THE 3-DIMENSIONAL STRUCTURE OF Ca-DEPLETED HUMAN C-REACTIVE PROTEIN FROM PERFECTLY TWINNED CRYSTALS

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C-reactive protein is a member of the pentraxin family of oligomeric serum proteins which has been conserved through evolution, homologues having been found in every species in which they have been sought. Human C-reactive protein (hCRP) is the classical acute-phase reactant produced in large amounts in response to tissue damage and inflammation, and is used almost universally as a clinical indicator of infection and inflammation. In order to clarify the structural rearrangements associated with calcium-binding, the reported affinity of calcium-depleted hCRP for polycations and other ligands, and the role of calcium in protection against denaturation and proteolysis, the structure of calcium-depleted hCRP has been determined by x-ray crystallography. Crystals of Ca-depleted hCRP are invariably twinned and those suitable for analysis are merohedral type II twins of point group 4 single crystals. The structure has been solved by molecular replacement using the calcium-bound hCRP structure (Shrive et al., Nature Structural Biology 3, 346-354, 1996). It reveals two independent pentamers which form a face-to-face decamer across a dyad near-parallel to the twinning 2-fold axis. Cycles of intensity deconvolution, density modification (10-fold ncs) and model building, eventually including refinement, give a final R-factor of 0.19 (R-free = 0.20). Despite poor definition in some areas, arising from the limited resolution of the data and from the twinning and disorder, the structure reveals the probable mode of twinning and the conformational changes, localized in one of the calcium-binding loops, which accompany calcium binding.

Keywords: TWINNING, DECONVOLUTION, C-REACTIVE PROTEIN

SOLUTION AND REFINEMENT OF A MEROHEDRALLY TWINNED SINGLE CRYSTAL

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Merohedral twinning is a frequently observed and often undetected complication in single crystal X-ray crystallography. It usually causes severe problems in both structure determination and refinement. The merohedrally twinned structure of 2-(Diphenylphosphinoyl)-2-phenylethenol (1) was solved and refined with SHELX-97 [1] and will be presented.
Compound 1, $C_{20}H_{17}O_2P$, crystallizes in the space-group $P3_1$ (Rint = 0.039) pretending to be $P3_112$ (Rint = 0.041) as a result of merohedral twinning with nine molecules (Z' = 3) in the unit cell of a = 19.755(1) and c = 11.236(1) Å. Merohedrally twinned crystals of compound 1 were measured on a Hilger & Watts (Y290) and on a Bruker APEX CCD diffractometer. After a very crude deconvolution of the data into contributions from the different twin domains, the program SHELXS [1] succeeded in solving the structure. All atoms of the three crystallographically independent molecules were automatically located in the first electron density map. Starting from this model, the refinement was performed against the original twinned data set using the program SHELXL [1]. Unlike L-threitol [2] compound 1 does not possess a twofold axis symmetry. The final crystallographic R-value is 4.6% for all reflections.
Each molecule of the asymmetric unit contains a hydroxyl group. Therefore, all molecules are held together by a system of strong hydrogen bonds, which consists of infinite chains in the direction of the shorter c axis. All oxygen atoms are involved as donors and acceptors.
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Keywords: **MEROHEDRAL TWINNING SOLUTION AND REFINEMENT CRYSTAL STRUCTURE**

DIFFICULT PHASING IN SOLVING THE STRUCTURE OF EUKARYOTIC GLUTAMINE SYNTHETASE II

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Glutamine synthetase (GS) catalyzes the ATP-dependent synthesis of glutamine in all organisms. GS I from bacteria is a dodecamer. GS II from eukaryotes has long been believed to be an octamer. Our reexamination of the subunit structure of eukaryotic GS II by X-ray crystallography and electron microscopy reveals a tetradecameric organization. A self rotation function calculated from the alfalfa GS crystal diffraction data showed that the molecule has 7-fold symmetry. The electron micrographs of both human GS and alfalfa GS showed a tetradecameric structure with two stacked rings of seven subunits. The conclusion of the tetradecameric subunit structure of eukaryotic GS II contradicts the conventional octameric model widely held for over thirty years. Currently we are determining the structure of GS II at atomic resolution. Alfalfa GS was crystallized in four different forms. The crystal form with smallest unit cell is in space group $P222_1$ with unit cell dimensions a = 137.7 Å, b = 170.4 Å, c = 383.4Å. There are 21 subunits (820 kDa) per asymmetric unit. Our attempts of heavy metal derivatization were unproductive due to the fact that heavy metals by themselves do not have enough scattering power for such a large unit cell. To overcome the problem, we are working on derivatizing the crystals with heavy metal clusters. As an alternative method of solving the phase problem, we are using simplified models: a spherical model and a model based on the cryo-EM reconstruction of human GS to obtain low-resolution phases by molecular replacement.

Keywords: **GLUTAMINE SYNTHASE, LARGE OLIGOMERIC PROTEIN STRUCTURE, PHASING**

SPACE GROUP CHOICE BY STATISTICAL TESTING

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The problem of a proper lattice parameters assignment and correct space group choice is often difficult to solve even in the case of single crystal data available. It is usually solved by checking the appropriate relations between the metric tensor components and in the intensity data set. Such a procedure is performed, as a rule, with an arbitrary confidence level (lepage, [1]). Another possibility is an inspection of the already determined crystal structure, e.g. Missym, [2]. We proposed a more detailed procedure [3] leading to quantitative probabilistic analysis, which, unlike the published approach [4], gives the results as a function of the cut-off level. Our procedure was applied to three types of problems:
a) differentiation between possible space groups;
b) checking the choice of lattice types, lattice parameters and laue class suggested by the software of a diffractometer;
c) detection of twinning. Some examples concerning the above cases solved using the hos program package [5] will be presented.
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