FLUORESCENCE STUDY OF PROTEIN ENGINEERED HIV-1 INTEGRASE

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ABSTRACT

Viral infection and replication is due, in part, to the activity of three essential enzymes, reverse transcriptase, protease and integrase, which encoded by the *pol* gene of HIV [1-4]. HIV-1 Integrase accomplishes a complex, yet essential step in the progression of viral infection. This enzyme is required for processing and integration of the viral DNA into the host chromosome by mechanisms known as 3' processing and strand transfer [5-8]. HIV integrase recognizes specific sequences in the long terminal repeat regions of viral DNA [9]. Subsequent to recognition, the DNA cleavage reaction, termed 3' processing, involves the removal of two nucleotides (GT), from the 3' ends (CAGT) of the viral DNA by a series of transesterification reactions [3, 10]. The product of this reaction has a 3'-OH which act as nucleophiles and covalently join to host's chromosomal DNA.

HIV-1 integrase is a 32 kD protein that consists of three functional domains. The N-terminal domain is classified as residues 1-50 contains a zinc finger motif that is thought to be involved in protein-protein interactions that lead to the multimerization of enzyme into its active complex [7]. The catalytic core, comprising residues 50-212, appears to be related to the family enzymes known as polynucleotidyl transferases. Integrase, as well as the other enzymes of this family, conserve the acidic residues Asp64, Asp 116, and Glu152 creating a "D,D-35-E" motif which is required for catalytic activity [5, 11]. The final domain is the DNA binding domain, which recognizes and interacts with the HIV LTR DNA region [9]. Although the structure of the holoenzyme has been somewhat contiguously solved by NMR and X-ray crystallography techniques [12,13], the conformations this protein takes on during these intricate and dynamic processes are not well understood.

In an attempt to understand the conformational changes in this enzyme during these orchestrated mechanisms, the protein was subjected to PCR site-directed mutations that would allow for time-resolved study via fluorescence spectroscopy. This protein has three functional domains (multimerization, catalytic core, and host DNA binding) where seven tryptophan residues are strewn within. The newly constructed mutants contained one tryptophan residue per enzyme, and this residue was localized to a specific domain so that each of the three domains could be observed during this enzyme's operation. This trytophan residue served as the donor of fluorescence to the acceptor, marina blue, which was located on a viral DNA substrate in Förster resonance energy transfer (FRET) experiments. These experiments allowed for the calculation of a mean distance between this donor/acceptor pair during protein-DNA interactions, which would indicate any changes in molecular distances during DNA processing. It was observed that the W19 (multimerization domain) did not undergo significant conformational changes in the presence of DNA because it showed no changes in fluorescence activity. Alternatively, both the W61 (catalytic core domain) and the W235 (DNA binding domain) were directly quenched by the unlabeled nucleotide. When comparing the FRET distances of each donor/acceptor pair is was seen that the distances of the W19 and W235 residues to the 3' end of the substrate agreed with previously determined distances found by crystal structures, while the distance between W61 and the DNA did not. The distance between W61 and the 3' end of the substrate was greater than crystal structure determinations, which hints at the idea that the DNA may not remain proximate to the active site after processing. Unfortunately, these mutants showed decreased solubility, which impeded long-term study of this mechanism. Thus, further mutations were made on the W61 mutant to enhance its solubility for future study. The F139D mutant has been expressed and identified by protein characterization techniques and will be the candidate for fluorescence study, pending light-scattering experiments to confirm its decreased aggregation in solution.

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