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

coding sequence of Zymomonas mobilis QC (ZmQC) was cloned and expressed. The recombinant ZmQC shows QC activity and its 1.7Å resolution crystal structure confirmed its close relationship to the plant QC enzymes. The bacterial ZmQC protein exhibits a five β-propeller fold with a cation, presumably calcium, in its core. The β-propeller consists of a five-fold repetition of four stranded antiparallel β-sheets arranged around a central axis to form a central tunnel connecting both sides of the molecule. The propeller is stabilized through a ¨ Velcro ¨ motif that tethers the toroidal structure but lacks any disulfide bridges present in the related plant enzymes. The putative active center of ZmQC, occupied in the crystal by a glycerol molecule, is lined by the residues E46, E90, W104, W130, W175, N175 and K244. The three trytophans form a square pocket juxtaposed by the other catalytically relevant residues E46, E90 and K244.

Keywords: Alzheimer disease, bacterial glutaminyl cyclase, pyroglutamate

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Optical separation of rimantadine and *in silico* prediction of chiral selectivity of M2 protein

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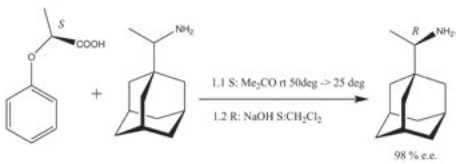
Racemic rimantadine possesses some N-methyl-D-aspartate (NMDA) antagonistic properties and is used as an antiparkinsonic drug. Rimantadine is also an orally administered antiviral drug used to treat and prevent influenza A infection. Genetic studies suggest that the virus M2 protein, a proton channel, plays an important role in the susceptibility of influenza A virus to inhibition by rimantadine. The production of chiral amines has great importance in the pharmaceutical industry, such as chiral switch. An aqueous solution of racemic compound and optically pure 2-phenoxypropionic acid [PPA] has been applied in the diastereomer salt separation.[1)] In this study, we focus on synthesis of chiral rimantadine, and clarify the inherent structures of R-rimantadine HCl and its diastereomer salt with R-PPA by X-ray analysis. From in silico prediction study of the enantiomers, it reveals that R-rimantadine has the higher inhibition activity of M2 protein than S-rimantadine and its derivative, adamantadine.



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Keywords: molecular mechanics dynamics, chiral drugs, single-crystal X-ray analysis

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Validation of charge density refinements and application to molecules of biological interest

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The electronic properties of the macrolide antibiotic roxithromycin (1) that interacts with the nucleotides of the peptidyl transferase cavity of the bacterial ribosome [1] are investigated. A high resolution dataset of (1) was collected with synchrotron radiation. The experimental charge density was determined by least-squares refinements (program XD [2]) using the rigid pseudoatom formalism of Hansen and Coppens [3]. The multipole model was based on one generated by the program INVARIOMTOOL [5]. Differences between experimental- and invariom model [4] electron density were calculated on a grid. The refined multipole parameters model the electron density in the crystal (including packing effects and hydrogen bonding), but can also model disorder. (1) provides a good example that minor disorder can easily escape the attention of the examiner. However, slight differences in the electronic density tend to result in pronounced differences in the electrostatic potential. Since we hope to shed light on biological function of the molecule on the electronic level, the calculated difference density is used to validate the experimental charge density. A closely related problem in charge density refinements is over-parametrisation, which can be indicated by RFree. Results for both validation procedures are reported.

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Keywords: charge density analysis, electrostatic properties, disorder

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High-pressure crystallisation of antibiotic molecules

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The application of high pressure is a powerful method for exploring the polymorphic behaviour of simple molecular compounds.[1, 2] Direct compression of either single crystals or powders, and crystal growth from the melt are two methods that have been successfully used to prepare new polymorphs.[3] The recent development of the experimental technique for in situ high-pressure growth of single crystals from solution has allowed a wider range of compounds to be studied including small-molecule pharmaceuticals and energetic materials, and has enabled the preparation of new solvates and hydrates.[2] We have tested and extended the technique to the study of larger, more complex compounds of biological importance, namely antibiotics, with chemical formulae comprising more than 15 non-H atoms. Materials crystallised in the 0.1 - 2.0 GPa pressure range have been identified and characterised by in situ X-ray diffraction. Monitoring and understanding the effects of pressure on antibiotics is an important step towards understanding the phenomenon of polymorphism in terms of observed and favourable intermolecular