were from New England Biolabs (Beverly, MA, USA). Dynabeads M-280 streptavidin (SA) magnetic particle and the Dynal MPC-96B concentrator were purchased from Dynal Biotech (ASA, Oslo, Norway). The monoclonal anti-DIG alkaline phosphatase (AP) conjugate antibody and monoclonal anti-DIG fluorescein isothiocyanate (FITC) conjugate antibody were purchased from Sigma (St Louis, MO, USA). The nickel-chelating column (Chelating Sepharose Fast Flow) was purchased from Amersham Pharmacia Biotech (Piscataway, NJ, USA). HEPES, imidazole, β-mercaptoethanol, isopropyl-beta-*D*-thiogalactopyranoside, bovine serum albumin (BSA) and

piperazine-*N, N'*-bis-2-ethanesulfonic acid (PIPES) were obtained from Merck (Hohenbrunn, Germany). Baicalein was purchased from Fluka (Buchs SG, Switzerland). All other chemicals were from Amresco (Solon, OH, USA).

Protein expression and purification The plasmid containing the HIV NL4-3 IN gene was used as the PCR template DNA. Site-directed mutagenesis was done by overlapping PCR to bring the F185K/C280S double mutations for the purpose of enhancing the protein solubility. The IN gene was modified to contain an *Nde* I site at the 5' end and a *BamH* I site and a termination codon (TAG) at the 3' end. The PCR product was digested with *Nde* I and *BamH* I and ligated with *Nde* I–*BamH* I-digested pET-28a to construct a pNL-IN recombinant plasmid. After confirmation by sequencing (Shanghai Sangon Bio, China), correctly constructed pNL-IN was expressed in Escherichia coli strain BL21 (DE3) as a soluble N-terminal 6-histidine tag fusion protein and purified by a nickel affinity column as described[13]. After elution from the nickel column, the purified protein was dialyzed against 1 mol/L NaCl, 20 mmol/L HEPES, pH 7.5, 0.1 mmol/L EDTA, 1 mmol/L dithiothreitol, and 10% (*w/v*) glycerol at 4 °C overnight and stored at -80 °C. SDS-PAGE was employed to analyze the expression and purification of the IN protein, and the IN concentration was measured by the Bradford method (Bio-Rad, Hercules, CA, USA).

Microplate ST assay For high-throughput purpose, ST assays were performed in a 96-well microplate (Corning, New York, NY, USA) in a final volume of 50 μL. The wells were washed once with 1×reaction buffer (25 mmol/L PIPES, pH 7.0, 10 mmol/L β-mercaptoethanol, 5% (*w/v*) glycerol, 0.1 g/L BSA, and 10 mmol/L MnCl₂). In total, 800 ng IN was added and pre-incubated in reaction buffer. Subsequently, 1.5 pmol donor DNA and 15 pmol target DNA were added and the reaction was initiated. After incubation for 1 h at 37 °C, 1.5 mL magnetic particles ($6.7×10^8$ beads/mL) and 51.5 μL binding buffer (10 mmol/L Tris-HCl, pH 7.6, 2 mol/L NaCl, 20 mmol/L EDTA, and 0.1% [*w/v*] Tween 20) were added and incubated at 20 °C for 15 min. Then the wells holding the mixture were placed in a magnetic concentrator, the supernatant was discarded, and the wells were washed 3 times with phosphate-buffered saline (PBS) containing 0.1% Tween 20 (PBST). Subsequently, 100 μL of 1:5000 diluted AP conjugate anti-DIG antibody was added and incubated for 30 min at 37 °C. Finally, the wells were washed 3 times with PBST and the magnetic beads were transferred into fresh wells; 100 μL P-Nitrophenyl Phosphate (P-NPP) substrate (0.1 mol/L Na₂CO₃, pH 9.5, 6.7 mmol/L P-NPP, and 2 mmol/L MgCl₂) was added. The plates were read at 405 nm with a Model 680 microplate reader (Bio-Rad, USA).

Results

HIV-1 IN purification and characterization IN was purified from the soluble supernatant by nickel-affinity chromatography. The SDS-PAGE analysis indicated that the expression of recombinant IN exceeded the expression of any other cellular protein and IN was highly soluble. The molecular weight of the recombinant IN was approximately 33 kDa, with a purity of approximately 95% (Figure 1).
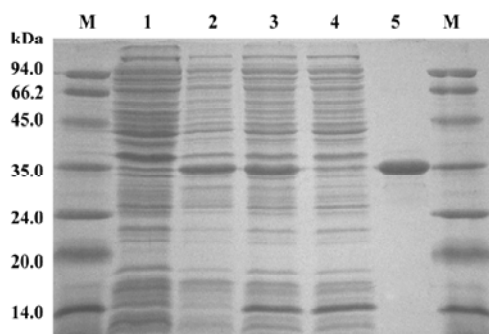

Figure 1. Coomassie-stained 15% SDS-PAGE analysis of protein expression and purification. Lane 1, *Escherichia coli* cell control; lane 2, induced pNL-IN in *Escherichia coli*; lane 3, cell suspension containing pNL-IN after induction; lane 4, column flow-through; lane 5, fraction of IN eluted from column. M, molecular weight markers.

Principle of the HTS assay for HIV-1 IN ST reaction In this HTS assay, the 31 bp duplex donor DNA substrate was designed to mimic the U5 end of the HIV-1 LTR. After annealing, there was a 3 nucleotides overhang at the 5' end of oligo I for better interaction of BIO-SA in the following DNA capture step, and the 3' GT dinucleotides were removed from oligo I. The donor DNA was labeled at the 5' end of oligo I with BIO, and the target DNA was labeled at the 3' end of oligo III with DIG. The ST reaction resulted in a 5' BIO and 3' DIG-labeled covalent DNA product. The DNA product was captured by SA-coated magnetic beads through the specific BIO-SA interaction, followed by the addition of the AP conjugate anti-DIG antibody. Therefore, the product was measured by the AP-coupled enzyme reaction (Figure 2).

Optimization of the ST activity assay To optimize this HTS assay, we varied the concentrations of donor DNA, target DNA, metal ion, and the IN protein to obtain the optimal reaction conditions. The optimal concentration of donor DNA was 30 nmol/L. The IN concentration varied between 17 and 1000 nmol/L, and 300 nmol/L was determined to be optimal. As for the target DNA, we varied the concentration
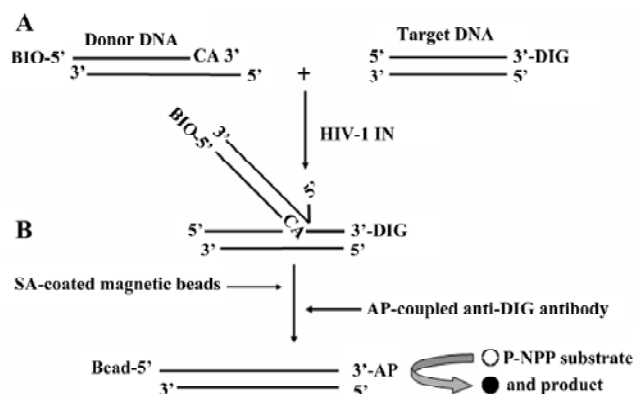

Figure 2. Schematic diagram showing the principle of the HTS assay for IN ST activity. (A) duplex donor DNA mimicking the post-processed U5 end of LTR is labeled at its 5' end with BIO. Target DNA is labeled at its 3' end with DIG. IN catalyzes the ST reaction and gives rise to a 5' end BIO and 3' end DIG-labeled DNA product. (B) SA-coated magnetic beads and the AP conjugate anti-DIG antibody were added subsequently to capture the reaction product and mark it with the AP enzyme, which is reactive with the P-NPP substrate.

from 10 to 750 nmol/L, and determined that the 300 nmol/L target DNA to be added was optimal. Furthermore, we investigated the performance of our assay in the presence of either cationic cofactor, Mg^{2+} and Mn^{2+}. Using this assay, ST activity peaked at 10 mmol/L Mn^{2+} and 5 mmol/L Mg^{2+}, respectively. Accordingly, the presence of Mn^{2+} instead of Mg^{2+} increased IN activity by almost double (Figure 3). Here, 300 nmol/L IN, 30 nmol/L donor DNA, 300 nmol/L target DNA, and 10 mmol/L Mn^{2+} were determined to be the optimal reaction conditions and used throughout the subsequent study.

To test the sensitivity and specificity of this assay, we measured the signal of all reactions under optimal reaction conditions. Furthermore, 2 widely-used essential parameters to indicate the quality of an assay: the signal-to-noise ratio (S/N) and signal-to-background ratio (S/B) were defined and calculated as:

$$S/N = \frac{\text{mean signal - mean background}}{\text{standard deviation of backgroud}}$$

and

$$S/B = \frac{\text{mean signal}}{\text{mean backgroud}}$$

As a result, the negative controls in the absence of either IN, donor DNA, or target DNA showed background readings measured as absorbance at 405 nm (A_{405}), lower than 0.05,
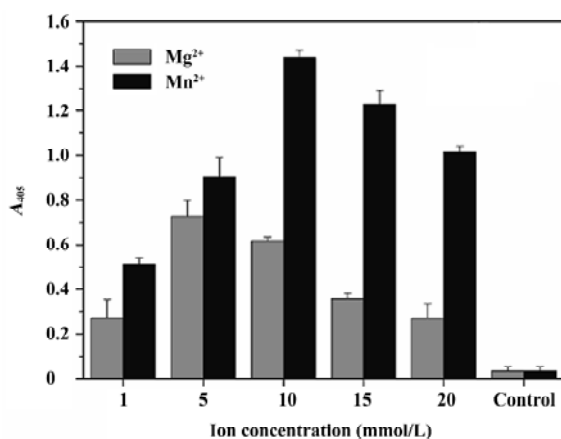
Figure 3. Effects of Mn^{2+} and Mg^{2+} on the performance of the ST reaction using the HTS assay. Metal ion concentrations varied from 1 to 20 mmol/L. A_{405} is shown with the different metal ion concentrations. Error bars represent SD from 3 replicate values.

whereas the ST reaction gave a signal of more than 1.4 (Figure 4A). Accordingly, the S/N and S/B ratios of the assay were 86 and 30, respectively.

To further demonstrate the robustness of this assay, we changed the AP conjugate anti-DIG antibody to the FITC conjugate anti-DIG antibody. After capturing the reaction product by magnetic beads, 100 mL of 1:500 PBS-diluted FITC conjugate antibody was added and incubated for 30 min at 37 °C. Finally, the microplate was washed as described and the plates were read with a 1420 Multilabel counter VIC-TOR reader (Perkin Elmer, Boston, MA, USA) at 485 nm excitation and 535 nm emission. The results were similar to the assay in which the AP conjugate antibody was used. The ST reaction showed a fluorescence signal as high as almost 1400, whereas the signals of negative controls were lower than 40 (Figure 4B). The S/N and S/B ratios were 61 and 32, respectively.

HIV-1 IN ST reaction character study The HIV-1 IN 3P reaction character was studied using the gel-based assay involving the radioactively-labeled oligonucleotide. Since IN pre-incubated in the presence of the DNA substrate was more active in the 3P reaction than IN pre-incubated alone or with Mg^{2+} [14], we were interested in the ST reaction character of IN under different pre-incubation conditions using our assay. We set 3 reaction groups: (i) IN pre-incubated alone in the reaction buffer without both DNA and Mn^{2+}; (ii) IN pre-incubated with Mn^{2+} in the reaction buffer without DNA; and (iii) IN pre-incubated with DNA in the reaction buffer, but without Mn^{2+}. The pre-incubation lasted for 30 min at 37 °C; subsequently, the ST reactions were induced by the addition of both the Mn^{2+} and DNA substrate in (i), only the
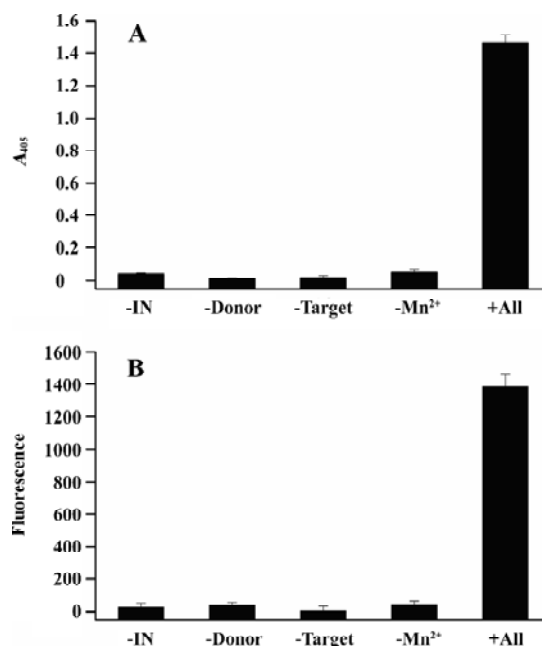

Figure 4. Assay performance under optimal reaction conditions using different detection strategies. Assay components marked (−) indicated below the bar graph were left out of the indicated assays. (A) AP conjugate anti-DIG antibody was involved, and a subsequent P-NPP substrate detection strategy was used. Signal was obtained as A_{405}. (B) FITC conjugate anti-DIG antibody was involved, and the signal was shown as fluorescence after excitation at 485 nm and emission at 535 nm. Error bars represent SD from 3 replicate values.

DNA substrate in (ii), and only Mn^{2+} in (iii). Aliquots were taken after 5, 10, 15, 20, 30, 40, and 50 min, and the product of the formation of the reactions versus the time plot were analyzed (Figure 5A). The result showed that Mn^{2+} strongly enhanced the ST activity of IN, with a signal 1.5 times that of IN pre-incubated alone. IN pre-incubated in the presence of DNA was almost the same active as IN pre-incubated alone. We subsequently performed the experiments in the presence of Mg^{2+} instead of Mn^{2+}. The time plot curves of the Mg^{2+}-dependent ST reactions were partially different from previous Mn^{2+}-dependent reactions. Compared with IN pre-incubated alone, the pre-incubation of IN with Mg^{2+} also enhanced the ST activity notably, but not as significant as that of the Mn^{2+}-dependent reaction. Unlike the Mn^{2+}-dependent reaction in which the pre-incubation of IN with DNA had no effect on ST activity, the pre-incubation of IN with DNA enhanced the Mg^{2+}-dependent ST activity remarkably (Figure 5B).

Antiviral compounds screening efficiency Baicalein and L-708906 were diluted in DMSO to a final concentration of 10% DMSO into the reaction volume and pre-incubated with
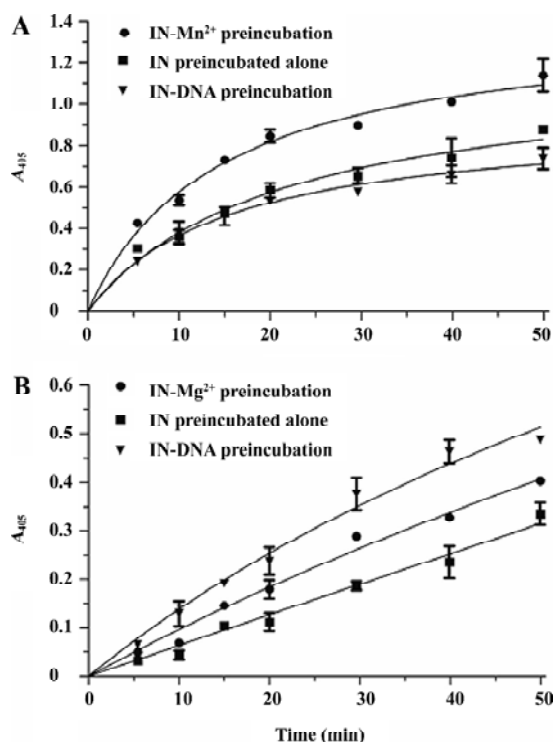
Figure 5. Time plots of the ST reaction after different pre-incubation procedures in the presence of either Mn^{2+} (A) or Mg^{2+} (B) as the metal ion cofactor. IN was pre-incubated at 37 °C in the reaction buffer alone without the metal ion (■), with only the metal ion without DNA (●), and with DNA without the metal ion (▼), respectively. After 30 min, other necessary reactants were added and the ST reactions were performed. In all cases, the ST reactions were allowed to proceed for 5, 10, 15, 20, 30, 40, and 50 min. Error bars represent SD from 3 replicate values.

IN at 37° C in the reaction buffer in the absence of Mn^{2+} for 10 min, followed by the addition of Mn^{2+} and the DNA substrates. The reactions were carried out at 37 °C for 1 h and the AP-coupled anti-DIG antibody and subsequent detection procedure was applied to detect the assay signals. The inhibition percentage and IC_{50} values were calculated based on the assay results using a non-linear regression curve fit. The IC_{50} values of baicalein and L-708906 for the IN ST reaction in this assay were 1.06 and 0.77 μmol/L, respectively (Figure 6).

Statistical evaluation of assay performance The screening window coefficient, Z (or Z') factor, has been defined to evaluate effective HTS assays[15]. It is capable of reflecting the assay signal dynamic range as well as the data variation associated with the signal measurements. In the present assay, all Z' factors were determined as described[15]:
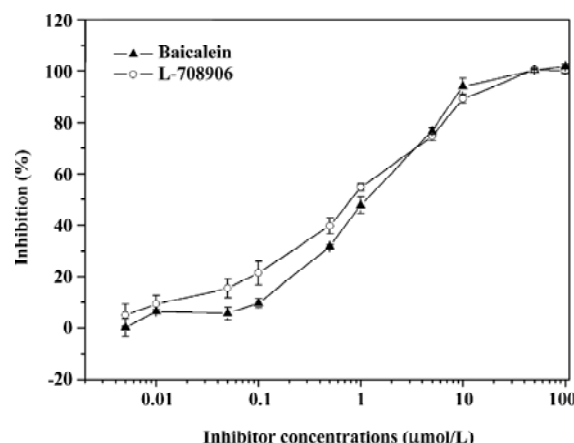
$$Z'=1-[3\times(SD_{POS}+SD_{NEG})/(M_{POS}-M_{NEG})]$$

Figure 6. Inhibition of the ST reaction with L-708906 and baicalein using the HTS assay. Compounds were diluted in DMSO to a final concentration of 10% (*v/v*) DMSO in the reaction volume. No compound, but only DMSO, and no IN reactions were set as the drug-free and negative controls, respectively. Error bars represent SD from 3 replicate values.

Calculations were based on the standard deviations (SD) and intensity means (M) of the controls. The highest values were used as the positive control and the no IN reactions (or no metal ion reactions) were used as the negative control. An analysis of the data resulting from the experiments present in these Figures showed that the assay we developed for IN ST activity had a Z' score ranging from 0.6 to 0.9 (Table 1). Together with the S/N and S/B ratios, these statistical parameters reflect the high sensitivity, specificity, and robustness of the assay.

Table 1. Z' factor for experiments presented.

Experiment	Z' factor
1. Figure 3A	0.894
2. Figure 3B	0.773
3. Figure 4A	0.893
4. Figure 4B	0.615
5. Figure 5A	0.744
6. Figure 5B	0.726
7. Figure 6A	0.871
5. Figure 6B	0.833

Z' factors were calculated using the formula described by Zhang *et al*[15]. Highest values in each Figure were used as positive controls and the lowest values as negative controls.

Discussion

In the present study, we describe a novel assay that can

be employed to measure the ST activity of IN as well as both the 3P and ST activities together. It is reported that ST reaction is the primary key to the effective suppression of viral replication, and compounds inhibiting the IN activity *in vitro* by interfering the 3P reaction alone lack antiviral activity[11,12]. Therefore, we applied this assay only in the ST reaction measurement in this work.

Several improvements have been acquired in this assay. First, the BIO-DIG combination was used and the assay was conducted in a 96-well microplate. This could be conducted in all kinds of multiwell microplate formats if desired, ensuring no radioactive waste and a high-throughput format. Second, the magnetic beads were used to capture the reaction product, therefore, all the reagents were freely suspended in solution instead of solid-phase assays in which donor or target DNA was attached to the microplate. Without the immobilization of DNA or the protein, each reagent can be added at any given time; it is more flexible to investigate the interaction of all reagents and it is easy to study the pharmacology of inhibitors targeting IN. Furthermore, with the application of magnetic beads, neither the precoating nor the blocking of microplate was required; it was less laborious and time consuming. After the last wash before the detection, the magnetic beads were easily transferred into fresh wells, and the background caused by the non-specific binding of DNA or the antibody was almost totally eliminated. Third, we applied this assay in 2 different detection systems. Both the AP conjugate and FITC conjugate anti-DIG antibodies and their following detection strategies were used. The result from both absorbance and fluorescence proves that the assay is specific and sensitive. Furthermore, the application of the fluorescent antibody needs no extra enzyme-coupled detection procedure, and the product formation is directly quantified by a fluorescent reader or flow cytometer. The entire assay can be accomplished in approximately 2 h; it is fast and convenient.

Divalent ion is necessary for both *in vivo* and *in vitro* activities of IN. *In vitro* assays use either Mg^{2+} or Mn^{2+} as the cofactor in reactions. Mg^{2+} is widely considered to be the biological relevant divalent cation cofactor for IN activity *in vivo*[16–19], but IN shows very low activity with Mg^{2+} as a cofactor, and Mn^{2+} is frequently used in most in vitro assays[8,10,20,21]. We optimized this assay in the presence of either Mg^{2+} or Mn^{2+} and found that the presence of Mn^{2+} instead of Mg^{2+} doubled the ST activity, but not as significantly as other reported works in which the Mn^{2+}-dependent activity was approximately 4–6 times that of the Mg^{2+}-dependent activity[13,20,21]. Using Mg^{2+} as the cofactor still showed a signal as high as 0.7 of A_{405}. Lee et al reported that

the 3P activity of IN alters depending on the structure and length of the oligonucleotide substrates, and increases in the length of the substrate cause alterations in the efficiency of Mg^{2+}-dependent ST activity[22]. Hwang *et al* optimized their ST activity assay in the presence of Mg^{2+} using 35 bp donor DNA and suggested that the lengthening of donor DNA allowed Mg^{2+} as the cofactor[7]. It is clear that an increase in the length of the DNA substrate could enhance the Mg^{2+}-dependent IN activities as suggested[7,22–24]. With the lengthening of donor DNA to 31 bp, the assay can be conducted in the presence of either cationic cofactor and extends the assay utility.

Previous *in vitro* studies for IN 3P reactions using Mg^{2+} as a cationic cofactor have reported that the pre-incubation of IN with DNA increases enzymatic activity, and IN-DNA interaction does not require a metal ion cofactor[14]. In contrast, Vink *et al* used Mn^{2+} in their study as the cationic cofactor and reported that the stable binding of IN to DNA requires Mn^{2+} and IN shows increased activity upon pre-incubation with Mn^{2+}[25]. We observed complicated interactions between IN, DNA, and divalent ions in the ST reaction. When Mg^{2+} was used as the reaction cofactor, the pre-incubation of DNA with IN enhanced the ST activity remarkably, as compared with IN pre-incubated alone (Figure 5B). This indicates an IN-DNA binding step before ST reaction. The same observation has been reported and the DNA-binding step has been determined by steady-state fluorescence anisotropy[14,26]. However, when Mn^{2+} was used as the reaction cofactor, no difference was observed after pre-incubation of DNA with IN, as compared with IN pre-incubated alone (Figure 5B). The result indicates that the IN-DNA interaction requires Mn^{2+} when Mn^{2+} is involved as the cationic cofactor. As previously suggested by Vink *et al*, Mn^{2+} is necessary for the effective binding and correct positioning of DNA in the active site of IN[25]. Furthermore, when either Mn^{2+} or Mg^{2+} was used as the cofactor, pre-incubation of metal ion with IN increased the ST activity, but pre-incubation of IN with Mn^{2+} instead of Mg^{2+} changed the ST activity more significantly (Figure 5). This information indicates the existence of the metal ion-IN interaction and metal ion coordination step before the ST reaction, and the interaction is more important for the Mn^{2+}-dependent ST reaction. Yi *et al* reported that the affinity of IN for DNA is increased in the presence of metal ions. The relative order of this effect is $Mn^{2+}>Mg^{2+}$, and it is suggested that the different metal ion effect may explain why Mn^{2+} is generally more effective than Mg^{2+} as a cofactor for catalysis in the *in vitro* assays [27]. Taken together, the information presented from our experiments indicates that the Mg^{2+}-dependent and Mn^{2+}-de-

pendent ST activity is not equivalent. The difference of IN activities in the presence of either 2 cations has been reported and widely studied[23,25–30]. Engelman et al reported that IN displays more non-specific nuclease activity and less ionic strength in the presence of Mn^{2+} than in the presence of Mg^{2+} [23]. Moreover, it has been demonstrated that mutations located in the IN binding site significantly affect the Mg^{2+}-dependent IN activity, but not the Mn^{2+}-dependent activity[28], suggesting that the IN-DNA contact is quite different with these 2 cations and IN coordinates these 2 cofactors differently. The coordination property differences of Mg^{2+} and Mn^{2+} may result in different conformational changes of IN, therefore causing differences in the specificity of IN-DNA interactions and accounting for differences in the Mg^{2+}-dependent and Mn^{2+}-dependent IN activities as suggested[26,28,30]. It is confirmed that our assay can be adapted to study the ST reaction mechanism in a high-throughput format.

The assay we developed was designed to screen antiviral compounds targeting IN. Therefore, we tested the inhibitor screening efficiency of the microplate assay using 2 known integrase inhibitors: baicalein and 1 compound of the diketo acid family L-708906. The IC_{50} values are comparable to previous experiment data using other assays[8,20], indicating that this microplate assay is efficient and reliable in antiviral compound identification. This assay is optimized in multiwell microplate format, therefore has the potential of screening antiviral drug candidates in a high-throughput format.

In summary, the assay we presented here can be used for the rapid and specific detection of HIV-1 IN ST activity as well as for the efficient identification of drug candidates targeting IN. Furthermore, this assay can be also adapted to study the reaction character in a high-throughput manner. Future efforts will focus on using this assay to screen compound libraries to test HTS efficiency of the assay and identify new IN inhibitor candidates.

Acknowledgements

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