

**CRYSTALLOGRAPHIC STUDIES OF SITE I DRUGS BOUND TO THE HUMAN SERUM ALBUMIN-MYRISTATE COMPLEX**

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Human serum albumin (HSA) is an abundant plasma protein that is responsible for the transport of a range of insoluble endogenous compounds including fatty acids, hormones and toxic metabolites such as bilirubin. Albumin also binds an impressive array of drugs in two primary sites (I and II) and much of the clinical and pharmaceutical interest in the protein derives from its effect on drug pharmacokinetics.

HSA is a monomer that contains three homologous helical domains (I-III) each divided into two subdomains (A and B). Previously we determined the first high-resolution structures of HSA complexed with fatty acids and revealed the molecular interactions between HSA and its primary ligand. Here we present a crystallographic study of site I drugs and drug-analogues complexed with HSA-myristate. The structures confirm that phenylbutazone, oxyphenbutazone, azapropazone, iodipamide, dansyl-L-arginine, indomethacin, warfarin, triiodobenzoic acid and di-iodosalicylic acid all bind at site I (subdomain IIA) in the presence of fatty acids. Our studies have also revealed the presence of additional drug sites for some of these compounds in subdomains IB and IIIB.

The crystal structures throw new light on the molecular details of HSA-drug interaction and provide a new understanding of the structural basis of the very broad drug-binding specificity of the protein. By using a range of site I drugs we have been able to probe both common and distinct modes of interaction at site I; it is apparent that side-chain flexibility is important for accommodation of a range of different compounds in this binding site.

**Keywords: PROTEIN DRUG INTERACTIONS**

**THE DERIVATION OF NON-MEROHEDRAL TWIN LAWS**

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Data sets from non-merohedral twins contain large numbers of reflections that are unaffected by twinning. It is our experience that their structures can be solved without difficulty. Problems such as large, inexplicable difference peaks and a high R-factor may indicate that twinning is a problem during refinement. Careful analysis of poorly fitting data reveals that they belong predominantly to certain distinct zones in which  $|F_{\text{obs}}|^2$  is systematically larger than  $|F_{\text{calc}}|^2$ . If twinning is not taken into account it is likely that these zones are being poorly modelled, and that their indices may provide a clue as to a possible twin law. We have written a computer program, called ROTAX, which makes use of this idea to identify possible twin laws. A set of data with the largest values of  $[F_o^2 - F_c^2]/\sigma(F_o^2)$  is identified and the indices transformed by two-fold rotations about possible direct and reciprocal lattice directions. Matrices which transform the indices of the poorly fitting data to integers are identified as possible twin laws. The user then has a set of potential matrices which might explain the source of the refinement problems described above.

**Keywords: TWINNING ROTAX REFINEMENT**

**RIBOSOMAL ANTIBIOTICS: THE STICKS IN THE WHEEL**

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Resistance to antibiotics is a major problem in modern therapeutics. Ribosomes of pathogenic bacteria are main targets for antibiotics. Ribosomes are cellular organelles catalyzing the translation of genetic code into proteins. They are protein/RNA assemblies arranged in two subunits that associate for performing protein biosynthesis. The larger creates the peptide bonds and provides the path for emerging proteins. The smaller has key roles in controlling the fidelity of codon-anti-codon base pairing and in initiating the biosynthetic process.

Two eubacterial source, *Thermus thermophilus* and *Deinococcus radiodurans* are suitable pathogen models and were used as references that allowed unambiguous localization of six clinically relevant and one universal antibiotics. These were shown to inhibit ribosome function by limiting ribosomal motion, interference with substrate binding and hindrance of the progression of nascent proteins. All were found to bind primarily to ribosomal RNA and their binding did not cause major conformational changes. Among them tetracycline and chloramphenicol targets the A-site tRNA in the decoding and peptidyl transferase cavity, respectively; clindamycin interferes with both A- and P-site tRNA; the macrolides: erythromycin, clarithromycin and roxithromycin bind to the entrance of the protein exit tunnel and physically block it; and edeine prevent platform mobility. The latter is a universal antibiotic, the only one studied by us that introduces allosteric effects – the production of a new base pair in a rather remote location.

**Keywords: RIBOSOMES, ANTIBIOTICS**

**TWINS AND MULTIPLE POLYMORPHS OCCURRING IN ENERGETIC ORGANIC MATERIALS**

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Multiple polymorphs occur in important energetic materials (four in HMX & CL20), and lead to substantial changes in performance. A new polymorph is sometimes thought to be a twin, if it is difficult to solve and the unit cell resembles a known crystal form. Picryl bromide (2,4,6-trinitrobenzene) is such a compound. The authors discovered many polymorphs for it, with asymmetric units ranging from 3 to 18 molecules! All display identical packing motifs, although they belong to four space groups:  $P1$ ,  $P-1$ ,  $P3_1$ , &  $P6_5$ . Five were eventually solved and refined to R's less than 0.040. A sixth refines poorly; it may be a twin or a composite of the other polymorphs. Twinning is a major problem in analytical crystallography. Fortunately, it is more often recognized and successfully confronted now. An initial rough solution of a twin may be elusive, especially if a large fraction of the data is close-overlapped. However, once a hint of the molecular shape and assembly is known, modern software is usually capable of providing a good data reduction and molecular refinement. If twinning is present but NOT recognized, some consequences may be: (a) no solution, but an incorrect new polymorph is reported, (b) a solution is found, but it has a poor R factor, or (c) an incorrect structure is found, which you report! Examples will be described, including several well-refined twin solutions, a partial disorder/twin solution (in progress), and the false solution of a feasible, but incorrect molecule (later corrected by twinning analysis).

**Keywords: TWINNING POLYMORPHISM ENERGETIC MATERIALS**