

## Driving Parts of Krebs Cycle in Reverse through Mineral Photochemistry

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How prebiotic metabolic systems could have functioned in the absence of enzymatic networks is a critically important unanswered question about the origins of life.<sup>1</sup> Such prebiotic cycles are considered as necessary platforms for the development of more advanced self-replicating biotic systems.<sup>2</sup> The prebiotic reductive tricarboxylic acid (rTCA) cycle (also called the reverse Krebs cycle or the reductive citric acid cycle; Figure 1) has received much attention because it provides a core mechanism for the synthesis of useful biomolecules from CO<sub>2</sub>.<sup>3</sup>

Experiments were conducted to test the hypothesis that the reactions of the rTCA cycle can be driven by photocatalysis on semiconductor particles suspended in water. A colloid of ZnS particles was prepared by mixing 50 mM ZnSO<sub>4</sub> with 50 mM Na<sub>2</sub>S.<sup>4</sup> In the first experiment, the suspension (diluted to 2.3 g L<sup>-1</sup>) was augmented by 1.5 mM sodium oxaloacetate and 8 mM Na<sub>2</sub>S. The pH was adjusted to 6.3 by the addition of sulfuric acid, causing the S(-II) concentration to decrease to 2 mM (assayed) because of H<sub>2</sub>S outgassing. The oxygen-free aqueous suspension was placed in a water-jacketed photoreactor at 288 K. After irradiation by wavelengths of 200–410 nm ( $2.2 \times 10^{-5}$  Ein s<sup>-1</sup>), samples were periodically withdrawn, filtered, and analyzed by ion chromatography. Calibration was done by pure standards.

The oxaloacetate concentration decreased whereas its reduction product malate increased (Figure 2), with a stoichiometric yield of up to 70% and an initial quantum efficiency of 3.9%. The malate concentration also subsequently decreased because of its further reaction. In a control run in the absence of irradiation, the oxaloacetate concentration did not decrease. In the absence of the ZnS particles but in the presence of irradiation, the oxaloacetate concentration decreased because of homogeneous photochemistry,<sup>5</sup> but pyruvate and not malate was the product. When ZnS particles were present, however, this homogeneous photochemistry was masked by photon absorption by the particles. A complete set of control experiments unequivocally established that heterogeneous photochemistry was the mechanism for oxaloacetate-to-malate conversion (Table 1).

In further experiments, the set of five reductive steps in the rTCA cycle was evaluated. Reactions 2 and 4 also proceeded (Figure 3). The stoichiometric yield of succinate from fumarate (reaction 2) approached 100%, with an initial quantum efficiency of 1.9%. Oxoglutarate, however, was a minor product of 2.5% stoichiometric yield from succinate (reaction 4). The controls again demonstrated that heterogeneous photochemistry was the mechanism of the conversions (Table 1). For reactions 3 and 5, although the concentrations of the reactant species decreased in time, the concentrations of the Krebs cycle products did not increase above the analytical detection limit, setting an upper limit of 1% for the possible yields of these products. For reactions 3 and 4 CO<sub>2</sub> bubbling, instead of H<sub>2</sub>SO<sub>4</sub> addition, was used to adjust pH (except in control E) and to add HCO<sub>3</sub><sup>-</sup> to the solution because this species is required for the carbon-chain lengthening reactions that occur in tandem with the reduction (Figure 1).

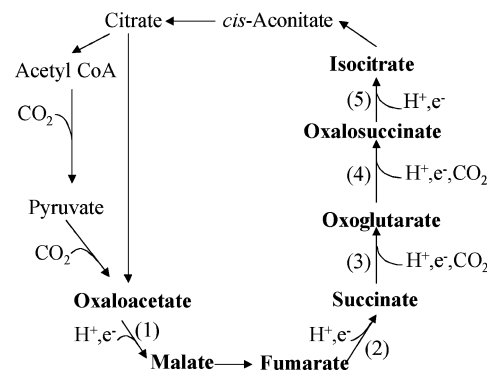


Figure 1. Reverse Krebs cycle. Highlighted are the five reduction reactions (labeled 1–5).

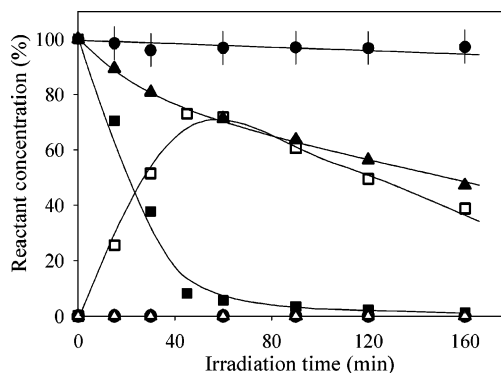
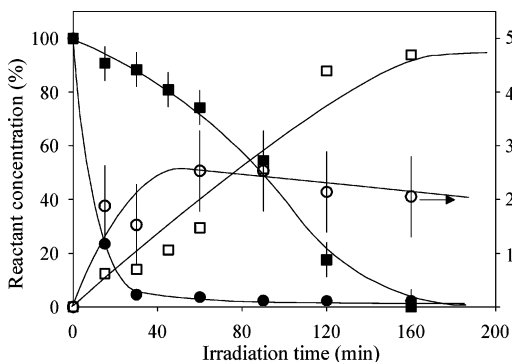


Figure 2. Photoreduction of oxaloacetate to malate. Legend: (■) oxaloacetate and (□) malate irradiated in the presence of ZnS colloid; (●) oxaloacetate and (○) malate in the absence of irradiation; and (▲) oxaloacetate and (△) malate irradiated in the absence of ZnS colloid. Uncertainties are shown for the upper data. See text for further reactions conditions. Lines are to guide the eye and do not represent a model fit.

The observed reactions were driven by the reducing power of the conduction-band electrons of ZnS,<sup>4,6</sup> which are poised at  $-1.0$  V vs NHE in the absence of band bending and adsorbates.<sup>7</sup> In comparison, the reduction potentials of reactions 1 to 5 vary from  $-0.2$  to  $+0.15$  vs NHE.<sup>8</sup> Ultraviolet light was required to drive the reactions because the band gap of ZnS is 3.6 eV (344 nm). In addition, to complete the electrochemical circuit, a scavenger was necessary for the valence-band hole poised at  $+2.6$  V vs NHE. In our experiments, the sulfur-based hole scavenger made from acidified Na<sub>2</sub>S contained both aqueous H<sub>2</sub>S and HS<sup>-</sup> as well as other sulfur oxyanions, originating either as impurities in solid Na<sub>2</sub>S or from rapid oxidation of HS<sup>-</sup> by residual O<sub>2</sub>. Measurements with a sulfide-selective electrode and ion chromatography showed that the initial reaction mixture had 2 mM HS<sup>-</sup>, 1 mM S<sub>2</sub>O<sub>3</sub><sup>2-</sup>, and 0.1 mM SO<sub>3</sub><sup>2-</sup>. Although the high concentrations of the sulfur-based hole scavenger minimized photooxidation of the organic molecules, control runs C<sub>i</sub> demonstrated that the rTCA reactions also proceeded at reduced sulfur concentrations (Table 1).



**Figure 3.** Photoreduction of (i) fumarate to succinate and (ii) oxoglutarate to oxalosuccinate. Legend: (■) fumarate is lost and (□) succinate is formed for irradiation in the presence of ZnS colloid. Likewise, (●) oxoglutarate is lost and (○) oxalosuccinate is formed, albeit at lower yield (right-hand axis). Uncertainties shown for the upper data apply to the other data points for which explicit uncertainty bars are omitted. Reaction conditions are as described for Figure 2.

**Table 1.** Control Experiments for Reactions  $i = 1, 2$ , and 4 to Demonstrate Heterogeneous Photochemical Generation of Products  $P_i$  in the Presence of Ultraviolet Irradiation  $h\nu$ , ZnS Colloid, Sulfur-Based Hole Scavenger  $S_T$ , Reactant  $R_i$ , and Carbon Dioxide  $\text{CO}_2$ .

	variables					obs'n
	$h\nu$	ZnS	$S_T$	$R_i$	$\text{CO}_2^a$	$P_i$
expt'l $i$	YES	YES	YES	YES	YES	YES
control $A_i$	NO	YES	YES	YES	YES	NO
control $B_i$	YES	NO	YES	YES	YES	NO
control $C_i$	YES	YES	minor <sup>b</sup>	YES	YES	<sup>c</sup>
control $D_i$	YES	YES	YES	NO	YES	NO
control $E_4^a$	YES	YES	YES	YES	NO	NO

<sup>a</sup> This column and row apply only to tests conducted for reaction 4. <sup>b</sup> ca. 100  $\mu\text{M}$  sulfur-based hole scavenger is present as a residual from the preparation method of the ZnS colloid from  $\text{ZnSO}_4$  and  $\text{Na}_2\text{S}$ . <sup>c</sup> Yes for reactions 1 and 2 and no for reaction 4.

Why reactions 1 and 2 are driven rapidly and at high yield, why reaction 4 is driven but more slowly, and why reactions 3 and 5 are apparently not driven at all offer no easy chemical explanations. For instance, the reactions are reduction of a carbonyl group (1 and 5), reduction of a double bond (2), introduction of  $\text{CO}_2$  to give a  $\beta$ -ketoacid (3), and introduction of  $\text{CO}_2$  and reduction to give an  $\alpha$ -ketoacid (4). Although explanations for the inefficiency of reaction 4 compared to reactions 1 and 2 might be that the transition state of reaction 4 is intrinsically much more elaborate, requiring the addition of  $\text{CO}_2$ , or alternatively that  $\text{CO}_2$  competes with the organic reactant for the conduction-band electron (yielding 360  $\mu\text{M}$  formate; data not shown), neither explanation provides insight into why reaction 3 does not also then proceed. A further challenge is to explain why reactions 1 and 5, which both involve a similar chemical reaction, have high and low yields, respectively. A speculation is that the three  $\text{COO}^-$  groups in the case of reactant 5 more strongly disfavor its association and thus reaction with the ZnS surface (isoelectric pH of 4<sup>4</sup>) than that of the two  $\text{COO}^-$  groups of reactant 1. Nevertheless, a thorough explanation for the differ-

ences in the efficiencies of the different reactions remains an open research question and may involve the particular details of molecular scaffolding on the surface.

In summary, the performed photochemical experiments demonstrate that three of the five reduction reactions in the rTCA cycle occur abiotically at the surface of the mineral ZnS. Reactions 1 and 2 have high stoichiometric yields (75 and 95%, respectively). Reaction 4 has a yield of 2.5%. For the rTCA cycle to be the first self-sustained cycle on early Earth, however, every reaction in the cycle must have a relatively high yield, including both the redox and nonredox reactions. In general, a single mineral surface arguably will not drive the entire suite of reactions. The role of ZnS on early Earth therefore could have been as a component of a more complex mineral assemblage, possibly driving the rTCA cycle by abiotic forces without the requirement of enzymes or early genetic material such as RNA molecules.

The experimental conditions of circumneutral pH and 288 K, as well as the ZnS semiconductor photocatalyst particles (sphalerite), are believed to have been prevalent in the waters of early Earth.<sup>4</sup> Although thermal reaction pathways on mineral surfaces, such as on FeS or  $\text{FeS}_2$ , have long been hypothesized as important in prebiotic chemistry as possible natural scaffolds for primitive catalysis of slow reactions,<sup>9</sup> absent thus far have been demonstrations of rapid and high efficiency conversions within putative prebiotic metabolic systems. The importance, therefore, of the described photochemical results is that new reaction pathways are opened by the interactions of excited-state species and radicals, that the sun's photons are harvested so that highly endoergic redox reactions can be driven, and that because of high overpotentials these reaction rates can be very rapid with high yields at moderate temperatures. These findings therefore both establish and constrain the plausibility of the occurrence of heretofore difficult chemical conversions in prebiotic metabolic systems.

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