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# 05-Molecular Modelling and Design for Proteins and Drugs

PS-05.01.20 DESIGN OF METALLOTHIONEIN ALPHA DOMAIN POLYMER. By J. Luo*, A. Pan, S. Yin, J. Sun, B. Kuang, L. Li, B. Ru and X. Gu, National Lab of Protein Engineering and Plant Genetic Engineering, Departemnt of Biology, Peking University, Beijing 100871, China

Metallothioneins(MTs) are low molecular weight, cysteine-rich and metal binding proteins. A native MT molecule contains two distinct domains, an α-domain which is characterized by cadmium-binding, and a β-domain which binds preferentially to zinc. Reconstruction and transformation of MTα domain polymer gene in plants may provide a valuable method for reclamation of wastelands and mine spoils rich of cadmium. Based on this conception, a gene which encodes human liver MT-IA(hMT-IA) α domain dimer was synthesized and is to be introduced into plants using the multiple copy cloning technique so that the host can yield more MT. In order to ensure the efficiency of expression of the gene in host, computer aided molecular design was employed in studying the structure and conformation both of native and mutant MT. The EMBL molecular biology data base was searched to retrieve protein sequence information of metallothionein molecules. Sequence analysis was then carried out which indicated that mammalian MTs are very conservative not only in the amino acid composition and sequence homology, but also in the domain arrangement. A three dimensional model of hMTI-A was built up taking the crystal structure of Rat MT-II and NMR data of Human MT-II, Rat MT-II and Rabbit MT-II as templates. Conformation study of this model indicates that a tripeptide of -Lys-Lys-Ser- serves as a linker between the two domains which are apparently independent. According to this investigation, a model of hMT-IAα domain dimer was constructed and the tripeptide -Lys-Lys-Ser- was used as the domain linker. A ten residue peptide -Glu-Leu-Asp-Gly-Pro-Lys-Lys-Ser-Gly-Ser- was finally chosen to link the dimers to form various α domain polymers. On designing of this linkage peptide, special consideration was given to the conformation flexibility and hydrophobicity of the peptide, the influence of the peptide on the dimer, as well as the ease of gene manipulation. This model was inspected by means of sequence analysis and molecular graphics, which gave some indication that various α domain polymers may fold in several patterns. Further experiments on expression proteins are underway.

## 05.02 - HIV Proteins and Drug Design

DS-05.02.01 STRUCTURE OF HIV REVERSE TRANSCRIPTASE by J. Wang*, S. J. Smerdon, L. A. Kohlstaedt, J. Jacger, P. A. Rice, J. M. Friedman, T. A. Steitz, Department of Molecular Biophysics & Biochemistry, Howard Hughes Medical Institute, Yale University, New Haven, CT 06511

The X-ray crystal structure of the reverse transcriptase (RT) from human immunodeficiency virus type-I co-crystallized with a non-nucleoside inhibitor, Nevarapine, has been determined at 3.2A resolution. The structure of the heterodimeric p66/p51 molecule shows a marked and somewhat unexpected degree of asymmetry with respect to domain and subdomain organization. The p66 subunit has a large cleft and resembles that of the Klenow fragment of E. coli DNA polymerase I while the equivalent cleft in p51 is filled by its connection domain. An A-form RNA-DNA hybrid can be model-built into the deep cleft that extends between the polymerase and RNase H active sites. This places the 3' end of the primer strand next to the conserved polymerase active-site carboxyls and the 3' end of the template strand 20 nucleotides upstream, next to the two metal ions of the RNase H active site. The relative positions of the metal ions and the modelled template terminus suggest a mechanism of RNA hydrolysis similar to that of the 3'-5' exonuclease of Klenow fragment. The presence of three conserved carboxylic acid residues in the pol active sites suggest that a two metal ion catalysis may also be involved in the polymerization reaction. Although these metals are not observed in electron density maps of RT at this stage, they are clearly seen in the structure of the Klenow polymerase. Solvent accessible surface calculations on the basis of a partially refined RT model indicate that interactions between the connection domains play a central role in the formation of all possible reverse transcriptase dimers (p66/p66, p66/p51, p51/p51). Furthermore, contacts with p51 involving the RNase H domain of p66 contributes substantially to the overall stability of the heterodimer. After the connection domain interactions are assumed to be in the asymmetric form seen in the X-ray structure, four main events must then take place to form the mature heterodimer which can be summarised as the unfolding and cleavage of one of the RNase H domains along with subdomain rearrangements.

DS-05.02.02 STRUCTURE OF HIV-1 REVERSE TRANSCRIPTASE/dsDNA/Fab COMPLEX: PROTEIN-DNA INTERACTIONS AND STRUCTURE OF THE POLYMERASE ACTIVE SITE. By J. Ding*, A. Jacobo-Molina, R.G. Nanni, X. Lu, C. Tantillo, A.D. Clark Jr., S.H. Hughes†, and E. Arnold, Center for Advanced Biotechnology and Medicine and Rutgers University Dept. of Chemistry, 679 Hoes Lane, Piscataway, NJ 08854-5638, †ABL-Basic Research Program, NCI-Frederick Cancer Research and Development Center, Frederick, MD 21702-1201.

The crystal structure of a ternary complex of human immunodeficiency virus type 1 reverse transcriptase (HIV-1 RT) heterodimer p66/p51, a 19 base/18 base dsDNA template-primer and a monoclonal antibody Fab fragment has been determined at 3.0 Å resolution (7 Å resolution structure reported in *Nature* 357:85-89, 1992). The polymerase domains of both p66 and p51 contain four subdomains, which are named fingers, palm, thumb and connection (Kohlstaedt, L.A., Wang, J., Friedman, J.M., Rice, P.A. & Steitz, T.A., *Science* 256:1783-1790, 1992). Although the structures of the individual subdomains within p66 and p51 are similar, their relative spatial arrangements within the two subunits are dramatically different. The template-primer binds in a large cleft formed by the fingers, palm, and thumb of p66. The structure of the template-primer is a hybrid resembling A-form DNA near the polymerase active site and B-form DNA towards the RNase H active site, with a significant bend (40-45°) at the A-/B- junction. The most numerous interactions of HIV-1 RT and the dsDNA occur primarily along the sugar-phosphate backbone and involve amino acid residues of the palm and thumb of p66. Highly conserved regions are located in the p66 palm near the polymerase active site and include a β-hairpin, denoted as

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