double-bromodomain of the CCG1 subunit in the TFIID complex (DBD(CCG1)). This interaction should be a key to connecting the histone acetylation and the nucleosome structure change. Here we report the crystal structure of the CIA-DBD(CCG1) complex at 3.3 Å resolution. Although we crystallized the complex by mixing equimolar amounts of CIA and DBD(CCG1), the crystal structure showed that one DBD(CCG1) molecule binds two CIA molecules in two distinct sites. The GST pull-down assay with point mutants of DBD(CCG1) at either binding site also suggested that DBD(CCG1) has two binding sites for CIA in solution. The crystal structure of the CIA-DBD(CCG1) complex suggested that an acetylated lysine residue can interact with DBD(CCG1) in the complex. In addition, the superposition of the CIA/ASF1 in the CIA-histone-H3-H4 complex [Natsume *et al.*, Nature (2007)] onto the CIA in the present complex showed that the histone H3-H4 dimer competes with DBD(CCG1) for CIA through steric hindrance. These observations suggest that (i) the recruitment of the CIA-DBD(CCG1) complex to the acetylated histone and (ii) the histone acetylation-induced nucleosome structure change are mediated by interactions among CIA, DBD(CCG1), and acetylated histones.

Keywords: histone chaperone, bromodomain, nucleosome assembly/disassembly

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Structural studies of human PCNA mutant, REV6-1

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All cells are continually exposed to a large variety of external and internal agents that damage their DNA. DNA replication in eukaryote is a highly coordinated process involving many proteins that work cooperatively to ensure the accurate and efficient replication of DNA. Although replicative DNA polymerases δ/ε synthesize genomic DNA in normal condition, the replicative polymerases are not able to synthesize DNA using damaged nucleotide as a template because of their high fidelity. In that situation, translesion synthesis (TLS) polymeases alternatively synthesize DNA by error-free or error-prone manners. PCNA, a member of sliding clamp family and ring-shaped homotrimer, not only stimulates processive DNA synthesis by the replicative polymerase, Polδ, but also provides the central scaffold to which TLS polymerases bind to gain access to the replicative machinery stalled at the lesion site and to execute lesion bypass synthesis. Actually, PCNA is characterized as one of *REV* genes' products affecting UV-induced mutagenesis in *Saccharomyces cerevisiae*. Furthermore, the *rev6-1* mutant strain produces a G178S substitution in PCNA (REV6-1) and shows deficiency in TLS. Although the side chain of substituted serine appears to show an unfavorable steric interaction because the G178 residue is located in a β-sheet at the interface between the monomers composing trimer, structural features of REV6-1 are still unclear. To clarify the influence to oligomerization of PCNA by only one-residue-substitution, we investigate the difference of structural and functional features between human PCNA and REV6-1 by several methods including crystallography, and discuss the comparisons of their behaviors in solution and crystal.

Keywords: DNA replication, structural biology, protein crystallography

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Uranyl mediated photocleavage in proteins

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Uranyl ions absorb light in the UV-visible region and have been found to bind to and cause photo-cleavage in biomolecules such as proteins and nucleic acids. In DNA, the uranyl ion binds to the phosphate backbone of the molecule and can potentially cleave the molecule between any base pair along the chain. In proteins, this binding and thereby the induced structural changes, are much more specific. One of many uranyl-binding proteins is porcine pancreatic elastase (PPE). In this study, elastase-crystals have been soaked in a uranyl-containing buffer and several data sets have been collected in house and at the synchrotron at both room temperature and at 100K. The uranyl binds with high occupancy in a single position close to the well-known calcium/sodium binding-site. Only small conformational changes compared to the native structure are seen upon ligand binding. These changes originate from amino acids moving either to accommodate space for (Tyr71) or to co-ordinate the ligand (Glu59, Glu69). However, the sodium ion is not moving from its native site. In addition, two acetates bind to the uranyl. After irradiation (400nm, 2h), the observed structure is still that of the native structure. However the density of one amino acids in the direct proximity of the uranyl ion, Asn63, has completely disappeared. The densities of the acetates have also degraded and only the positions of the coordinating oxygens are visible.

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Keywords: photo-induction, protein-ligand complexes, protein X-ray crystallography

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Water-soluble chlorophyll-binding protein from *Chenopodium album*

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Water-soluble chlorophyll-binding proteins (WSCPs) have been found in some terrestrial plants. WSCPs can be categorized into two classes according to photoconvertibility. The absorption spectra of class I WSCPs change drastically on exposure to visible light, whereas those of class II WSCPs do not change at all. The amino acid sequences of classes I and II WSCP show no similarity to each other. The physiological function of WSCPs is not known although it is supposed to be a chlorophyll (Chl) carrier. So far, only the structures of class II WSCP have been determined because of high sensitivity

of class I WSCP to visible light [1]. WSCP from *Chenopodium album* (*C. album* WSCP), belonging to class I WSCP, was extracted from leaves.The crystals were obtained using a reservoir solution containing 3.0 M KSCN and 26% PEG monomethyl ether 2,000. All of the experiments were performed in a dark room to avoid the photoconversion. X-ray data were collected at beamline BL-6A of the Photon factory (Tsukuba, Japan). The structure was determined at 2.0 Å by the MAD method with a reconstituted *C. album* WSCP with Chl containing Zn instead of Mg. *C. album* WSCP is a homotetramer assembled with crystallographic 222 symmetry. Each subunit consists of 147 residues and contains one Chl molecule. The Chl binding mode of classes I and II WSCP are completely different from eath other. As for class II WSCP, four Chl molecules reside at the subunits' interface and at the center of a tetramer, therefore the four Chl molecules interact intimately, whereas each Chl molecule of *C. album* WSCP is accommodated in the subunit, and isolated from each other.

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Keywords: water-soluble chlorophyll protein, photoconvertibility, pigment protein

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Multiple coordination and quaternary states of fish hemoglobin re-open the root effect question

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The Root effect is a widespread property in fish hemoglobins (Hbs) that produces a drastic reduction of cooperativity and oxygen-binding ability at acidic pH. Up to now, the structural explanation of the Root effect has been based on the two-state model, and is related to an over-stabilization of the T quaternary structure. Here, we report the crystal structure of the deoxy and carbomonoxy form of the non-Root effect major component Hb isolated from the Antarctic fish Trematomus newnesi (Hb1Tn). In the deoxy state, the inter-aspartic hydrogen bond at the $\alpha 1\beta 2$ interface between Asp95α and Asp101 β is observed. In the carbomonoxy Hb1Tn crystals, both a T-like state and a R/T intermediate quaternary structure are observed. In these crystals, three of four independent CO coordination states are not assisted by the hydrogen bond with the distal histidine, that goes out of the heme pocket. This un-assisted CO coordination states are associated with unusually small thermal fluctuations which characterise both α and β CD corners. The accessibility of ligated states within three different quaternary structure (T, R and R/T intermediate) suggests a novel structural explanation of protein allostery based on a three state Edelstein's model. Grant Sponsor: PNRA.

Keywords: allostery, hemoglobins, Raman scattering

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Crystal structure of human cystathionine gamma lyase: A key enzyme in hydrogen sulfide production

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Impairment of the formation or action of hydrogen sulfide (H_2S), an endogenous gasotransmitter, is associated with various diseases such as hypertension, diabetes mellitus, septic and haemorrhagic shock and pancreatitis. Cystathionine-beta-synthase (CBS) and cystathionine-gamma-lyase (CSE) are two pyridoxal-5-phosphate (PLP)-dependent enzymes largely responsible for the production of H_2S in mammals. CBS is expressed predominantly in the central nervous system and the regulation of CBS has been well studied whereas CSE is mainly responsible for the production of H_2S outside of the nervous system and its regulatory mechanisms are less well understood. Here we report the crystal structure of human CSE at 2.4 A resolution. Structural characterization, combined with literature provides new insights into the CSE-mediated production of H_2S. Structure of the different forms of CSE reveal an open form, a hitherto not reported for any PLP dependent enzymes, and closed conformation of human CSE. Our results will be a starting point to facilitate structure-based design of novel inhibitors to aid in the development of therapies for diseases involving derangement of sulfur metabolism.

Keywords: hydrogen sulfide, cystathionine gamma lyase, crystal structure

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Structural characterization of the bacterial glutaminyl cyclase from *Zymomonas mobilis.*

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N-terminal pyroglutamate (pE) formation is an event catalyzed in biological systems by glutaminyl cyclases QC, (EC 2.3.2.5), which can be classified into two families, the plant and the mammalian family of QCs. Strong evidence exists for a direct participation of human QC in the onset and progression of Alzheimer's disease by generation of pE modified amyloid peptides. The plant enzymes, which show no sequence homology to the mammalian enzymes, have been implicated in defense mechanisms. Analysis of microbial genomes reveals a series of genes with peptide sequences homologous to plant QC enzymes. Here we show that these bacterial sequences indeed code for glutaminyl cyclases. The putative