2.14: Steady-State and Equilibrium Kinetics of Carbonic Anhydrase-Catalyzed C O 2 /HC O − 3 CO2/HCO3− Interconversion

The $CO_2 \rightleftharpoons HCO_3^-$ interconversion catalyzed by CA is extremely fast. The usual kinetic parameters describing an enzymatic reaction are the turnover number or kinetic constant for the reaction, k_{cat}, and the Michaelis constant K_m. In the simple catalytic scheme

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P, \tag{2.14.1}$$

where E stands for enzyme, S for substrate, and P for product, K_m^{-1} is given by $k_1/(k_{-1} + k_2)$. If k_2 is small, $k_{cat} = k_2$ and $K_m^{-1} = k_1/k_{-1}$, the latter corresponding to the thermodynamic affinity constant of the substrate for the enzyme. The pH dependences[46] of k_{cat} and K_m for CO_2 hydration for the high- and low-activity isoenzymes have been determined (Figure 2.2).[33,36] It appears that K_m is pH-independent, whereas k_{cat} increases with pH, reaching a plateau above pH 8. For bicarbonate dehydration (the reverse of Equation 2.6), H^+ is a cosubstrate of the enzyme. The pH dependence of k_{cat}/K_m for HCO_3^- dehydration is also mainly due to k_{cat}, which shows the same pH profile as that for CO_2 if the experimental kinetic data are divided by the available concentration of the H^+ cosubstrate.[47,48] Further measurements have shown that the pH dependence of k_{cat} reflects at least two ionizations if the measurements are performed in the absence of anions.[49] The value of k_{cat} reaches its maximum at alkaline pH only when buffer concentrations exceed 10^{-2} M.[50] In other words, the exchange of the proton with the solvent is the rate-limiting step along the catalytic pathway if relatively high concentrations of proton acceptors and proton donors are not provided by a buffer system. This limit results from the high turnover of the enzyme, which functions at the limit imposed by the diffusion rate of the H^+ cosubstrate. At high buffer concentration, k_{cat} shows an isotope effect consistent with the occurrence of an internal proton transfer as the new rate-limiting step.[51]

Measurements of the catalyzed reaction performed at chemical equilibrium starting from mixtures of ^{12}C-^{18}O-labeled HCO_3^- and ^{13}C-^{16}O-labeled CO_2 have shown the transient formation of ^{13}C-^{18}O-labeled species (both CO_2 and HCO_3^-) before ^{18}O-labeled water appears in solution.[52] These experiments provided evidence that, at chemical equilibrium, an oxygen atom can pass from HCO_3^- to CO_2 and vice versa several times before being released to water. Furthermore, maximal exchange rates are observed even in the absence of buffers.

Under chemical equilibrium conditions, ^{13}C NMR spectroscopy is particularly useful in investigating substrate interconversion rates, since the rates pass from a slow-exchange regime in the absence of enzyme to fast exchange at sufficient enzyme concentration. In the absence of enzyme two ^{13}C signals are observed, one for CO_2 and the other for HCO_3^-. In the presence of enzyme only one averaged signal is observed (Figure 2.6). Starting from the slow exchange situation, in the absence of enzyme, the increase in linewidth ($\Delta\nu$) of the substrate (A) and product (B) signals (caused by exchange broadening that is caused in turn by the presence of a small amount of catalyst) depends on the exchange rate and on the concentration of each species, according to the following relation:

$$\Delta\nu_A[A] = \Delta\nu_B[B] = \tau_{esch}^{-1} \tag{2.9}$$

Therefore, the exchange rate τ_{esch}^{-1} can be calculated.[53] The appearance of the NMR spectrum for different τ_{esch}^{-1} values is illustrated in Figure 2.6 under the condition [A] = [B]. For the high-activity enzyme it was found that the maximal exchange rates are larger than the maximal turnover rates under steady-state conditions; the ratio between k_{exch} of the high-activity (type II) and low-activity (type I) forms is 50, i.e., larger than the ratio in k_{cat}.[49,54] This result is consistent with the idea that the rate-limiting step in the steady-state process is an intramolecular proton transfer in the presence of buffer for type II enzymes, whereas it may not be so for the type I enzymes. The exchange is pH-independent in the pH range 5.7 - 8, and does not show a proton-deuteron isotope effect. The apparent substrate binding (HCO_3^-) is weaker than steady-state K_m values, indicating that these values are not true dissociation constants. Chloride is a competitive inhibitor of the exchange.[49]

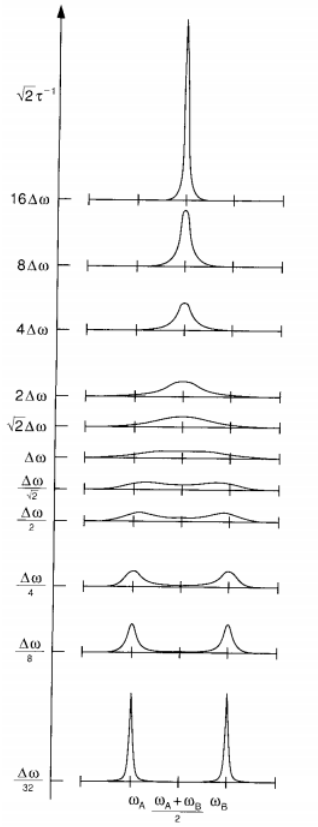

Figure 2.6 - Calculated lineshape for the NMR signals of nuclei equally distributed between two sites ([A] = [B]), as a function of the exchange rate τ^{-1}. $\Delta\omega$ is the peak separation in rad s^{-1}.

A similar investigation was conducted for type I CoHCA at pH 6.3, where the concentrations of CO_2 and HCO_3^- are equal.[55] The two lines for the two substrates were found to have different linewidths but equal T_1 values. Measurements at two magnetic fields indicate that the line broadening of the HCO_3^- resonance is caused by substrate exchange and by a paramagnetic contribution due to bonding. The temperature dependence of the linewidth shows that the latter is determined by the dissociation rate. Such a value is only about 2.5 times larger than the overall $CO_2 \rightleftharpoons HCO_3^-$ exchange-rate constant. Therefore the exchange rate between bound and free HCO_3^- is close to the threshold for the rate-limiting step. Such an exchange rate is related to the higher affinity of the substrate and anions in general for type I isoenzymes than for type II isoenzymes. This behavior can be accounted for in terms of the pK_a of coordinated water (see Section C).