A wheel invented three times

The molecular structures of the three carbonic anhydrases

The need for an enzyme to catalyze the slow conversion between carbon dioxide and bicarbonate enabled physiologists to predict the existence of carbonic anhydrase almost 70 years ago. Shortly thereafter the enzyme was purified from erythrocytes (Meldrum and Roughton, 1933; Stadie and O'Brien, 1933). The enzyme, now called α-carbonic anhydrase (α-CA), was found to contain a zinc ion (Keilin and Mann, 1940).

A large number of α-CA isoenzymes, primarily from mammalian species, have subsequently been identified and characterized, and several of their molecular structures have been elucidated, starting with that of human α-CAlII (Liljas et al., 1972). When carbonic anhydrase from plants and certain bacteria was characterized and sequenced, it turned out to be oligomeric and its amino acid sequence had no similarity to the previously studied enzymes (Hewett-Emmett and Tashian, 1996). This form is called β-carbonic anhydrase (β-CA). Subsequently, a carbonic anhydrase from archaea was identified (γ-carbonic anhydrase, γ-CA). In this case, the amino acid sequence was again strikingly different (Alber and Ferry, 1994). In spite of the differences in sequence, however, all forms of carbonic anhydrase have an essential zinc ion in the active site. It became evident that only a comparison of the molecular structures might clarify the relationships between the different enzymes.

Light was shed on this problem by recent papers in The Journal of Biochemistry and The EMBO Journal, which reported the structure of β-CA from plant and algae, respectively (Kimber and Pai, 2000; Mitsuhashi et al., 2000), following the earlier elucidation of the structure of γ-CA (Kisker et al., 1996). These publications illustrate that all of the carbonic anhydrases are completely different from one another at the level of their tertiary and quaternary structures, but that the active sites show essential features of remarkable similarity.

The differences between the various carbonic anhydrases are illustrated in Figure 1. Notably, γ-CA has a primitive β-helix structure (shown in yellow) in which a short repeated amino acid sequence produces the main part of the fold, whereas the α- and β-CAs have folds that are constructed by different arrangements of α-helices and β-strands. Differences in quaternary structure exist even within the class of β-CA (Figure 1). Thus, in the two β-CAs for which structures have been elucidated, the plant enzyme forms an unusual octamer structure, which is not based on 4-fold but on repeated 2-fold symmetry, while the algal polypeptide has an internal repeat [the unique (monomer) fold is highlighted]; pea β-CA octamer; γ-CA trimer.

Fig. 1. The tertiary structures of the three forms of carbonic anhydrase. From top to bottom: α-CA monomer; algal β-CA dimer with internal repeat [the unique (monomer) fold is highlighted]; pea β-CA octamer; γ-CA trimer. For the oligomeric forms of the enzyme, one monomer is highlighted with the β-strands in yellow and the α-helices in blue. The other monomers are in light gray. The zinc ions are indicated by red spheres.
that have essential functions are different, the catalytic mechanisms are probably very similar, since in all cases, an obligate hydrogen bond acceptor is within hydrogen bonding distance of the available ligand position at the zinc ion, and the OH of bicarbonate would necessarily be the metal ligand (Figure 2). Carbonic anhydrases provide an excellent example of convergent evolution. It is of significant interest to clarify how the same function has evolved three times completely independently, to generate enzymes that appear to be different superficially but that actually have great underlying similarities. The species distribution for the three classes of protein does not reveal a pattern that could explain how they are related. What was the driving force that created three versions of this wheel?


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