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The Viability of a Nonenzymatic Reductive Citric Acid Cycle – Kinetics and Thermochemistry

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Abstract The likelihood of a functioning nonenzymatic reductive citric acid cycle, recently proposed as the precursor to biosynthesis on early Earth, is examined on the basis of the kinetics and thermochemistry of the acetate → pyruvate → oxaloacetate → malate sequence. Using data derived from studies of the Pd-catalyzed phosphinate reduction of carbonyl functions it is shown that the rate of conversion of pyruvate to malate with that system would have been much too slow to have played a role in the early chemistry of life, while naturally occurring reduction systems such as the fayalite–magnetite–quartz and pyrrhotite–pyrite–magnetite mineral assemblages would have provided even slower conversions. It is also shown that the production of pyruvate from acetate is too highly endoergic to be driven by a naturally occurring energy source such as pyrophosphate. It is thus highly doubtful that the cycle can operate at suitable rates without enzymes, and most unlikely that it could have participated in the chemistry leading to life.

Keywords citric acid cycle · nonenzymatic · citrate · kinetics

Introduction

Morowitz et al. (2000) recently proposed a nonenzymatic reductive citric acid cycle as the foundation for biosynthesis, and thus as a basis for life's origins. The scheme, offered as an alternative to the protein- and RNA-first routes to life, utilizes a series of reductions, carboxylations, and dehydrations through a sequence of 11 carboxylic acids, and the net reaction

$$\text{citrate} + 6\ CO_2 + 9\ H_2 = 2\ \text{citrate} + 5\ H_2O \tag{1}$$

reflects both the cycle's conversion of CO_2 to the organic realm and its autocatalytic nature.

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In a frank evaluation of the proposal, Orgel (2000) listed a number of issues that had to be addressed. He began with the requirement that "each step of a proposed cycle must proceed at a reasonable rate, and that this will often depend on the availability of a suitable catalyst." This suggestion is eminently sound and would seem to be the basis of any judgment as to the value and appeal of the proposed cycle. It echoes the concluding statement in a commentary accompanying the paper by Morowitz et al. (2000) noting that the cycle's existence and persistence require a demonstration of the cycle without enzymatic assistance (Schuster, 2000).

In response to Orgel, Smith and Morowitz (2004) offered that a reasonable rate "must be defined relative to the rates of competing reactions with the same inputs and outputs and need not be competitive with the rates in a modern, life-rich world." While the suggestion of a reasonable rate does not limit discussion solely to modern world chemistry, the authors went no further in delineating just what those inputs and outputs in an ancient, lifeless world might have been. They left the matter unresolved.

Orgel's cautionary note underscores a potential shortcoming to the proposition, and deserves serious consideration. In line with his comments the prospective kinetics of some elements of the cycle are examined here. The cycle is inspected further from a thermodynamics perspective where there appear to be additional questions of viability.

Discussion

It is clear that since the cycle involves a series of carboxylates, decarboxylation must be considered a prime competitive process. The kinetics of the decarboxylation of acetate, the direct precursor of pyruvate in the cycle, have been thoroughly studied over a range of conditions by Bell et al. (1994), and one of their findings in the work was catalysis of the reaction by some common mineral materials. The acetate half-life at 25 °C in the presence of Ca-montmorillonite is on the order of 25,000 years, for example. Other acids in the proposed cycle should be similarly vulnerable, and it is immediately apparent that a viable cycle would have required a series of early Earth catalysts boosting the rates of their respective reactions sufficiently to overcome destructive decarboxylation.

But could such naturally occurring catalysts actually have existed? We can explore that question by considering the portion of the cycle that includes the sequence

$$\text{pyruvate} + CO_2(aq) \underset{-2}{\overset{2}{\rightleftarrows}} \text{oxaloacetate} \tag{2}$$

$$\text{oxaloacetate} + AH_2 \xrightarrow[\text{cat.}]{3} \text{malate} \tag{3}$$

where AH_2 is some reducing agent. Oxaloacetate is known to undergo rapid decarboxylation in aqueous solution at ambient temperatures (Ochoa, 1948; Gelles, 1956), and we can safely presume that its decarboxylation takes place much faster than its hydrogenation. That is, $k_{-2} >> C_{AH_2}k_3$ where k's are rate constants and C represents concentration. Thus Equation (2) is a true equilibrium with an equilibrium constant K_2, and Equation (3) is rate controlling. The observed rate constant for pyruvate conversion to malate is then

$$k_{obs} = C_{CO_2}K_2k_3 \tag{4}$$

where k_3 a second order rate constant that includes the reducing component.

Table I Selected reduction potentials at 25 °C

System	Half-cell	E^o/V[a]
Phosphinate–phosphate	$H_2PO_2^- + 2\ H_2O \leftrightarrows H_2PO_4^- + 2\ H^+ + 2\ e^-$	0.77
"Pyrite-pulled"	$H_2S + FeS \leftrightarrows FeS_2 + 2\ H^+ + 2\ e^-$	0.13
H_2	$H_2 \leftrightarrows 2\ H^+ + 2\ e^-$	0.00
FMQ	$3\ Fe_2SiO_4 + 2\ H_2O \leftrightarrows 3\ SiO_2 + 2\ Fe_3O_4 + 2\ H^+ + 2\ e^-$	−0.10
PPM	$6\ FeS + 4\ H_2O \leftrightarrows Fe_3O_4 + 3\ FeS_2 + 4\ H^+ + 4\ e^-$	−0.13

[a] Developed from the values in the database in the thermodynamics software package SUPCRT92 (Johnson et al., 1992).

Smith and Morowitz (2004) note that pyruvate to oxaloacetate is uphill by 62.1 kJ/mol, and accordingly $K_2 = 1.5 \times 10^{-11}$ in concentration terms. (Although the temperature is unstated, we presume the Gibbs energy value is for 300 K.) Then for an atmosphere at 300 K with a CO_2 partial pressure of 1 atm, employing the CO_2 Henry's law constant (Pandis and Seinfeld, 1989) and presuming a nominally pH neutral aqueous medium

$$k_{obs} = 5.2 \times 10^{-13} k_3 \tag{5}$$

and an estimate for k_3 is required.

It is notable that the reduction in Equation (3) has to be quite selective, converting the ketone function to an alcohol while not affecting the carboxyl groups. While this degree of specificity is not uncommon in some organic laboratory operations with specialized reducing reagents, the nonenzymatic conversion of oxaloacetate to malate has not been reported. We can gain some rough sense of a limiting value for k_3 by considering the work of Brigas et al. (2006) who reported on the palladium-catalyzed reduction of aromatic carbonyl compounds by phosphinate ($H_2PO_2^-$) under conditions where carboxylic acids were not reduced. The results of that study should safely reflect an upper limit value for k_3 since as is shown in Table I, the reducing potential for the phosphinate–phosphate couple is substantially greater than those for naturally occurring redox systems including the fayalite–magnetite–quartz (FMQ) and pyrrhotite–pyrite–magnetite (PPM) mineral assemblages and the "pyrite pulled" reduction system proposed by Wächtershäuser (1990, 1993), and is greater than that for H_2 itself.

The study by Brigas et al. was conducted at 20 °C in aqueous systems that included an organic solvent to assure complete solution of the carbonyl compound. The 10% Pd-on-carbon catalyst had a substantial metal surface area of about 26 $m^2\ g^{-1}$, and depending upon the starting carbonyl compound the reductions proceeded from about 50% to greater than 90% completion in 5 min. From the quantities of the starting materials (in the range 0.05 M carbonyl substrate and 0.2 M phosphinate), the conversion levels, and the reaction time, a second order rate constant for that catalytic system can be estimated to be on the order of $4 \times 10^{-2}\ M^{-1}\ s^{-1}$.[1] Then introducing this value as k_3

$$k_{obs} = 2 \times 10^{-14} M^{-1} s^{-1} \tag{6}$$

This value for k_{obs} is obviously quite small; a system at 1 atm CO_2 employing commercial-grade Pd/C catalyst and a steady-state 1 mM phosphinate concentration would require about 1 Gyr to convert half the pyruvate to malate. An interval of that magnitude not only

[1] Since phosphinate >> carbonyl substrate, 90% conversion in 5 min with 0.2 M phosphinate yields a second order rate constant of $-(\ln 0.1)/(300 \times 0.2)$.

exceeds the acetate half-life, it is clearly out of line for any chemical process considered for a role in life's beginnings. Lower phosphinate levels would of course result in even longer reaction periods, while naturally occurring redox systems would require still longer intervals. On this basis it seems clear that on an enzyme-free early Earth the pyruvate → oxaloacetate → malate conversion would have been a fatal choke point in the cycle's flow.

It can be noted further that there is a parallel progression later in the cycle involving the series succinate → ketoglutarate → oxalosuccinate → isocitrate. The sequence employs the same reaction string that drives the acetate-to-malate sequence, carbonylation/carboxylation/reduction, and there must be barriers to successful functioning in that portion of the cycle as well. But since oxalosuccinate decarboxylates even more rapidly than does oxaloacetate at 300 K by a factor of seven to eight (Ochoa, 1948), the kinetic and thermochemical restraints in that case should be even more severe.

There are still other shortcomings evident from a thermodynamics perspective. In the proposed cycle pyruvate is generated from acetate with the stoichiometry

$$CO_2(g) + H_2(g) + \text{acetate} \rightarrow H_2O + \text{pyruvate} \tag{7}$$

The reaction is endoergic by about 92 kJ/mol and it therefore requires a significant source of energy.[2] Smith and Morowitz (2004) suggest pyrophosphate hydrolysis as an energy supply, but that route offers only about 14 kJ/mol and is clearly inadequate to power the formation of pyruvate. In addition, energy transfer requires a direct mechanistic link between pyrophosphate conversion to phosphate and the oxidation state changes in Equation (7). While a link bridging the two routes utilizing early Earth materials cannot be ruled out in principle, it is difficult to see how simple hydrolysis can be joined mechanistically to redox chemistry in an enzyme-free environment.

Conclusion

It seems quite clear from this discussion that early Earth resources could not possibly have included the conditions necessary for the proposed sequence, and in fact in retrospect the defects in the proposition should have been evident by inspection. First, Equation (2) represents a nonenzymatic reverse decarboxylation which, while considered theoretically (Zhan et al., 2001), has not been reported.[3] Central to the issue is the highly positive Gibbs energy of the reaction, which is a lower limit to the severe Arrhenius activation energy barrier which would curb the nonenzymatic reaction kinetically.

Second, a nonenzymatic reducing system that could reduce oxaloacetate to malate, if it had existed, would have reduced pyruvate to lactate as well and the cycle could then go no further. In fact it is likely that any system capable of overcoming the severe endoergic hurdle of Equation (2) would in all likelihood reduce all of the carboxylic acids in the cycle to their respective alcohols, if not their alkanes.

It is thus apparent that an enzyme-free reductive citric acid cycle would be so challenged both kinetically and thermodynamically as to be unattainable. The proposed cycle could not have existed as a precursor to biosynthesis on early Earth. Indeed, the very existence of enzymatically promoted reactions such as carboxylations, which are otherwise inaccessible,

[2] The Gibbs energy values for the solid acids taken from Stull et al. (1969) were used here.

[3] Obviously not included in that statement is the reaction of CO_2 with carbanionic species including Grignard and organolithium reagents in the laboratory systhesis of organic carboxylates.

reflects the vital role enzyme-like catalysis must have played in the earliest chemistry of life's beginnings.

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