Dehydrogenases involved in Reduction of CO_2 to CH_3OH

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CO2 reduction | cofactor regeneration | enzyme immobilization

1. The *Dehydrogenases*

The three dehydrogenase enzymes involved in the CO_2 to methanol conversion are: *Formate dehydrogenase*, *Formaldehyde dehydrogenase* and *Alcohol dehydrogenase*. Dehydrogenases are enzymes that catalyze the “proton coupled to electron transfer” (PCET, $H^+ + e^-$) from a molecule that acts as “electron + proton” donor (reductant) to another one that acts as an acceptor (oxidant). During the redox reaction, NADPH/$NADP^+$ or NADH/NAD^+ (nicotinamide adenine dinucleotide) is employed as an essential cofactor. The three enzymes above manage the reduction in the oxidation state of carbon dioxide to carbon-based energy-carrier resources. [1]

2. Formate *Dehydrogenase*

Formate dehydrogenases are categorized as metal-independent and metal-dependent based on the presence of metals (molybdenum-Mo or tungsten-W) in the active sites. The former catalyzes the reaction from HCO_2H to CO_2 irreversibly; the latter catalyzes the reduction of CO_2 to HCO_2H reversibly and, because of this, is also employed in the enzymatic CO_2 to HCO_2H conversion. [2] The redox potential for the enzymatic reduction of CO_2 to HCO_2^- is $E°' = −420$ mV, but both types of *Formate dehydrogenase* ($F_{ate}DH$) mainly catalyze HCO_2^- oxidation, hence their *dehydrogenase* designation. The difference in catalytic reaction reversibility is due to differences in the catalytic reaction mechanism and the enzyme structure governing the energy reorganization during catalysis. [3] Among these $F_{ate}DHs$, the one extracted from *Candida boidinii* (EC 1.2.1.2, $CbF_{ate}DH$) is commercially available and can be easily handled as a catalyst for CO_2 reduction. $CbF_{ate}DH$ is a homodimer [79 kDa (6 nm × 6 nm × 10 nm)] with two independent active sites catalyzing the NAD^+-dependent oxidation of formate to CO_2 via an irreversible hydride transfer from formate to NAD^+. [4]

The conversion of CO_2 to formate is a process thermodynamically unfavorable and which somehow needs to be “helped” to happen. **Table 1** shows the *Km* values with reference to the substrates of the reaction in the two possible directions. The *Km* is the index of the affinity between enzyme and substrate: the lower its value, the higher the affinity of the substrate for the enzyme. As reported in **Table 1**, such value is lower for CO_2 than for

HCO_2^-, confirming that the formate oxidation reaction is favored. Nevertheless, the formate reduction reaction can be “forced” to take place by working with an excess of substrate and cofactor, i.e., with high amounts of NADH and CO_2.

Table 1. *Km* values for the CO_2 reduction–formate oxidation reactions.

Enzyme	Reaction	*Km*	Ref.
$F_{ate}DH$	$CO_2 \rightarrow HCO_2^-$	30–50 mM	[5]
	$HCO_2^- \rightarrow CO_2$	0.5 mM	[5]

While for NADH, the excess is reached by adding the cofactor in the solution, for CO_2, it is not so simple due to its low solubility and its interaction with water. Moreover, one must consider that depending on the pH of the solution, CO_2 in water is found in three forms: carbon dioxide, hydrogencarbonate, and carbonate, as shown in **Figure 1**.

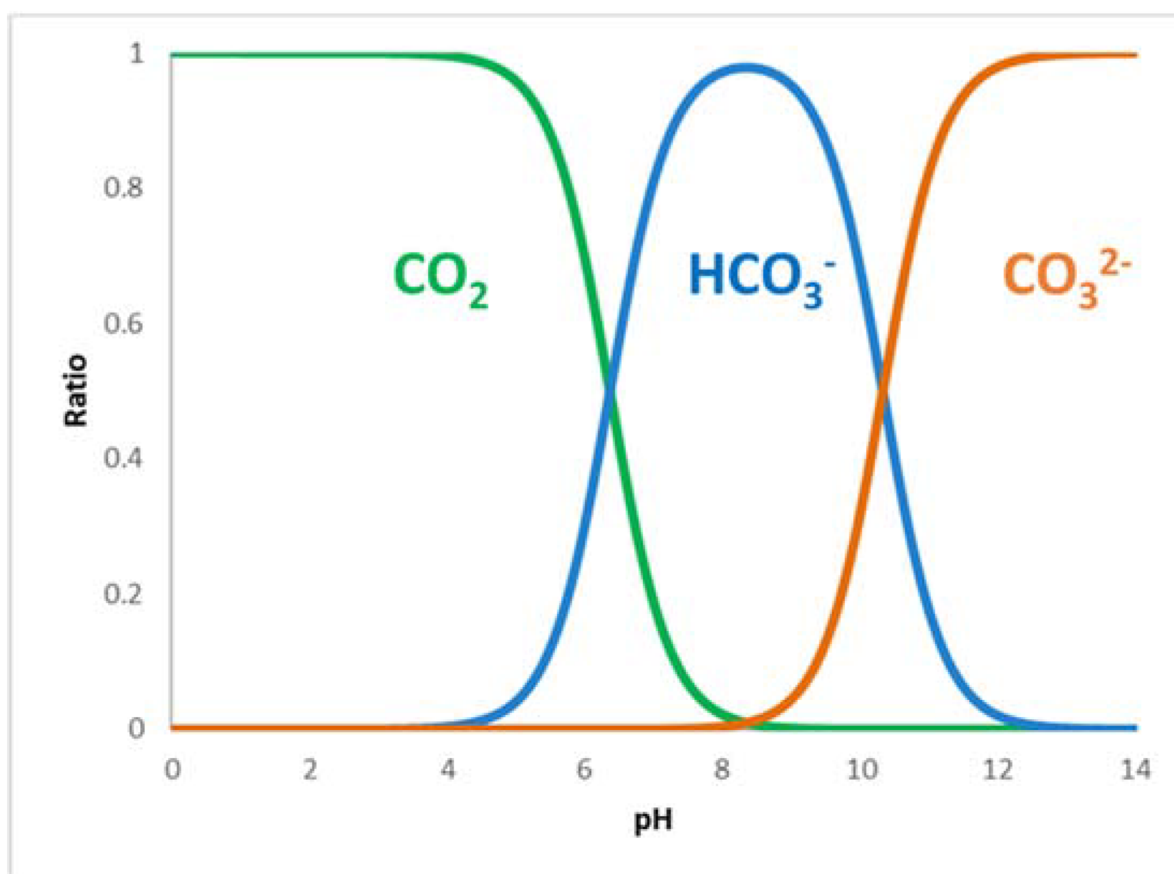

Figure 1. Distribution of species derived from CO_2 according to pH in water solution. [6]

There are conflicting opinions in the literature on the role of the CO_2 hydration reaction. Sato et al. [7] affirmed that as carbonate and hydrogencarbonate concentrations increase in solution, there is a suppression of the formate formation reaction due to the fact that such species are competitive inhibitors of carbon dioxide for the formate

production with $CbF_{ate}DH$. Other authors, see Wang et al.,[8] consider hydrogencarbonate to be the active species, and in some cases (Cazelles et al. [5]), $KHCO_3$ is dissolved in water rather than bubbling CO_2 for better reproducibility. In order to increase the presence of CO_2 in the solution, this is left to bubble for at least half an hour before starting the reaction, and in some cases, a pressurized system is also used. However, the low solubility of CO_2 remains one of the reasons for the low yields of such a reaction. In order to improve the yield, a fourth enzyme is added to the system, i.e., *Carbonic anhydrase*, which very quickly (k_{Cat} = 10^6 s^{-1}) catalyzes the hydration–dehydration of CO_2. (Equation (1))

$$CO_2 + H_2O \leftrightharpoons H^+ + HCO_3^- \quad (1)$$

According to Wang et al., [8] with the catalysis of *CA*, CO_2 molecules are rapidly hydrated and transformed into hydrogencarbonate, which is converted into formate, while the cofactor NADH is oxidized. Another possibility to increase CO_2 availability is to use materials such as MOFs able to both immobilize enzymes and absorb CO_2.

Although *Formate dehydrogenase* from *C. boidinii* is the most widely used enzyme for testing the reduction reaction, $F_{ate}DHs$ are also derived from other microorganisms and have been tested for their ability to reduce CO_2. Nielsen et al.[9] compared the catalytic efficiency of *Formate dehydrogenase* of various organisms by highlighting the type of electron donor, the *Km* for CO_2, and the working conditions. Amongst the various enzymes, the activity of $DdF_{ate}DH$ (*Desulfovibrio desulfuricans*) is particularly interesting, with a K_m of 0.02 mM and a catalytic efficiency K_{cat}/K_m of 2968. [10]

Other $F_{ate}DHs$ such as, for example, $F_{ate}DH$ from *Thiobacillus* sp. KNK65MA,[11] $F_{ate}DH$ from *Myceliophthora thermophila*,[12] $F_{ate}DH$ from *Clostridium carboxidivorans*,[13] on the other hand, have a low K_m (0.95, 0.44, and 0.05 mM, respectively) but present a not very high catalytic efficiency (K_{cat}/K_m = 0.34, 0.23, 1.6, respectively).

3. Formaldehyde *Dehydrogenase*

The second step of the reaction is the reduction of formate to formaldehyde. The enzyme involved in such a reaction is *Formaldehyde dehydrogenase*. Again, as observed in the first step, the enzyme has more affinity for formaldehyde than formate, and this affects the reaction yield (**Table 2**). The kinetic parameters of $F_{ald}DH$ for the reduction reaction ($HCO_2H \rightarrow HCHO$) have not been determined yet, mainly due to the difficulty of measuring the reaction rates at different formic acid concentrations while keeping the pH constant. [14] *Formaldehyde dehydrogenase* ($F_{ald}DH$, EC 1.2.1.46) used in the reduction of formic acid is extracted from *Pseudomonas putida* and is a homo-tetramer of approximately 168 kDa [4 × 42 kDa (5 nm × 6 nm × 10 nm)]. [5]

Table 2. *Km* values for the formate reduction–formaldehyde oxidation reactions.

Enzyme	Reaction	*Km*	Ref.
$F_{ald}DH$	$HCO_2^- \rightarrow HCOH$	n.d	[5]

Enzyme	Reaction	*Km*	Ref.
	HCOH → HCO_2^-	0.09 mM	[5]

It was shown that the formate reduction step in the enzymatic cascade from CO_2 to methanol is the bottleneck of the reaction, as this enzyme has low activity and is sensitive to pH, substrate, and product concentration. [5],[14],[15] Luo et al. [16] reported that if a minimum concentration of 10 mM of formate is not reached, the reaction does not proceed, and higher concentrations at the same time do not improve the speed of the reaction. Therefore, they defined 10 mM as the optimum concentration for the reduction to occur. Such information is obtained by a step-by-step study of the cascade reaction. Given this, it is possible to state that in the cascade reaction, the slow accumulation of formate affects the low yield of the second reduction step from formate to formaldehyde. Starting from formate instead of CO_2 would, thus, help the process.

4. Alcohol *Dehydrogenase*

The third enzyme involved in this reaction is *Alcohol dehydrogenase*. *Alcohol dehydrogenase* is present in many organisms, but the mainly used is the one from *Saccharomyces cerevisiae* (*Alcohol dehydrogenase* (*ADH*), EC 1.1.1.1, a homo tetramer of about 141–151 kDa with a size of 7 nm × 10 nm × 11 nm), which is commercially available. *ADH* normally acts on primary or secondary alcohols, but the enzyme oxidizes methanol much more poorly than ethanol. In the cascade reaction, it is used in the final step of the multienzyme process, i.e., the reversible reduction of formaldehyde to methanol. [17] The forward reaction (formaldehyde → methanol) is much more efficient than the reverse one (methanol → formaldehyde) given the affinity of the substrates for the enzyme (see the km value in **Table 3**); the reduction in formaldehyde is considered almost irreversible. This step is the only one favored in the direction of the cascade reaction from CO_2 to methanol. However, *ADH* is not a very stable enzyme for industrial use. Indeed, when the three *dehydrogenases* catalyzing the cascade reaction from CO_2 to CH_3OH were employed as free enzymes, *ADH* was by far the least stable one. [18]

Table 3. *Km* values for the formaldehyde reduction–methanol oxidation reactions.

Enzyme	Reaction	*Km*	Ref.
ADH	HCOH → CH_3OH	6 mM	[5]
	CH_3OH → HCOH	100 mM	[5]

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