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Photocatalytic reversible amination of α -keto acids on a ZnS surface: implications for the prebiotic metabolism[†]

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We report the enzyme-like reversible amination of four intermediates pertinent to the reductive tricarboxylic acid cycle on a photo-irradiated surface of mineral sphalerite (ZnS). Given its prevalence in the waters of early Earth, we suggest that the mineral-based photochemistry might have catalyzed the homeostasis of prebiotic metabolic systems.

Redox homeostasis is a set of enzyme-assisted metabolic reactions that happen in living organisms to maintain life. How such equilibrium systems could have evolved and functioned before the emergence of enzymatic networks remains a critically important challenge in the context of the origins of life. An intriguing scenario claimed that the outgrowth of the primordial metabolism may be postulated as a continuum from Earth's geochemical processes to chemoautotrophic biochemical procedures on a sulfide mineral surface.¹⁻⁷ It makes sense and rapidly gained ground partly because in all extant life forms transition metal sulfide cluster complexes play a crucial role in certain enzymes.^{8–10} In order to reconstruct the possible outline, some surface reactions in aqueous metal sulfide systems have been developed to mimic basic biochemical steps, and many interesting results have been achieved. However, the previous studies have mainly focused on the fixation of gas molecules.^{11–13} the reduction of metabolic intermediates,^{5,6} and the extension of molecular chains.^{5,14–16} Up to now, little attention has been paid to the archaic redox homeostasis.7

Herein we describe a model for the prebiotic redox chemistry, *i.e.*, the photocatalyzed reversible amination of α -keto acids on ZnS particles. The mineral sphalerite (ZnS) is a common semiconductor and is believed to have been a typical constituent of shallow-water hot springs on early Earth, within reach of solar light.^{17,18} Previous research on ancient rocks demonstrated that there was negligible oxygen and resulting ozone in the primordial atmosphere.¹⁹ So the solar light reaching Earth contained a UV component that was several orders of magnitude stronger than it is today.¹⁷ ZnS can adsorb the UV light with a wavelength



Scheme 1 The reversible amination of α -keto acids on a photo-irradiated ZnS semiconductor particle.

shorter than 344 nm to trigger prebiotic redox reactions *via* the interaction of an adsorbate with a conduction band (CB) photoelectron (e^-) or a valence band (VB) hole (h^+ , Scheme 1).

For example, the reductive tricarboxylic acid (rTCA) cycle, which is employed by a number of extant chemoautotrophs for carbon fixation, has been suggested to be the central part of the primordial metabolism.^{20,21} Through a series of photodriven reactions, not only can ZnS play a role in abiotic anaplerosis and replenish the rTCA cycle using CO_2 as the initial inorganic material, but also drive the cycle forward (Scheme 2).

Once a steady matter flow enters into the rTCA cycle, it is equally important to remove the anions to avoid their accumulation.²² An archetypical pathway for this so-called cataplerotic metabolism in extant organisms is the reductive amination of α -ketoglutarate (α -KG, 1d) catalyzed by glutamate dehydrogenase (GDH) (Scheme 2),²³

NH₃ +
$$\alpha$$
-ketoglutarate + NAD(P)H + H⁺
 \leftrightarrow glutamate + NAD(P)⁺ + H₂O (1)

This is a reversible process of homeostasis. The back reaction can utilize external glutamate (3d) to produce the intermediate α -KG for the rTCA cycle and make the pathway homeostatic.

The aim of this work is to simulate such an enzyme-assisted process on a photo-irradiated ZnS surface. ZnS colloidal suspensions were *in situ* prepared in a quartz conical flask by mixing O₂-free Na₂S and ZnSO₄ solutions. For the reductive amination experiments, one mmol α -KG and 100 mmol NH₄Cl were added. Na₂SO₃ was used as the hole scavenger when required. The pH value was adjusted to 9 since the seepage water from sulfide minerals was suggested to be ultramafic.^{1,24}

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Scheme 2 The rTCA cycle (thin solid line) coupled to its anaplerotic reactions (dashed line). The simulated reversible amination of keto acids in this study (thick solid line) may balance the entry and exit to the antique cycle and contribute to the prebiotic redox homeostasis.

Photocatalytic reactions were carried out under an argon atmosphere by using a 500 W mercury–xenon lamp (see details in Fig. S1, ESI†). The results are presented in Table 1 and Fig. S2 (ESI†). It can be found that only 0.5% glutamate was formed within 4 h (run 1) when no Na₂SO₃ was added, while in the presence of the sacrificial agent, the yield was enhanced by nearly six times (run 2). In the control runs without exposure to irradiation (run 3) or in the absence of ZnS (run 4), no trace of glutamate was found. Therefore, it can be concluded that the photoelectron transfer from a ZnS surface to Schiff base intermediate 2d was the mechanism for the formation of glutamate (Scheme 1).

In hot springs water is always overheated, reaching a maximum value of about 350 $^{\circ}$ C.¹⁷ We therefore examined the influence of thermal treatment on the photocatalytic activity of ZnS. The fresh ZnS precipitates were filtered and

Table 1 Reversible amination of α -KG assisted by the electron shuttle on a ZnS surface

Run	ZnS^{a} (2 mmol)	$Na_2SO_3^{\ b} (0.2 \text{ mM})$	$h\nu$	Yields ^c (%)
Amination of α-KG (1 mmol)				Glu
1	ZnS _{is}	_	+	0.5 (0.0)
2	ZnS_{is}	+	+	2.9(0.3)
3	ZnS_{is}	+	_	ud ^d
4	_	+	+	ud
5	ZnS_{100}	+	+	7.4 (0.7)
6	ZnS_{200}	+	+	6.8 (0.6)
7	ZnS_{300}	+	+	0.6(0.1)
8	ZnS_{400}	+	+	1.5 (0.1)
9	ZnS_{100}	_	+	3.8 (0.2)
10	ZnS_{100}	+	_	ud
Deamination of Glu (1 mmol)			α-KG	
11	ZnSie	_	+	2.8(0.2)
12	ZnS100	_	+	18.3 (1.0)
13	ZnS_{100}	+	+	ud
14	ZnS_{100}	_	_	ud
15		_	+	ud

^{*a*} For the subscripts of ZnS, please refer to the experimental section. ^{*b*} "+" and "-" indicate the presence or absence of a species, respectively. ^{*c*} Each value is the average of three independent repeat experiments; numbers in parentheses are standard deviations of the mean. Reaction conditions: pH 9, 30 °C, 4 h. ^{*d*} Undetectable.

then heat-treated under a nitrogen atmosphere at different temperatures. It was found that the calcination process significantly improved the catalytic efficiency of ZnS. In the presence of Na₂SO₃, the maximum yield of glutamate was observed for a catalyst heated at 100 °C (runs 5-8). It is more than 2.5 times that formed by the *in situ* precipitated ZnS (run 5 vs. run 2). This difference in efficiencies may be due to the improved crystallinity of the calcined ZnS with fewer crystal defects (Fig. S3, ESI[†]), but is not a result of the difference in specific surface area (Table S1, ESI[†]). Such an improvement decreases the possibility of photoelectron-hole recombination, thereby leading to higher catalytic activity. This explanation can be more unequivocally conformed by the controls in the absence of a hole scavenger. Without the use of Na₂SO₃, the yield of glutamate catalyzed by calcined ZnS (run 9) was almost eight times higher than that formed by in situ precipitated ZnS (run 1). The control in the absence of irradiation again demonstrated that heterogeneous photochemistry was the reaction mechanism (run 10). That the activities of ZnS samples were reduced with the further enhancement of treated temperature may be due to their surface alteration at higher temperatures (>300 °C) (Fig. S4, ESI[†]).

Alpha-KG is one cataplerotic exit point out of the rTCA cycle (Scheme 2). In biochemistry, this tunnel is not one-way but bidirectional to guarantee the homeostasis of metabolic intermediates. In other words, α -KG, as the deamination product of external glutamate, may revisit the cycle through the reverse pathway. ZnS can catalyze such an anaplerotic reaction (Fig. S5d, ESI[†]). A summary of the results is presented in the lower moiety of Table 1. Thermal treatment remarkably raised the deamination efficiency of glutamate by ZnS (runs 11 and 12), just as it has behaved in the amination reaction. Unlike the reduction process, however, the oxidative deamination reaction occurred at the hole sites on the surface of ZnS particles (Scheme 1). The presence of Na₂SO₃ entirely inhibited the formation of α-KG (run 13), since it forcefully competed with glutamate to be oxidized by the hole. The complete set of control experiments implies that the oxidation of glutamate is driven by the ZnS-assisted photochemical reaction.

In further experiments, a set of other three reversible redox steps pertaining to the rTCA cycle (Scheme 2) was evaluated, *i.e.*, the reductive amination of glyoxylate (1a), pyruvate (1b), and oxaloacetate (1c), and the corresponding oxidative deamination of glycine (3a), alanine (3b), and aspartate (3c) (Fig. S5–S8, ESI⁺). In Fig. 1, the amount of each product formed during the photoreaction is expressed as a function of irradiation time. The time-dependent results of 1d and 3d are also represented. A general trend is that the stoichiometric yields of the products increased along with time, and subsequently reached a constant level or decreased because of their further reactions. Special note should be taken of the data that the main reaction products from 1c and 3c were 3b and 1b but not 3c and 1c, respectively. For the amination of 1c, the yields of 3c did not increase above the detection limit and were up to 0.1% even after a prolonged time (24 h) (Fig. S7, ESI[†]); while in the deamination products of 3c, no trace of 1c was detected (Fig. S5c, ESI[†]). A decarboxylation process of 1c to 1b is unequivocally involved in both reactions²⁵ and may help to explain the unexpected results. However, this question still remains



Fig. 1 Main reaction products from the reductive amination of 1a-1d (a) and the deamination of 3a-3d (b) on ZnS_{100} surface *vs.* the irradiated time. Uncertainties are shown by using error bars. For the experimental conditions please refer to runs 5 and 12 in Table 1, respectively.

to be specified. Another challenge is to explicate the differences in the reaction efficiencies of different reactants. A possible explanation is that the higher the nonpolarity, the more accessible the reactants are to the hydrophobic surface of ZnS and then the more available to participate in the photochemical reaction. As a result of the differences in the side chain groups, the nonpolarity of the reactants seems to follow the trend 1b/3b > 1d/3d > 1a/3a > 1c/3c.

In summary, the simulated GDH-like chemistry (eqn (1)) demonstrates that the reversible amination of several α -keto acid intermediates pertinent to the rTCA cycle can occur on the photo-irradiated surface of mineral sphalerite. The yields are not very high and studies upon reactivity changes of the system as a function of a full range of experimental conditions have not been investigated to determine the optimized yield. However, since the presented experimental conditions were carefully designed to simulate the primeval hot spring environments as closely as are presently known, the results can be related to how an extant metabolic mechanism might have evolved on early Earth. Not only do they provide a new pathway for the prebiotic synthesis of amino acids, but also an abiotic archetype for the primeval redox metabolism. The ZnS-photo-assisted redox chemistry could

balance the entry and exit of rTCA cycle metabolites (Scheme 2). The equilibrium point is controlled by the concentration of the hole scavenger. This scenario envisages a primary role of mineral-driven catalysis for the archaic redox homeostasis. Taken together with the results in previous studies,^{5,6} the minerals in the submarine hydrothermal systems make the whole homeostasis possible by modulating a series of cascading reactions (Scheme 2). Later on in the evolutionary context, the enzyme-like chemistry of the minerals was replaced by extant enzymatic networks.¹

Given its activity in both reductive amination and oxidative deamination, the ZnS-based model presented in this study implies that it may also perform a similar function to that of transaminase. Work along this line is in progress in this laboratory.

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