β-carbonic anhydrase active site architecture is a mirror image of α-carbonic anhydrases

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Carbonic anhydrases, enzymes that catalyse the reversible hydration of CO_2 to HCO_3^-, occur in three families (designated α, β, and γ) that are evolutionarily unrelated to one another. The structures of α- and β-carbonic anhydrase have previously been determined, and here we report the structure of the β-carbonic anhydrase from the dicotyledenous plant *Pisum sativum* at 1.93 Å resolution. The structure was determined using a combination of multiple anomalous diffraction off the active site zinc ion and noncrystallographic symmetry averaging. The molecular fold is novel with a four-stranded Rossman-fold-like module being supplemented by a five helical subdomain and an extra antiparallel β-strand. The molecule assembles into an octamer using a novel dimer of dimers of dimers arrangement that necessitates one interaction surface to mediate two different types of interaction. The active site is located at the interface between two monomers with Cys160, His220, and Cys223 binding the catalytic zinc ion and residues Asp162 (oriented by Arg164), Gly224, Gln151', Val184, Phe179', and Tyr205' interacting with the bound substrate analogue, acetic acid. The active site is buried below the surface of the enzyme, connected to bulk solvent by a channel that is extremely narrow and requires the movement of Tyr205' to allow substrates access. Within β-carbonic anhydrases, two distinct patterns of conservation of critical active site residues are observed, implying two potentially mechanistically distinct classes of β-carbonic anhydrases. Comparison of the β-carbonic anhydrase active site with that of α-carbonic anhydrase reveals that the substrate binding groups have a one-to-one correspondence. Eleven atoms with corresponding functionality in the two active sites can be superimposed with an rmsd of 0.4 Å, but only if the mirror image of one of the active sites is used. The residues in each molecule that form a hydrophobic path along one half of the active site also superimpose well. Therefore, despite differing folds, α- and β-carbonic anhydrase have converged upon a very similar active site design and likely share a common mechanism.

Acknowledgments

Use of the Advanced Photon Source was supported by the U.S. Department of Energy Basic Energy Sciences, Office of Science, under Contract No. W-31-109-Eng-38. Use of the BioCARS sector 14 was supported by the Medical Research Council.