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Reinforcing carbon fixation: CO_2 reduction replacing and supporting carboxylation

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Carbon dioxide enters the biosphere via one of two mechanisms: carboxylation, in which CO_2 is attached to an existing metabolite, or reduction, in which CO_2 is converted to formate or carbon monoxide before further assimilation. Here, we focus on the latter mechanism which usually receives less attention. To better understand the possible advantages of the 'reduction-first' approach, we compare the two general strategies according to the kinetics of the CO_2-capturing enzymes, and the resource consumption of the subsequent pathways. We show that the best CO_2 reducing enzymes can compete with the best carboxylases. We further demonstrate that pathways that fix CO_2 by first reducing it to formate could have an advantage over the majority of their carboxylation-only counterparts in terms of ATP-efficiency and hence biomass yield. We discuss and elaborate on the challenges of implementing 'reduction-first' pathways, including the thermodynamic barrier of CO_2 reduction. We believe that pathways based on CO_2 reduction are a valuable addition to nature's arsenal for capturing inorganic carbon and could provide promising metabolic solutions that have been previously overlooked.

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Introduction

Carbon fixation is arguably the single most important biochemical process in the biosphere, providing the elemental backbone for the cellular building blocks of all organisms. When discussing carbon fixation, carboxylation reactions usually take the spotlight. This is reasonable, as in almost all carbon fixation pathways inorganic carbon enters cellular metabolism via a carboxylase, for example, in the Calvin Cycle, Rubisco is the entry point for CO_2 and the rest of the pathway serves, in broad terms, to regenerate the substrate of Rubisco, ribulose 1,5-bisphosphate. However, inorganic carbon can enter metabolism via a different route, which is frequently overlooked: CO_2 reduction (Figure 1). Inorganic carbon can be reduced to formate or carbon monoxide, which can then be assimilated into central metabolism in a number of ways. The best known example is the reductive acetyl-CoA (rAcCoA) pathway (i.e. Wood–Ljungdahl pathway [1]) — the only carbon fixation pathway that produces ATP rather than consumes it (mostly due to electron bifurcating enzymes), making it the most efficient route for carbon assimilation. The rAcCoA pathway combines all possible assimilation strategies: the product of the pathway, pyruvate, is composed of a carbon that originates from CO_2 reduction to formate, a carbon that originates from CO_2 reduction to carbon monoxide, and a carbon that originates from a carboxylation reaction (pyruvate synthase).

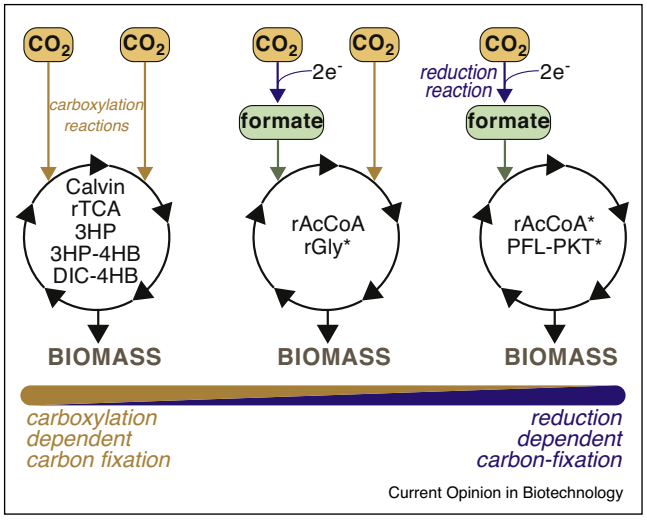
Many studies and reviews discuss carbon fixation pathways from different perspectives, e.g., ecological [2,3], biotechnological [4,5], evolutionary [6,7]. In this review, we focus on the opportunities provided by CO_2 reduction. We compare the two carbon fixation strategies — carboxylation versus reduction — in terms of the kinetics of the inorganic-carbon-capturing enzymes as well as the properties of the overall pathways, for example, ATP-consumption and oxygen tolerance. We show how carboxylation and CO_2-reduction can be combined (schematically shown in Figure 1) to enrich the solution space of carbon fixation and further discuss the challenges of implementing CO_2-reducing pathways in foreign hosts.

As carbon monoxide can be assimilated only via the rAcCoA pathway, we shall focus in this review on the metabolic opportunities that CO_2 reduction to formate can provide.

Carbon capturing enzymes: carboxylases versus CO_2-reductases

We start by taking a closer look at the enzymes that directly accept inorganic carbon — 'carbon capturing enzymes' — which either reduce CO_2 or attach it to an existing metabolite (carboxylation enzymes). We performed a comprehensive, manually curated literature search to identify kinetically superior carboxylating enzymes of all classes. Figure 2 shows the k_{cat} and k_{cat}/K_M (i.e. K_M^{CO2}) of the best carbon capturing enzymes, where the former parameter is relevant for the reaction

Figure 1



Schematics of carbon fixation strategies. Carbon fixation can be a purely carboxylation dependent process, as in the naturally occurring Calvin Cycle, reductive TCA cycle, 3-hydroxypropionate bi-cycle, 3-hydroxypropionate-4-hydroxybutyrate cycle, and the dicarboxylate-4-hydroxybutyrate cycle. Carbon fixation can integrate carboxylation and CO_2 reduction, as is the case in the natural reductive acetyl-CoA pathway and the synthetic reductive glycine pathway (Figure 3a,b, respectively). Carbon fixation can also be carboxylation-free, relying solely on carbon reduction, as is the case in a proposed variant of the reductive acetyl-CoA pathway (purple lines in Figure 3a) and in the synthetic PFL-PKT cycle (Figure 3c). Synthetic pathways are marked with '*'. We do not show CO_2 reduction to carbon monoxide, although it plays a central role in the reductive acetyl-CoA pathway, in order to keep the schematics simple to read.
Abbreviations: Calvin corresponds to Calvin Cycle, rTCA to the reductive tricarboxylic acid cycle, 3HP to 3-hydroxypropionate bi-cycle, 3HP-4HB to 3-hydroxypropionate-4-hydroxybutyrate cycle, DIC-4HB to dicarboxylate-4-hydroxybutyrate cycle, rAcCoA to the reductive acetyl-CoA pathway, rGly to the reductive glycine pathway, and PFL-PKT to the PFL-PKT cycle (PFL corresponds to pyruvate formate-lyase and PKT to phosphoketolase).

rate under CO_2 saturating conditions, and the latter is relevant for the reaction rate at low CO_2 concentrations. For each enzyme class we show the five enzymes closest to the Pareto front [8] in terms of both of these parameters: the enzymes with the best apparent tradeoff between k_{cat} and k_{cat}/K_M. Only enzymes that were measured at mesophilic conditions ($15°C < T < 40°C$) were considered. Some of these enzymes accept bicarbonate rather than CO_2; in these cases, we have converted the K_M for bicarbonate to represent the K_M for CO_2 — marked as K_M^{CO2} — assuming pH 7, ionic strength of 0.25 M and temperature of 30°C (and further assuming that bicarbonate and CO_2 are equilibrated, see Supplementary information). This enables us to directly compare the kinetics of CO_2-utilizing and bicarbonate-utilizing enzymes.

CO_2 is often described as a small, poor electrophile which is difficult to activate, hence resulting in poor kinetics of carboxylating enzymes. Yet, as shown in Figure 2, the best carboxylating enzymes are much better than the 'average' enzyme, having k_{cat} and k_{cat}/K_M^{CO2} orders of magnitude higher than the average parameters [9] (see Supplementary information for values and references). The best variants of PEP carboxylase, PEP carboxykinase, pyruvate carboxylase, and crotonyl-CoA carboxylase/reductase are even better than the average 'central metabolism' enzyme ($k_{cat} > 79\ s^{-1}$ and $k_{cat}/K_M > 410\,000\ s^{-1}\ M^{-1}$ [9]). With only very few exceptions, it seems that k_{cat} of carboxylating enzymes is limited to $\leq 100\ s^{-1}$ and k_{cat}/K_M^{CO2} is limited to $\leq 10^7\ s^{-1}\ M^{-1}$, where PEP carboxylase is by far the best enzyme in terms of this latter parameter.

How do CO_2-reducing enzymes, specifically formate dehydrogenases (FDHs), compare with the carboxylating enzymes in their ability to use CO_2? Metal-free FDHs are extremely slow in the CO_2 reduction direction [10–12], and are not the topic of this review. On the other hand, metal-dependent FDHs are quite efficient in reducing CO_2 [13••] and serve this purpose in various prokaryotes, such as acetogens and methanogens [14]. While data regarding the kinetics of these enzymes in the reductive direction is quite limited, recent studies have uncovered two highly performing enzymes, which are shown in Figure 2 (red squares): hydrogen-dependent carbon dioxide reductase from the acetogen *Acetobacterium woodii* [15•], featuring quite high k_{cat} but rather low k_{cat}/K_M (maybe since the organism usually grows at elevated $[CO_2]$); and FDH from *Desulfovibrio desulfuricans* [16••], with both high k_{cat} ($>46\ s^{-1}$) and high k_{cat}/K_M ($\sim 3\,000\,000\ s^{-1}\ M^{-1}$). Therefore, the best FDH variants are not necessarily worse than the best carboxylating enzymes, and could, in fact, surpass some of the major carboxylating enzyme classes; for example, *D. desulfuricans*'s FDH is considerably faster than the best variants of Rubisco, and is orders of magnitude better than the characterized 2-oxoacid synthases [17] (e.g. pyruvate:ferredoxin oxidoreductase, 2-keto:ferredoxin oxidoreductase) that play a central role in three of the six known carbon fixation pathways [2,3].

Carbon fixation pathways: carboxylation routes versus 'reduction-first' routes

Next, we analyzed the expected biomass yields of carbon fixation pathways, aiming to compare the performance of purely carboxylation pathways to those in which CO_2 is first reduced. To represent the former group, we chose the five naturally occurring carbon fixation pathways that harbor no CO_2-reducing reaction, i.e., Calvin Cycle, 3-hydroxypropionate (3-HP) bi-cycle, 3-hydroxypropionate-4-hydroxybutyrate (3-HP-4HB) cycle, dicarboxylate-4-hydroxybutyrate (DIC-4HB) cycle, and the reductive TCA (rTCA) cycle [2,3], as well as the recently (*in vitro*) established synthetic CETCH cycle [18••]. To represent the latter group, we chose the rAcCoA pathway,