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CRYSTALLINE WATER ADDUCTS OF DRUG COMPOUNDS

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About one third of the drug compounds show the ability to form adducts with water. These binary solids (i.e. co-crystals) are subsumed under the term hydrate. The existence of a hydrate is critical for many basic pharmaceutical processes which is particularly true when different anhydrous (polymorphic) forms exist beside the hydrate(s). Based on the changes of the crystal lattice upon dehydration and the course of the moisture sorption isotherm two main classes of water adduct can be classified: stoichiometric and non stoichiometric hydrates. In stoichiometric hydrates the crystal lattice collapses upon dehydration, resulting in a completely (amorphous phase) or partly disordered state, one ore more crystal forms with less (lower hydrate) or no crystal water (anhydrous form, polymorphic forms) or the chemical decomposition of the compound. In non-stoichiometric hydrates the crystal lattice type is essentially maintained upon dehydration and either an isomorphic desolvate or a disordered state is formed. The process is reversible which can be recognized by a more or less continuous change of the moisture sorption isotherm in contrast to the isotherms of stoichiometric hydrates, which exhibit a discontinuous course. For the assessment of structure-properties relationships in hydrates it is necessary to apply a wide variety of analytical methods and strategies and to reflect on the crystal structure and the arrangements and interactions of water molecules in the lattice. This presentation summarizes some representative examples from a more systematic approach to understand the complex nature of these binary crystals.

Keywords: HYDRATES, POLYMORPHS, DRUG COMPOUNDS

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MOLECULAR INTERACTIONS AND TRANSFORMATION KINETICS IN POLYMORPHIC FAT CRYSTALS

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The factors affecting structural and kinetic behavior of polymorphic fat crystals are discussed in terms of molecular interactions and crystallization conditions, by taking a model substance of triacylglycerol. As to the molecular interactions, the fatty acid compositions and their positions connected to glycerol carbon atoms are of primary significance. A general tendency is revealed that three typical forms, α/β'/β, are revealed in the fats that contain fatty acid moieties of similar chemical structures connected to the glycerol carbon atoms in a symmetric manner. By contrast, recent atomic-level single-crystal X-ray analyses have clarified that β' form can become more stable in the fats that contain mixed fatty acid moieties. In particular, the mixed-acid fats whose chains are connected to the glycerol carbon atoms in an asymmetric manner make the β -prime form most stable. Mixing of different fats also modifies the polymorphic occurrence and stability because of specific molecular interactions among glycerol-bone group, aliphatic chains and methyl end groups. For the kinetic properties of polymorphic crystallization, these molecular interactions also play important roles, in addition to external factors such as crystallization rate, shear stress, ultrasound irradiation and template effects.

Keywords: POLYMORPHISM, FAT CRYSTAL, CRYSTALLIZATION

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TIME-RESOLVED STRUCTURAL STUDIES OF HYDROXYMETHYLBILANE SYNTHASE (HMBS)

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Hydroxymethylbilane synthase (HMBS, EC 4.3.1.8) catalyses the conversion of porphobilinogen (PBG) to the tetrapyrrole hydroxymethylbilane. To investigate the mechanism of this reaction, a time-resolved Laue study was carried out. The reduced, catalytically active K59Q mutant of HMBS from *Eschericia coli* was chosen for this purpose because kinetic experiments had previously shown that various enzyme-substrate complexes are accumulated during the pre-steady-state period of the reaction with this mutant. During data collection, the crystals were immobilized in a flow cell and exposed to a continuous flow of a solution of PBG. Data were collected from several crystals prior to any contact of the crystals with PBG, and then at time points ranging from 7 s up to 2 h after the start of the flow of substrate solution. In one case, monochromatic data were collected at 12 h. A structural analysis of the processed data revealed that extended electron density appears in the active site region most prominently at 2 h. Detailed structural refinement on the 2-h data allowed a detailed omit-type difference map to be calculated. This shows electron density on the active site adjacent and above the side chain of Asp84, supposed to play a pivotal role throughout the catalytic reaction cycle. The elongated density commences at the putative binding site for PBG, extends up towards R149, past R155 (residues whose mutation also causes the build-up of intermediate enzyme-substrate complexes) and out towards the open solvent channel of the crystal. In summary, the position and shape of the elongated density favors the interpretation of the structure at 2 h as being that of a Michaelis complex of HMBS.

Keywords: LAUE CRYSTALLOGRAPHY FLOW CELL ENZYME-SUBSTRATE COMPLEX

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STRUCTURAL INTERMEDIATES IN THE PHOTOCYCLE OF E46Q MUTANT PHOTOACTIVE YELLOW PROTEIN

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Photoactive Yellow Protein (PYP) is a blue light photoreceptor from *Ectothiorhodospira halophila* involved in negative phototaxis. The E46Q mutant is especially interesting since it is isosteric with the wild type protein but has significantly different photocycle kinetics. The E46Q mutant has an accelerated photocycle that is difficult to explain and underscores the need to further elucidate the photocycle mechanism in PYP. Room temperature time-resolved X-ray crystallography was used to visualize structural photocycle intermediates in the E46Q mutant of PYP. 59 Laue datasets were collected at 30 different time delays spaced logarithmically throughout the photocycle, from 500 ps to 24 ms. Extreme care was taken during data collection to reduce systematic error while maximizing percent photoactivation and signal to noise. Redundant datasets were collected in time regions where the structural signal was difficult to interpret. The redundancy, completeness in time, and overall quality of this Laue time series allowed accurate visualization of small structural changes occurring during the E46Q mutant PYP photocycle. The transitory structural intermediates observed in the E46Q mutant PYP photocycle were refined to 1.6 Å. A detailed comparison between structural photocycle intermediates in wild type PYP and E46Q mutant PYP will provide insight into the effects of protein microenvironment on the PYP photocycle.

Keywords: LAUE PYP TIME RESOLVED