

# Dehydrogenases involved in Reduction of CO<sub>2</sub> to CH<sub>3</sub>OH

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The three dehydrogenase enzymes involved in the CO<sub>2</sub> to methanol conversion are: *Formate dehydrogenase*, *Formaldehyde dehydrogenase* and *Alcohol dehydrogenase*.

Keywords: CO<sub>2</sub> reduction ; cofactor regeneration ; enzyme immobilization

## 1. The Dehydrogenases

The three dehydrogenase enzymes involved in the CO<sub>2</sub> to methanol conversion are: *Formate dehydrogenase*, *Formaldehyde dehydrogenase* and *Alcohol dehydrogenase*. Dehydrogenases are enzymes that catalyze the “proton coupled to electron transfer” (PCET, H<sup>+</sup> + e<sup>-</sup>) 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<sup>+</sup> or NADH/NAD<sup>+</sup> (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

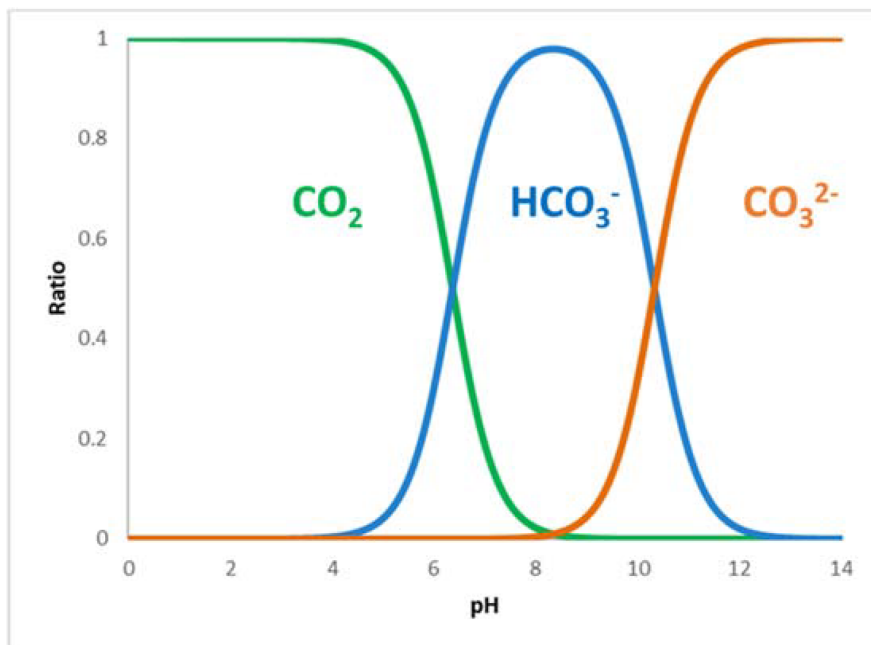
*Formate dehydrogenases* are categorized as metal-independent and metal-dependent based on the presence of metals (molybdenum-Mo or tungsten-W) in the active sites. The former catalyzes the reaction from HCO<sub>2</sub>H to CO<sub>2</sub> irreversibly; the latter catalyzes the reduction of CO<sub>2</sub> to HCO<sub>2</sub>H reversibly and, because of this, is also employed in the enzymatic CO<sub>2</sub> to HCO<sub>2</sub>H conversion. [2] The redox potential for the enzymatic reduction of CO<sub>2</sub> to HCO<sub>2</sub><sup>-</sup> is E°' = -420 mV, but both types of *Formate dehydrogenase* (*F<sub>ate</sub>DH*) mainly catalyze HCO<sub>2</sub><sup>-</sup> 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<sub>ate</sub>DHs*, the one extracted from *Candida boidinii* (EC 1.2.1.2, *CbF<sub>ate</sub>DH*) is commercially available and can be easily handled as a catalyst for CO<sub>2</sub> reduction. *CbF<sub>ate</sub>DH* is a homodimer [79 kDa (6 nm × 6 nm × 10 nm)] with two independent active sites catalyzing the NAD<sup>+</sup>-dependent oxidation of formate to CO<sub>2</sub> via an irreversible hydride transfer from formate to NAD<sup>+</sup>. [4]

The conversion of CO<sub>2</sub> to formate is a process thermodynamically unfavorable and which somehow needs to be “helped” to happen. **Table 1** shows the *K<sub>m</sub>* values with reference to the substrates of the reaction in the two possible directions. The *K<sub>m</sub>* 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<sub>2</sub> than for HCO<sub>2</sub><sup>-</sup>, 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<sub>2</sub>.

**Table 1.** *K<sub>m</sub>* values for the CO<sub>2</sub> reduction–formate oxidation reactions.

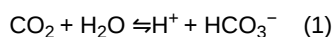
Enzyme	Reaction	<i>K<sub>m</sub></i>	Ref.
<i>F<sub>ate</sub>DH</i>	CO <sub>2</sub> → HCO <sub>2</sub> <sup>-</sup>	30–50 mM	[5]
	HCO <sub>2</sub> <sup>-</sup> → CO <sub>2</sub>	0.5 mM	[5]

While for NADH, the excess is reached by adding the cofactor in the solution, for CO<sub>2</sub>, 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<sub>2</sub> 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<sub>2</sub> according to pH in water solution. <sup>[6]</sup>

There are conflicting opinions in the literature on the role of the CO<sub>2</sub> hydration reaction. Sato et al. <sup>[7]</sup> 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<sub>ate</sub>DH*. Other authors, see Wang et al., <sup>[8]</sup> consider hydrogencarbonate to be the active species, and in some cases (Cazelles et al. <sup>[5]</sup>), KHCO<sub>3</sub> is dissolved in water rather than bubbling CO<sub>2</sub> for better reproducibility. In order to increase the presence of CO<sub>2</sub> 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<sub>2</sub> 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 ( $k_{Cat} = 10^6 \text{ s}^{-1}$ ) catalyzes the hydration–dehydration of CO<sub>2</sub>. (Equation (1))



According to Wang et al., <sup>[8]</sup> with the catalysis of CA, CO<sub>2</sub> molecules are rapidly hydrated and transformed into hydrogencarbonate, which is converted into formate, while the cofactor NADH is oxidized. Another possibility to increase CO<sub>2</sub> availability is to use materials such as MOFs able to both immobilize enzymes and absorb CO<sub>2</sub>.

Although *Formate dehydrogenase* from *C. boidinii* is the most widely used enzyme for testing the reduction reaction, *F<sub>ate</sub>DHs* are also derived from other microorganisms and have been tested for their ability to reduce CO<sub>2</sub>. Nielsen et al. <sup>[9]</sup> compared the catalytic efficiency of *Formate dehydrogenase* of various organisms by highlighting the type of electron donor, the  $K_m$  for CO<sub>2</sub>, and the working conditions. Amongst the various enzymes, the activity of *DdF<sub>ate</sub>DH* (*Desulfovibrio desulfuricans*) is particularly interesting, with a  $K_m$  of 0.02 mM and a catalytic efficiency  $K_{cat}/K_m$  of 2968. <sup>[10]</sup>

Other *F<sub>ate</sub>DHs* such as, for example, *F<sub>ate</sub>DH* from *Thiobacillus* sp. KNK65MA, <sup>[11]</sup> *F<sub>ate</sub>DH* from *Myceliophthora thermophila*, <sup>[12]</sup> *F<sub>ate</sub>DH* from *Clostridium carboxidivorans*, <sup>[13]</sup> 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<sub>ald</sub>DH* for the reduction reaction (HCO<sub>2</sub>H → 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. <sup>[14]</sup> *Formaldehyde dehydrogenase* (*F<sub>ald</sub>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)]. <sup>[15]</sup>

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

Enzyme	Reaction	<i>K<sub>m</sub></i>	Ref.
<i>F<sub>ald</sub>DH</i>	$\text{HCO}_2^- \rightarrow \text{HCOH}$	n.d	[5]
	$\text{HCOH} \rightarrow \text{HCO}_2^-$	0.09 mM	[5]

It was shown that the formate reduction step in the enzymatic cascade from CO<sub>2</sub> 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<sub>2</sub> 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 *k<sub>m</sub>* 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<sub>2</sub> to methanol. However, *ADH* is not a very stable enzyme for industrial use. Indeed, when the three *dehydrogenases* catalyzing the cascade reaction from CO<sub>2</sub> to CH<sub>3</sub>OH were employed as free enzymes, *ADH* was by far the least stable one. [18]

**Table 3.** *K<sub>m</sub>* values for the formaldehyde reduction–methanol oxidation reactions.

Enzyme	Reaction	<i>K<sub>m</sub></i>	Ref.
<i>ADH</i>	$\text{HCOH} \rightarrow \text{CH}_3\text{OH}$	6 mM	[5]
	$\text{CH}_3\text{OH} \rightarrow \text{HCOH}$	100 mM	[5]

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