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INTEGRASE, AS POTENTIAL TARGET IN HIV-1 THERAPY – A SPECTROSCOPIC APPROACH

Horea Porumb

UFR Médecine, Santé, Biologie Humaine, Université Paris 13, 93017 Bobigny Cedex et Laboratoire de Biotechnologies et de Pharmacologie Génétique Appliquée (LBPA), UMR CNRS 8113 Ecole Normale Supérieure de Cachan, 94235 Cachan Cedex, France

Integrase (IN) catalyses the insertion of the pro-viral DNA of HIV-1 into the human genome by a two-step reaction: i. the “activation” of the pro-viral LTR extremities by the excision of a GT dinucleotide in 3' of each strand and ii. the insertion of activated pro-viral DNA into the host chromosome, a process referred to as “strand transfer”. Recent integrase inhibitors, belonging to the DKA family, block the process at the strand transfer stage.

A helix-turn helix (HTH) motif, consisting in the $\alpha 4$ and $\alpha 5$ helices of IN, behaves like a multifunctional entity. Being involved in enzyme oligomerization (via its $\alpha 5$ helix), in LTR end recognition (via its $\alpha 4$ helix) and in binding LEDGF prior to the choice of the integration site (via the $\alpha 4$ - $\alpha 5$ turn region, which also acts as a strong epitope), HTH emerges as a central piece of IN structure and activity. The $\alpha 4$ helix and the loop that links it to the $\alpha 3$ helix is the target of the DKA inhibitors. Altogether, HTH constitutes a model for the study of new inhibitors acting at the IN-IN, the IN-DNA and the IN-LEDGF interfaces.

The above story will be the pretext for the description of a number of (mainly UV-VIS and NMR) physical chemical and biochemical techniques – enumerated here in the order of presentation: circular dichroism spectral deconvolution, fluorescence quenching, fluorophore conjugation, enzymatic assays, helicogenic substitution, ANS oligomerization test, Stern-Volmer assay, fluorescence temperature coefficient, secondary structure prediction, chemical shift index, trifluoroethanol titration, NMR temperature coefficients, diagnosis for coiled-coil formation, fluorescence anisotropy, etc.

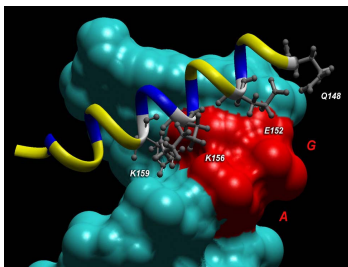


Image: the $\alpha 4$ peptide bound to the LTR DNA extremity

References:

1. Circular dichroism and fluorescence of a tyrosine side-chain residue monitors the concentration-dependent equilibrium between U-shaped and coiled-coil conformations of a peptide derived from the catalytic core of HIV-1 integrase. Horea Porumb, Loussinée Zargarian, Hayate Merad, Richard Maroun, Olivier Mauffret, Frédéric Troalen and Serge Femandjian (2004) *Biochim. Biophys. Acta - Proteins & Proteomics*, 1699, 77-86.
2. Merad H, Porumb H, Zargarian L, Rene´ B, Hobaika Z, et al. (2009) An Unusual Helix Turn Helix Motif in the Catalytic Core of HIV-1 Integrase Binds Viral DNA and LEDGF. *PLoS ONE* 4(1): e4081. doi:10.1371/journal.pone.0004081