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Folding, Cofactor Binding, Catalysis, Oligomerization and Function of 13000 Short Chain Oxidoreductase Enzymes

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The 13,000 member short-chain oxidoreductase (SCOR) family of enzymes includes at least 300 biochemically characterized enzymes in prokaryotes and eukaryotes. They catalyze oxidation, reduction, epimerization and synthase reactions. Over 70% (~7,900) of the putative family members belong to a subfamily that contains the signature sequence TGxxxGIG in the (2(3 turn of the Rossmann fold. The crystal structures of 50 unique SCORs, have now been reported. Although there is not one residue fully conserved, 40 fingerprint residues are conserved at 70% identity or greater. We are determining the precise roles of each of the fingerprint residues in controlling protein folding, cofactor binding, catalysis, and function. Cofactor selectivity is controlled by two adjacent residues in the β2α2 turn of the Rossmann fold, seven residues may be critical to catalysis, C-H..O hydrogen bonds may play a significant role in catalysis (Fig. 1a) and a 3_{10} kink and patterns of aromatic amino acid substitution on helix 5 may control dimer formation. The ϕ,ψ values of seven of the 11 Gly residues in the fingerprint fall in a region of the Ramachandran plot where the other 19 amino acids are rarely observed. Gly residues in these positions are indispensable for the maintenance of the Rossmann fold. The substrates for the family include steroids, sugars, prostaglandins, alcohols, acids, aromatics, dyes, and xenobiotics and none of the 40 fingerprint residues are substrate specific. Substrate binding specificity is determined by amino acids in three flexible loops. In crystal complexes, substrates and inhibitors make hydrogen or van der Waals contacts with amino acids in sequence specific locations on the three loops. A co-conserved set of amino acids (T,D,N,G,Q,F,M,T, and L) in nine positions on the three loops are found in 177 members of the SCOR family. Many of these 177 proteins have been biochemically characterized as β-keto [acyl carrier protein] reductases (Fig. 1b). Different combinations of amino acids in the same nine sequence positions on the three flexible loops identified 11 subgroups of SCORs that have different substrates. Supported by NIH Grant No. DK26546.

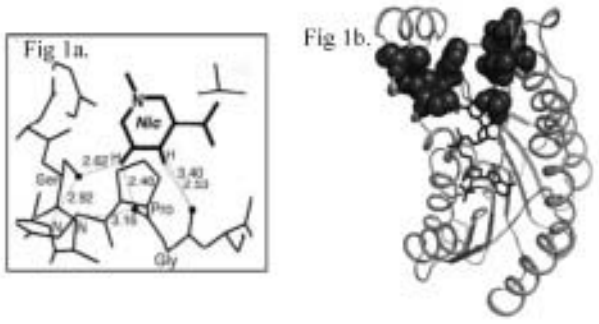

Fig1a) A conserved network of short contacts and apparent CH..O H-bonds around the nicotinamide ring (Nic) in the crystal structures of 33 TGxxxGIG enzymes. b) β*-ketoacyl ACPR (1Q7C) substrate contact residues.*

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Structural studies of chiral resolution

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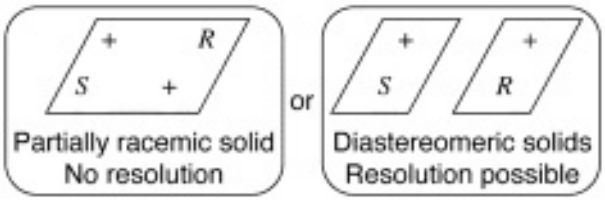
The two major crystallization alternatives for racemates of chiral compounds (*R, S)* comprise the formation of racemic crystals or conglomerates. The latter are often desirable for synthetic and economic reasons [1] and less frequent.[2-4] In the more popular case of a racemic compound yielding racemic crystals, chemists in charge of separating the enantiomers often recur to the precipitation of adducts with an enantiomerically pure reagent (+): Ideally, the resulting diastereomers (*R*+) and (*S*+) will crystallize separately and differ in their physical properties, *e.g.* in solubility. However, an unfavourable alternative outcome of this adduct formation shown below, namely the precipitation of a single partially racemic product containing both diastereomers in the same solid, has been discovered long ago,[5] but no systematicstructural studies have been published to date. We have investigated examples for both aspects of resolution, spontaneous resolution by precipitation of conglomerates and attempted separation by formation of diastereomeric adducts. In our approach, ligands and counter ions in ionic compounds with chiral cationic palladium complexes have been subjected to systematic variations. The crystallization products have been structurally characterized, and their lattice energy has been modeled with an empirical intermolecular force field. We find that conglomerates form with weakly coordinating small anions [6] whereas partially racemic solids are obtained from salts in which the ionic constituents interact *via* classical hydrogen bonds.[7] The partially racemic crystals are pseudosymmetric with respect to improper symmetry operations and closely related to the truly racemic structures.



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