Binding Residues of Intergrase (IN) and Its Host Cofactor Ini1

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ABSTRACT

Purpose Integration of viral DNA into its host chromosome is an important step in the HIV replication process (7). HIV integrase is an important enzyme which plays an essential role in inserting the vital DNA into the host chromosome and replicating HIV (6). It is one of the three enzyme will complement the therapeutic use of HIV protease and reverse transcriptase inhibitors. Though combining antiviral therapy with protease and reverse transcriptase inhibitors has proved the improvement of antiviral therapy for treatment of AIDS. However, because there are still some problems for drug resistance and toxicity, we still need to do some additional researches on the antiviral drugs. Integrase is a good target for antivirals due to its necessity for HIV replication. In addition, integrase can only use a single active site to accommodate two different configurations of DNA substrates, which will constrain the process of HIV to develop drug resistance to integrase inhibitors (7).

To achieve the goal of crystallizing IN, our lab has been focusing much attention on the proteins that are binding partners of IN or host cofactors known to alter the biochemical characteristics of HIV-IN. One such host cofactor is SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily B member 1 or also known as Ini1/SNF5. The goal of my research will be to express, purify, and characterize various Ini1 fragments as a potential chaperone in the crystallization of IN. And to find Ini1's correct residues which binds to IN.

Methods We cloned three different Ini1 constructs into the Sumo-Pro expression vector. We used PCR to get three lengths of fragments and inserted our construct into the SUMO vector which encodes a SUMO expression chaperone as well as a 6-Hisitidine affinity tag for IMAC purification. We tested for expression of the

Sumo-Ini1 fragments through common methods utilizing the T7 based expression systems engineered into the Sumo-Pro vector. After small scale expression (5ml or 100ml cultures) and lysis via sonication, Ini1 was affinity purified with Ni-NTA and then by size-exclusion chromatography. Once the best behaved Ini1 construct was identified, we produced the protein in multiple 1L scale and purified to >90% purity. The SUMO tag includes an N-terminal recognition site that is recognized by the SUMO protease and will be removed. The purified protein then will be screened for crystal conditions.

Results Size exclusion chromatography showed us a single, homogeneous, peak when Ini1 protein fragment from residue 170 to residue 250 was dialyzed with IN. In addition, after purification, both IN and Ini1 was contained in this single peak. This confirmed that we had a Ini1 fragment that was selectively binding to IN and forming a complex upon mixing and dialyzing. The discovery of this stable complex is the first step in our attempts to stabilize IN for our crystallographic pursuits.

KEY WORDS HIV1, Integrase, Integrase Interactor 1 (Ini1), protein complex, x-ray crytallography

INTRODUCTION

Retroviruses, such as HIV-1 that causes AIDS, have an RNA genome that is reverse-transcribed into a linear viral DNA upon entering the host cell. Integration of this viral DNA into host's chromosome is an essential step in the lifecycle of retroviruses, and is carried out by the virally encoded integrase (IN) protein. HIV IN folds as a 3-domain protein. Retroviral INs functions as a tetramer and catalyzes processing of the blunt-ended viral DNA ends as well as subsequent concerted insertions of these processed ends into the backbones of a target DNA. The central catalytic domain contains the triad of acidic residues (DDE motif) that coordinate Mg2+ or Mn2+ ions in the active site (4). These metal ions play critical roles in activating the attacking nucleophiles during both 3'-end processing and strand transfer

reactions. The catalytic domain alone forms a stable homodimer, but in the presence of N- and C-terminal domains IN is capable of forming higher order oligomers. Chemical cross-linking, AFM volumetric, and kinetic studies suggested that IN tetramer is the fully active for responsible for the concerted integration reaction in vitro (1). The IN tetramer juxtaposes the two viral DNA ends to form a synaptic complex, and following the 3'-end processing, captures a target DNA for performing the concerted strand-transfer reactions (2). Although the chemistry of the IN-catalyzed reactions is well understood, there is much unknown about how IN carries out these reactions. Despite the high medical relevance of retroviral IN, no crystal or NMR structure is available for any IN-DNA complex or a full-length three-domain IN protein responsible for the concerted integration reaction. The lack of structural information has been a significant limitation in our mechanistic understanding of the IN-catalyzed reactions (5).

MATERIALS AND METHODS

Materials

Bl21 (DE3) cells

Synthetically engineered Ini1 gene

HIV1 Integrase

Sumo-pro expression vector

6-Hisitidine affinity tag

Ni-NTA

LB, TB, Zym-5052

Methods

Molecular biology We cloned three different Ini1 constructs into the Sumo-Pro expression vector. The Aihara lab has already obtained the complete Ini1 gene. We used PCR techiniques to ‘fish’ out Ini1 fragements of various lengths for study. The

first fragment was the core IN binding domain (residues 183-243). We also designed primers to extend the size of the Ini1 fragment all the way to the C-terminus (residues183-385). Lastly, I made an intermediate sized construct (residues 183-275). The use of the Sumo-pro vector would place my gene of interest on the C-terminus of an expression chaperone (SUMO) and a 6-Hisitidine affinity tag for purification. A 6-Hisitidine affinity tag was added to the C-terminus of IN integrase (Ini was without 6-Hisitidine affinity tag.).

Expression of Ini1 in E.coli I tested for expression of the Sumo-Ini1 fragments through common methods used to maximize heterlogous expression levels. Initial testing was done in 5ml scale. First, I transformed my expression vector into two different E.coli cell lines: bl21 (DE3) and bl21 (DE3)-Rosetta2. The first cell line is a generic cell line used for expression and the second includes an expression vector (pRare) that produces rare codons that facilitates the tRNA requirements of a eukaryotic gene beings expressed in a proakyrote such as E.coli. In addition to testing 2 different cell lines, I used different expression temperatures (37C, 25C, 18C) to find the optimal temperature for maximum protein expression. Lastly, I used three different media (LB, TB, Zym-5052) to maximize expression levels. The initial expression will be assessed by SDS-PAGE gels and further characterized after purification.

Characterize solubility and oligomeric states of Ini Then I characterized the solubility and oligomeric states of Ini1. Once expression has been confirmed by SDS-PAGE, I will grow 1L cultures. After expression and lysis via sonication, protein will be affinity purified with Ni-NTA and then by size-exclusion chromatography. These last two purification steps not only purify our protein but will allow us to access the solubility and the oligomeric state of the purified protein.

Crystal screening of best Ini1 constructs At last, several crystal screening of best Ini1 were constructed. Once the best behaved Ini1 construct has been identified, we would continue producing the protein in multiple 1L scale and purified to >90% purity. The

SUMO tag included an N-terminal site that was recognized by the SUMO protease and was removed. The purified protein was screened for crystal conditions. And I tested over 1000 conditions through the crystallization facility at our department.

RESULTS

We sought to find the correct fragment of Ini1 which binds to integrase (IN) by running different parts of Ini1's size exclusion chromatography followed by running the fraction in SDS-PAGE gels.

Three lengths of Ini1 constructs were prepared. Using PCR techniques, the first fragment is the core IN binding domain (residues 183-243). Through designing primers, we are able to extend the size of the Ini1 fragment all the way to the C-terminus (residues 183-385). Lastly, an intermediate sized construct (residues 183-275) was made. The use of the Sumo-pro vector would place my gene of interest on the C-terminus of an expression chaperone (SUMO) and a 6-Hisitidine affinity tag for purification. A 6-Hisitidine affinity tag was added to the C-terminus of IN integrase. But we did not add tag to Ini1. However, after purification for Ini1 residue 170 to 250 with Ni-NTA, we found both IN and Ini1 existed in the fluid. From this result, it is obvious to see that IN and Ini1 banded each other.

From size-exclusion chromatography (Figure 1), we found that there is a clear and single peak for the IN core combined with Ini1 (170-250) protein. It means that Ini1 from residue 170 to residue 250 binds with IN very well (Figure 3).

In addition, after purification, we also ran the SDS-PAGE of Integrase (IN) and Ini1size exclusion fraction (residue 170 to 250) (Figure 2). The gel confirmed that contained in this single peak from the sizing column contained both the IN and Ini1 proteins (residue 170 to 250). On the eighth column, there are two unclear marks, one is integrase and the other is Ini1 size exclusion fraction (residue 170 to 250). This shows that IN and Ini1 were separated when running the gel.

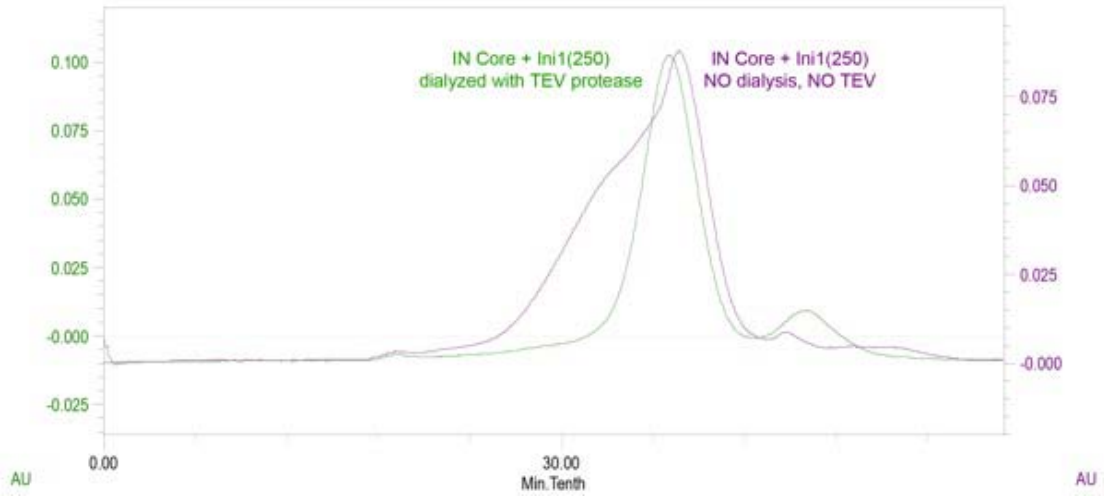

Fig. 1 The comparison between IN core with Ini1 (170-250) dialyzed with TEV protease and IN core with Ini1 (170-250) which is without dialysis and TEV protease.

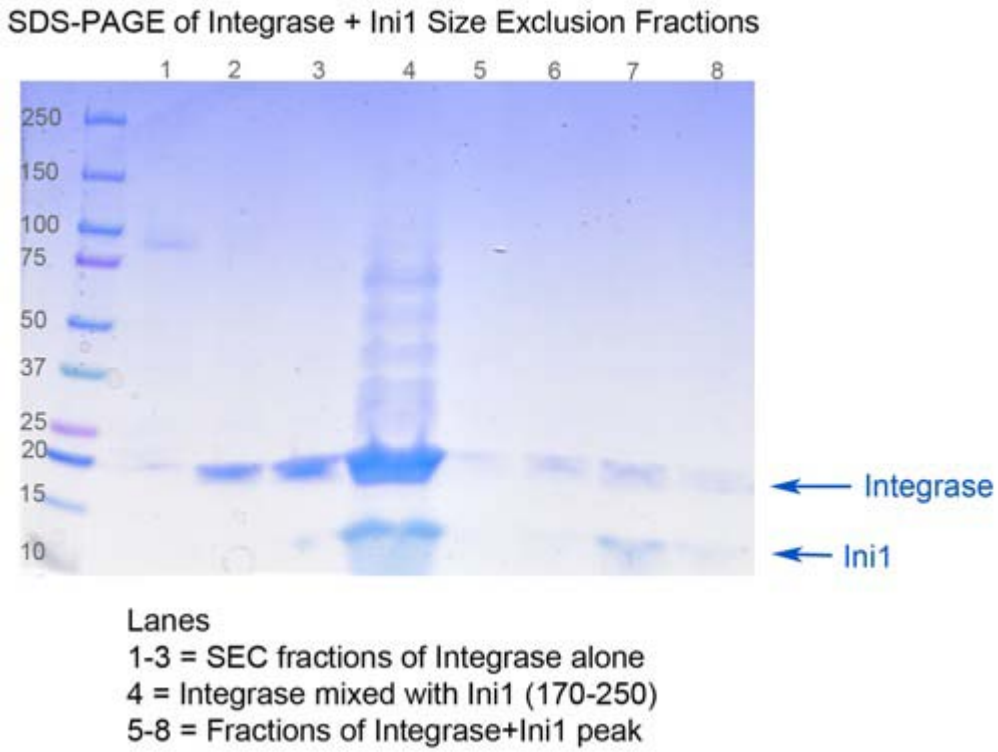

Fig. 2 The SDS-PAGE of IN with Ini1 size exclusion fractions (170-250). Column forth shows IN and Ini1 (170-250) binds together. Column eighth shows IN and Ini1 were separated when running the SDS-PAGE.

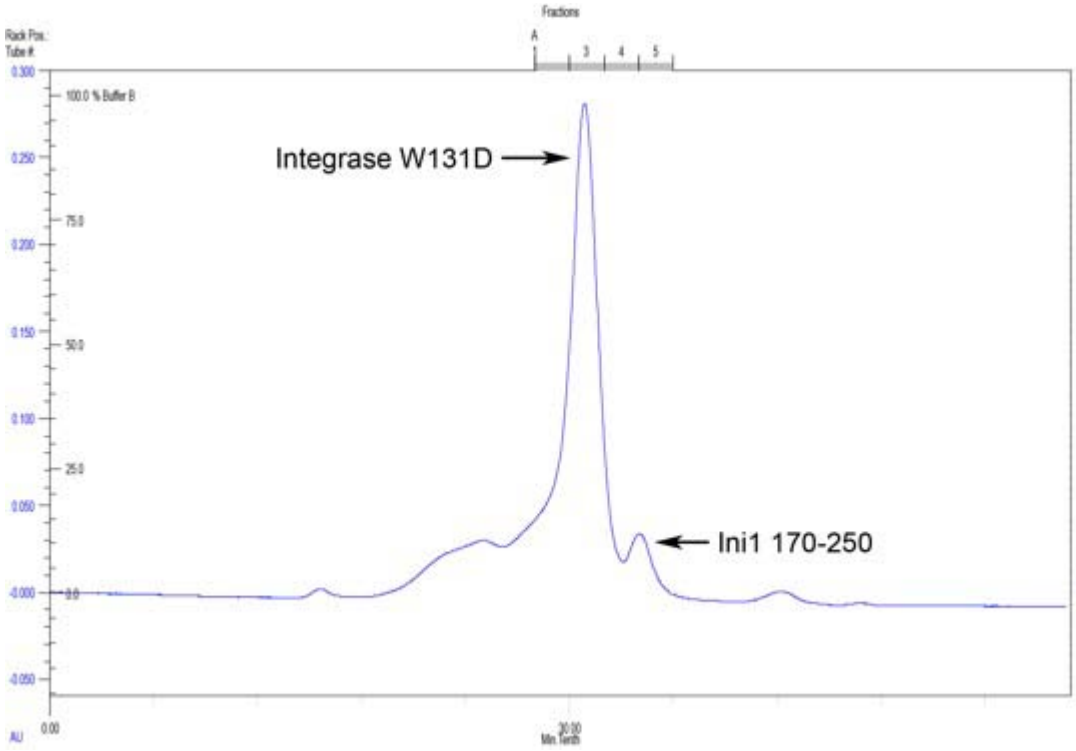

Fig 3. Size- Exclusion Chromatography Graph of IN W131 D and Ini1 170 -250. Ini1 did not bind to this mutant of IN which stabilizes IN but inhibited its binding effectiveness to Ini1.

DISCUSSION

We found that residue 170-250 of Ini1 cofactor binds to IN integrase very well. From SDS-PAGE gel, we can found that both IN and Ini1 comes off the column, untagged Ini1 coming for the ride. That means that Ini1 binds to IN. And also there is a clear and high peak for the IN core combined with Ini1 (170-250) protein. It means that Ini1 from residue 170 to residue 250 binds with integrase IN very well. It is obvious to see that IN and Ini1 binds together at the beginning, and then separated when running the SDS-PAGE. However, other fragments of Ini1 cofactor did not bind to IN intergrase very well.

In order to generate a more folded domain of Ini1 that binds to IN Integrase, new ini1 constructs generated. Those are p19.TEV-INI1 (170-230), p19.TEV-INI1 (170-240), p19.TEV-INI1 (180-230), p19.TEV-INI1 (180-240), p19.TEV-INI1

(180-250). These were all made with the natural human cDNA sequence. And all have a short TEV-cleavable 8xHis tag. But we hoped that p19 construct doesn't have his-tag.

However, it is still unknown that whether the concentration of Ini1 or IN integrase will also influence the result of this experiment. But in some other research, IN's concentration will also influence Ini1 both stimulates and inhibits *in vitro* integration (3). So, there are still some other factors that can influence the binding condition.

CONCLUSIONS

We have shown that residues 170 to 250 are the best constructs of Ini1 cofactor for the purposes of the Aihara Lab. Through getting different lengths of Ini1 fragments and added a 6-Hisitidine affinity tag for purification, a variety of different fragments have been prepared to do the Size- Exclusion Chromatography and SDS- PAGE. We have identified from Size-Exclusion Chromatography that IN and Ini1(170-250) forms a complex in solution. The SDS-PAGE gel shows that within the single peak of the sizing column contains both IN and Ini1, confirming the fact that in solution. IN and Ini1(170-250) forms a complex. Ultimately, the goal of the Aihara Lab is to crystallize IN in the presence of DNA. One of the main hindrances in achieving this goal is the instability of IN protein. It is our hope that the complex of IN and Ini1 will substantially stabilize IN to improve our chances of crystallizing IN.

REFERENCE

1. Bao, K.K., Wang, H., Miller, J.K., Erie, D.A., Skalka, A.M., Wong, I. (2003) Functional oligomeric state of avian sarcoma virus integrase. *J. Biol. Chem*. 278, 1323-1327.
2. Bera, S., Pandey, K.K., Vora, A.C., Grandgenett, D.P. (2009) Molecular Interactions between HIV-1 Integrase and the two viral DNA ends within the synaptic complex that mediates concerted integration. *J Mol Biol*. Apr 9. (Epub ahead of print).
3. Das, Supratik, Jennifer Cano, and Ganjam V. Kalpana. "Multimerization and DNA Binding Properties of INI1/hSNF5 and Its Functional Significance." The Journal of Biological Chemistry 284 (2009): 19903-9914. The Journal of Biological Chemistry. 27 Apr. 2009. Web.
4. Engelman, A., Craigie, R. (1992) Identification of conserved amino acid residues critical for human immunodeficiency virus type 1 integrase function in vitro. *J Virol*. 66, 6361-6369.
5. http://www.biomedcentral.com/content/pdf/1742-4690-5-114.pdf
6. Dyda, Fred, Alison B. Hickman, Timothy M. Jenkins, and Alan Engelman. "Crystal Structure of the Catalytic Domain of HIV-1 Integrase: Similarity to Other Polynucleotidyl Transferases." 266.5193 (1994): 1981-986. *Science*. Web. 28 Aug. 2011. <http://www.sciencemag.org/content/266/5193/1981.short>.
7. Goldgur, Yehuda, Robert Craigie, Gerson H. Cohen, and Tamio Fujiwara. "Structure of the HIV-1 Integrase Catalytic Domain Complexed with an Inhibitor: A Platform for Antiviral Drug Design." *PNAS* 23rd ser. 96 (1999): 13040-3043. *PNAS*. Web.