

ORGANIZATIONS OF INDIVIDUAL SUBUNITS WITHIN THE FULLY FUNCTIONAL HIV-1 INTEGRASE TETRAMER AS A THERAPEUTIC TARGET

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HIV-1 integrase (IN) functions as a tetramer and a correct organization of individual subunits within the nucleoprotein complex is essential for effective concerted integration. Interactions between individual subunits are highly dynamic in unliganded IN, while the stable synaptic complex (SSC) is formed upon association of a tetrameric protein with two viral DNA ends. A key cellular cofactor LEDGF also promotes IN tetramerization. However, the preformed IN-LEDGF complex is defective for concerted integration, while addition of LEDGF to the preassembled IN-vDNA complex ensures effective pair-wise integration. In the present study we have examined a hypothesis that direct binding of LEDGF differentially modulates structural conformations and functions of the pre-formed IN-vDNA complex and unliganded IN. We used protein-protein FRET to characterize how additions of LEDGF and vDNA modulate IN conformations. The obtained results suggest that IN forms “open” and “closed” conformations in the IN-LEDGF and IN-vDNA complexes. The “closed” conformation is consistent with the fully functional nucleoprotein complex, where two active sites in the context of the SSC are correctly positioned for effective concerted integration. The order of addition experiments indicated that addition of LEDGF to the preformed SSC does not significantly alter structural arrangements between IN and viral DNA. These findings provide insights into the organization of IN subunits in the functional nucleoprotein complex. At the same time modulation of the unliganded IN structure by LEDGF suggests a new allosteric mechanism for inhibiting the retroviral enzyme, where interacting IN subunits could be “locked” into the non-functional conformation.

In a related study we have identified and characterized an allosteric mechanism of action for a known IN inhibitor compound **1**. We have found that the inhibitor strongly modulates dynamic interactions between IN subunits and compromises formation of the fully functional nucleoprotein complex. Structure activity relationship studies of **1** indicated the importance of a central methyl ester group of the inhibitor for selective binding of the compound to HIV-1 IN dimer interface. The proposed mechanism of action and binding site for the small molecule inhibitor identified in the present study provides an attractive venue for developing allosteric inhibitors of HIV-1 IN.