

2.3: Catalytic Mechanism

All the above structural and kinetic information obtained under a variety of conditions with different metal ions can be used to propose a catalytic cycle for carbonic anhydrase (Figure 2.21). As shown by studies on the pH-dependent properties of native and metal-substituted CAs, both type-I and type-II proteins have two acidic groups, the zinc-coordinated water and a free histidine. At physiological pH the enzyme is essentially in the Zn—OH form (step A in Figure 2.21). A Zn—OH moiety is a relatively good nucleophile, poised for nucleophilic attack on carbon dioxide. It is possible that the hydrogen bond with Thr-199, which seems to be consistent with an sp^3 oxygen, orients the OH for attack at the substrate CO_2 . Studies of the copper derivative indicated that the concentration of CO_2 in the cavity is higher than in bulk solution (step B).

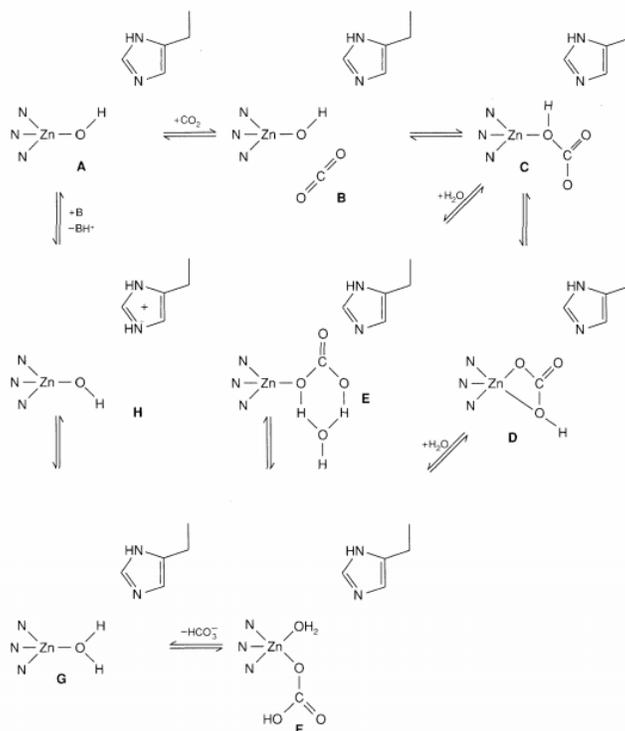


Figure 2.21 - Proposed catalytic cycle of CA.

Molecular dynamics calculations have shown that there are either three⁹⁶ or two⁹⁷ potential wells for CO_2 in the hydrophobic pocket. It was shown⁹⁸ that when Val-143 is replaced by the much larger Phe, the activity decreases by a factor of 10^3 . Apparently the large Phe residue does not leave space within the cavity to accommodate CO_2 .

It would also be nice if the enzyme were able to activate CO_2 . There is no evidence that it does, even though the positive charge around zinc and the NH of Thr-199 would represent two electrostatic attraction points that could activate CO_2 . It is well-known that CO_2 's interactions with positive charges activate the carbon for nucleophilic attack.^{99,100} The positioning of CO_2 between zinc and the peptide NH of Thr-199 would be ideal for the OH attack. Merz⁹⁷ locates it as shown in Figure 2.22.

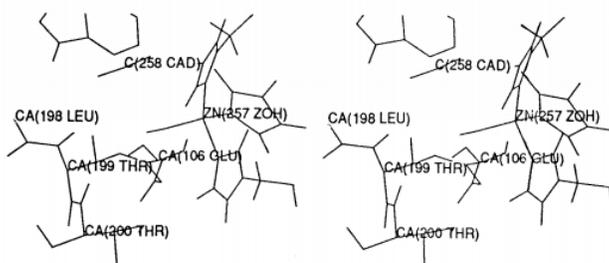


Figure 2.22 - Stereo view of the site of activation of CO_2 in the cavity of CA as proposed by Merz.⁹⁷

It was believed that, once bicarbonate is formed (C), the proton has to transfer to a terminal oxygen atom, either via an intermediate in which bicarbonate is bidentate (D) or via a hydrogen-bond network (E). Indeed, in model compounds one would expect HCO_3^-

to bind through a nonprotonated oxygen. However, the possibility of restoring the hydrogen bond with Thr-199 as in sulfonamide adducts could justify the presence of the hydrogen on the coordinating oxygen.²¹⁴ The bicarbonate derivative is presumably in equilibrium between four- and five-coordinate species (F), as shown by the electronic spectra of the cobalt derivative.⁵⁹ The five-coordinate species provides a low barrier for the substrate detachment step via an associative mechanism involving coordination of a water molecule (G). A possible five-coordinate species would contain bicarbonate in the B site and water in the C site (Figure 2.12). It is reasonable that the measured K_m for the reaction of bicarbonate dehydration is the thermodynamic dissociation constant of the $M-HCO_3^-$ species. Anionic or neutral inhibitors are competitive with bicarbonate because they tend to bind at the same site. At this stage the second substrate, which is H^+ , has to be released (H). It is reasonable that the water proton transfers to a group inside the cavity, e.g., the free histidine mentioned above, and subsequently to the solvent. In the absence of buffers the latter step is rate-limiting for the high-activity isoenzymes, since the diffusion rate cannot exceed the product of the concentration times the diffusion coefficient, i.e., $10^{-7} M \times 10^{11} M^{-1}s^{-1}$. Such a limit is then $10^4 s^{-1}$, whereas the turnover rate is $10^6 s^{-1}$. The presence of buffer can assist in proton transfer at this stage, in such a way that the rate-limiting step becomes the internal proton transfer. The release of H^+ from the $Zn-OH_2$ moiety is also the rate-limiting step for the low-activity CA III, as nicely shown by the electronic spectra of CoCA III. These spectra change from the basic form at the beginning of the reaction to the acidic form upon CO_2 addition (Figure 2.23).¹⁰¹ After the interconversion of CO_2 into bicarbonate, there is an accumulation of the $CoOH_2$ species, the deprotonation of which is slower than the release of HCO_3^- .

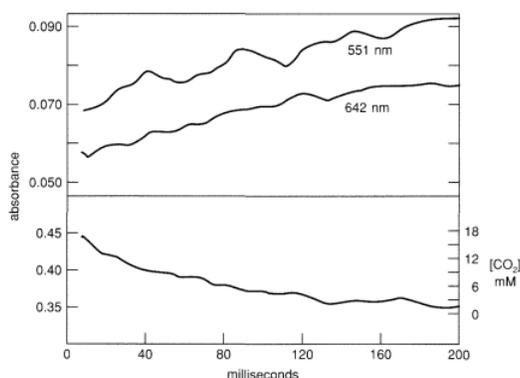


Figure 2.23 - Time dependence of $\epsilon_{15.6}$ and $\epsilon_{18.1}$ of cobalt(II)-substituted CA III after addition of CO_2 to a buffered enzyme solution at pH 8. The initial drop of absorbance reflects the accumulation of a $CoOH_2$ intermediate.¹⁰¹

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