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STRUCTURAL ASPECTS OF GENE EXPRESSION BY AML1/CBFβ, C-MYB, AMV V-MYB, C/EBPβ AND ETS-1

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Assembly of stereospecific, multiprotein complexes on enhancers and promoters is a key step in transcriptional activation and tightly controlled by sequence specific transcription factors. Physical interaction between these transcription factors is supposed to control their DNA bindings, which lead to synergistic activation of transcription. Here, we address this problem for three different mechanisms of cooperation for trans-activation of myeloid genes.

We determined the crystal structures of c-Myb(R1R2R3)-C/EBPβ(bZip)-DNA, AMV v-Myb(R1R2R3)-C/EBPβ(bZip)-DNA, AML1(Runt)-CBFβ-C/EBPβ(bZip)-DNA, AML1(Runt)-DNA and AML1-Ets-1-DNA. On the basis of these crystal structures, further supported by mutational studies, atomic force microscopy observations and various biophysical and biochemical methods, such as EMSA, GST pull-down assays, surface plasmon resonance, ITC, CD and UV melting experiments, and luciferase trans-activation assays, we propose mechanisms of AML1-CBFβ, c-Myb-C/EBPβ and AML1-Ets-1 cooperative trans-activation. In the case of AML1-CBFβ cooperation, CBFβ binds to AML1 and enhances its DNA binding affinity by allosteric mechanism without interacting with DNA [Tahirov et. al. Cell 104, 755-767 (2001)]. In the case of AML1-Ets-1 cooperation, these transcription factors bind to adjacent sites on DNA by counteracting the action of autoinhibitory modules. Contrary to these two cases, c-Myb interacts with C/EBPβ from distant sites on promoter and their cooperation is accompanied by looping of intervening portion of DNA [Tahirov et. al. Cell 108, 57-70 (2002)].

Keywords: TRANSCRIPTION, PROTEIN-DNA, PROTEIN-PROTEIN

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A PHOTORECEPTOR POISED FOR LIGHT ACTIVATION

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To collect light information, organisms from all kingdoms of life employ photoreceptors that consist of small molecule co-factors (chromophores) bound to specific proteins. The specific environment inside the protein often substantially alters, or tunes, the photochemical and photophysical properties of the chromophores to fit the organisms' specific sensing needs.
Here we present the 0.83 Å resolution structure of the bacterial photoreceptor PYP. The very precise structure reveals subtle distortions in PYP's 4-hydroxy cinnamic acid chromophore that are the result of the protein's tuning action. Analysis of these distortions has provided new insights into the molecular details of wavelength and photochemical tuning in these photoreceptors. Bonding geometry analysis shows that the chromophore exists as a hybrid between its quinonic and phenolic form when inside the active site of the protein. The chromophore also exhibits significant bowing. This bowing is implicated in the suppression of fluorescence in favor of double bond isomerization as PYP's main photochemical response. The chromophore also shows a vibrational mode, which anticipates the trajectory the chromophore takes during double-bond isomerization. The structure further provides a glimpse at long range concerted motions in PYP and suggest a link between the equilibrium structural dynamics in PYP and the structural variations within the family of PAS domain proteins of which PYP is a member.

Keywords: ULTRA-HIGH RESOLUTION, PHOTORECEPTOR, DYNAMICS

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XYLANASE AT ULTRA HIGH 0.89 Å (100 K) AND ATOMIC 1.11 Å (293 K) RESOLUTIONS: EVIDENCE FOR SALT BRIDGE PLASTICITY

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Thermoascus aurantiacus xylanase, a thermostable enzyme, hydrolyses xylan, a major hemicellulose component in the biosphere. Structure of the xylanase with Triosephosphtae Isomerase (TIM) barrel, (β/α) 8-fold has been solved to small molecule accuracy at atomic resolution 1.11 A (293 K) (RTUX) and ultrahigh resolution 0.89 Å (100 K) (CTUX) using X-ray diffraction data sets collected on a synchrotron source resulting in R/R_{free} (%) 9.16/11.67 and 8.85/10.41 respectively. Both the structures have been refined with anisotropic atomic displacement parameters. The 0.89 Å structure with 177476 observed unique reflections has been refined without any stereo chemical restrains during final stages. It is note worthy that the 0.89 Å structure represents one of the highest resolution structures of such size with one monomer molecule in the asymmetric unit and also the highest resolution TIM barrel fold structure to date. Interestingly, salt- bridge Arg124- Glu232, which is bidentate in RTUX is water mediated in CTUX suggesting plasticity of ion pairs in proteins with water molecules mediating some of the alternate arrangements. The availability of accurate structural information at two different temperatures has enabled the study of the temperature dependent deformations of the TIM barrel fold of the xylanase. Analysis of RMS deviation of C-α atoms between RTUX and CTUX suggests that interior β-strands are less susceptible to changes with temperature than α-helices which are on the outside of the barrel. The 0.89Å ultra high-resolution structure of the xylanase can be a useful template to theoretical modeling studies of the structure and dynamics of the ubiquitous TIM barrel fold.

Keywords: TIM BARREL ULTRAHIGH RESOLUTION SALT BRIDGE PLASTICITY

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ACORN AND ITS APPLICATIONS

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Acorn is a comprehensive and efficient procedure to determine any 3-dimension structure with atomic resolution data. A complete structure can be developed from a fragment composed of less than 5% of the scattering matter of unit cell. For example, acorn can solve a structure of 1093 atoms from only one sulphur or a structure of 4762 atoms from 9 sulphurs. In such applications the substructure will generally have been identified from their anomalous scattering data by Acorn or other direct methods and patterson method. A standard alpha-helix or a motif from protein data bank are effective fragments to be used and acorn uses the molecular replacement method based on the correlation coefficient between observed normalized structure factors and those calculated from the fragment to obtain correct orientations and positions. The initial phases from the fragment are refined using dynamic density modification, real space sayre equation refinement and patterson superposition method. The map from Acorn normally reveals more than 90% of the whole structure and the model is easily built up automatically by computer. Acorn has solved 5 new protein structures with sizes from 125 to 350 residues by users in the UK and France. In addition, Acorn can solve substructures, for instance those of anomalous scatterers from SAD or MAD data. Examples to date include 7 substructures from 10 phosphorus to 166 selenium atoms. Data to much lower resolution are of course sufficient for these applications.

Keywords: AB INITIO PHASING MACROMOLECULE ATOMIC RESOLUTION