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COMBINING ATOMIC RESOLUTION CRYSTALLOGRAPHY AND QUANTUM MECHANICS: A STUDY OF TRYPSIN FROM FUSARIUM OXYSPORIUM

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Macromolecular crystallography has become the method of choice for the investigation of protein function. However, in the average protein structure interesting details or deviations from standard geometry might sometimes be missed. Atomic resolution crystallography (< 1.2 Å) is a powerful tool for the elucidation of protein mechanisms. The atomic positions are accurately defined and fine details in the electron density become visible. Yet even at this stage not all of the electronic detail can be visualized and some properties remain undetermined: the protonation state and the oxidation state. The combination of atomic resolution crystallography and ab initio quantum chemical calculations enables to close the gap between the protein structure and the chemical properties. Using the crystal structure as a geometrical template, one can assign a chemical state and calculate an energy-optimized theoretical model. Comparison between the actual crystal structure and the theoretical structure allows the assignment of a protonation state or an electronic state and the determination of the charge distribution. Fusarium oxysporum trypsin is a serine protease cleaving peptides at the C terminus of arginines or lysines. Like all proteases, trypsin also shows autoproteolysis. In the atomic resolution crystal structure of the native enzyme, peptide fragments could be found in the active site. Further co-crystallization experiments with arginine and lysine were carried out in order to obtain a clear picture of the interaction with the substrate. Also, soaks in solutions either at low pH or containing an inhibitor were performed in order to determine which of the water molecules is involved in catalysis. The electron density observed in the active site was interpreted as a tri-peptide gly-ala-arg with the arginine binding to the P1 site and making close contacts with Ser195 of the catalytic triad. Three water molecules are observed in this area, one of which is in favorable position for involvement in catalysis. The structures show significant deviations from standard geometry and valence state of the bound substrate. Most notably, one of the carboxyl oxygen atoms is missing, leaving the carbon atom with a valence number of three. The carbon is located only 1.8 Å away from the Ser195 O^{-}. In order to explain these deviations and to assess the electronic situation in these structures, quantum chemical calculations were launched. The results indicate that a true reaction intermediate of considerable covalent character is trapped in the active site. The de-protonation of the catalytically active serine seems to be an important factor as is creates an environment that stabilizes this intermediate. The water molecule involved in catalysis was identified and the role of the oxyanion hole illustrated. Comparison of different structures confirms these results and proves the validity of the method

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CATARACT MUTANT AND NATIVE HUMAN γD CRYSTALLIN CONTACT AT 1.2 Å RESOLUTION

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Refraction and transparency of eye lenses derives from high protein concentration, although abrupt changes in the refractive index on the light wavelength scale will cause light scattering. A missense mutation in the 2-domain human γD crystallin (R58H) results in reduced solubility causing crystallization that is responsible for aculeiform cataract. The native and mutant proteins both crystallized in the same space group with one molecule/asymmetric unit. The most difference between the two lattices is that R58 in the native structure forms a strong ion-pair with symmetry related D156. The shorter histidine in the R58H mutant does not form this contact, or any other favorable protein-protein interaction. Surprisingly then, the less soluble mutant makes more favorable interactions with its neighbors in the lattice than does the native molecule. There is, however a significant difference between the two structures in the conformation of R168 situated on the partner domain l, but adjacent to the mutation site at residue 58. In the mutant structure there is only one conformation of R168 whereas in the native structure R168 clearly exists in two conformations with the double conformers mainly interacting with water molecules. Analysis of the two lattice structures indicates other areas where there is more static/thermal disorder of certain side chains in the native structure compared with the mutant.

Keywords: GAMMAD CRYSTALLIN, EYE LENS PROTEIN, DOMAIN INTERACTIONS

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***IN-SITU* OBSERVATION OF CRYSTALLIZATION PROCESSES OF N-ALKANES IN OIL-IN-WATER EMULSION BY SYNCHROTRON RADIATION SAXS/WAXS-DSC SIMULTANEOUS MEASUREMENTS**

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In recent years, crystallization processes occurring in encapsulated systems such as emulsions, vesicles and foams have widely been studied to synthesize functional organic and inorganic crystalline materials. Controlling crystal structures, crystal size and morphology has been elucidated in these encapsulated systems, in comparison to those occurring in bulk phases. The roles of interfacial membranes that construct encapsulated systems play critical roles, in particular, during nucleation processes that must be monitored in-situ with suitable techniques. However, due to the very nature of encapsulation, conventional optical techniques cannot be employed for the systems having micron- and nano-meter dimensions. We have done simultaneous analyses of small-angle and wide-angle X-ray scattering (SAXS/WAXS), using synchrotron radiation, and differential scanning calorimetric (DSC) techniques to monitor the crystallization processes in oil-in-water (O/W) emulsion droplets whose average diameter was smaller than 1 micron-meter. Normal alkanes having the numbers of carbon-atoms of 15 through 22 were employed as an oil phase, and three types of high-melting hydrophobic emulsifiers were introduced to the oil phase so that interfacial heterogeneous nucleation was enhanced. As a complimentary study, in-situ optical observation was also done for large-sized mono-dispersed emulsion droplets (34 μm). Interfacial heterogeneous nucleation processes involving the formation of template and accelerated nucleation by the template have been unveiled by these in-situ observations.

Keywords: SAXS WAXS DSC n-ALKANE CRYSTALS IN SITU OBSERVATION

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SOLVENT DEPENDENCY ON POLAR MORPHOLOGY

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Polymorphism is the ability of a substance to exist as two or more crystalline phases that have different arrangements and/or conformations of the molecules in the crystal lattice. Each polymorph possesses different physical properties and morphologies. Two polymorphic forms of a steroid were investigated by *in-situ* and *ex-situ* optical microscopy, RAMAN spectroscopy and X-ray powder diffraction. Crystal morphologies of both polymorphs are highly dependent on the solvent and growth conditions. This paper focuses on the unusual polar morphology common to both polymorphs that have been observed. A crystal has polar morphology if its habit does not exhibit inversion symmetry. As the two polymorphs occur in the same solvent system with the same polar morphology, it is hard to distinguish between them. Results from molecular dynamic simulations of surface interactions with the solvent predict the polar crystal morphology that is observed experimentally. This indicates that the polar morphology is caused by surface-solvent interactions affecting growth of specific faces.

Keywords: POLYMORPHISM POLAR MORPHOLOGY MOLECULAR DYNAMICS SIMULATION