The cradle chemistry of life: On the origin of natural products in a pyrite-pulled chemo-autotrophic origin of life

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Abstract. It is argued that life originated in an autocatalytic carbon dioxide fixation cycle. Specifically, an archaic version of the reductive citrate cycle is discussed. The reducing power for this process is seen as being provided by the formation of pyrite from iron sulfide and hydrogen sulfide. A variety of novel pyrite-pulled redox reactions will be discussed which support this proposal. It is suggested that the mechanism of oxidative pyrite formation gives rise to group activation in the form of thioacids and thioesters and to the formation of carbanions for condensation reactions. As examples of the evolution of biosynthetic pathways the joint origin of fatty and isoprenoid lipids is discussed as well as an archaic version of the tetrapyrrol pathways.

Scientific theories are designed to explain the world. To explain means to reduce known facts to unknown assumptions. This is Poppers formulation. And it is true. When we explain in chemistry we reduce known reaction products to unknown reaction mechanisms and unknown intermediates. When we explain in biology we reduce known organisms to unknown missing links. And if we wish to explain in biochemistry we have to reduce known natural products and their biosynthetic pathways to an unknown primordial metabolism.

THE PRIMORDIAL ENERGY SOURCE

From a chemical point of view evolution is a gigantic reaction mechanism, stretching over some four billion years. Very simply speaking I see this reaction mechanism as an autocatalytic chain reaction with branchings, where some of the branch products ignite ever new chain reactions, drawing in ever new types of starting materials. How will the chemist approach the mechanism of these earliest chain reactions? He will begin by asking a most simple question. What are the earliest starting materials and what are the earliest end products?

Todays biosphere gives us the most fundamental alternative to this question. It is the alternative between two radically different ways of life: autotrophy and heterotrophy. Autotrophs are capable of producing all their constituents from inorganic scratch. Heterotrophs are dependent on taking up organic compounds as food. For now more than 60 years biology has been dominated by the notion of a heterotrophic origin of life in a prebiotic broth. Gerald Joyce wrote as late as 1988: "It is almost inconceivable that [the first living system] could have been anything other than a heterotroph"(ref. 1). I shall now invite you to conceive of the inconceivable and to explore with me some of the chemical consequences of a hypothetical chemo-autotrophic origin of life.

In a chemo-autotrophic origin of life the starting material is carbon dioxide or an equivalent C1-unit. This means that the reactions must be reductive. For such a reductive metabolism we need a reducing agent. It must be strong enough for reducing carbon dioxide and carboxylate groups; geochemically plausible; and with an explanatory connection with todays biochemistry. It appears to me that there is only one energy source to satisfy these requirements. With this I come to my main thesis (ref. 2,3). It identifies the first energy source of life with the formation of pyrite from ferrous ions and hydrogen sulfide.

$$FeS + H_2S \longrightarrow FeS_2 + 2H^+ + 2e^- \qquad (1)$$
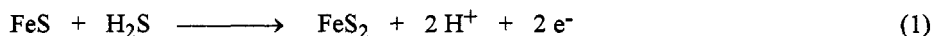
This energy source is indeed powerfull enough for all biochemical reducing reactions. It has a standard potential of -620 mV. Pyrite formation is geochemically ubiquitous under anaerobic conditions. Pyrite is a deep thermodynamic sink. It is extremely stable. In fact, it does not dissolve in hot concentrated hydrochloric acid.

EVOLUTIONARY CONSEQUENCES

We now come to the main consequence of pyrite formation as the earliest energy source for life. Its end product is a particulate entity, a crystal. The surface of a pyrite crystal is cationic. The products of carbon fixation are anionic.Therefore, they will become bonded to the pyrite surface *in statu nascendi*. Here they undergo recursive reactions. They establish a surface reaction system, a surface metabolism. This has several important consequences:

(1) It gives rise to a process of selection by the selective detachment of organic constituents from the pyrite surface.

(2) Strong surface bonding tends to stabilize polyanionic polymers. This is the physical basis for the appearence of structural complexity.

(3) Given the possibility of optically active pyrite, the origin of biochirality may find an easy explanation in a chiral transfer from the pyrite crystal to the surface bonded reaction products (ref. 4).

(4) Most importantly, the process of surface bonding *in statu nascendi* gives rise to the particulate entities which King postulated as a requirement for metabolic evolution (ref. 5).

(5) The accumulation of surface-bonded, anionic lipids will lead automatically to the development of membranes and of closed cellular structures with internal pyrite.

(6) Lastly, pyrite comes in a unique crystal form: raspberry pyrite. It is unique in this respect among minerals. This raspberry structure must be due to a surface phenomenon in conjunction with secondary nucleation.

I propose that this mechanism is the inanimate placeholder for cell division (ref. 4).

THE MAIN CHEMICAL CONSEQUENCE

My theory is based on the assumption that the first metabolism is reductive and that the system FeS/H_2S can form pyrite with a variety of inorganic and organic electron acceptors. When it was first published, this theory was in conflict with a standard doctrine of geochemistry (ref.6). It was believed that under geochemical conditions pyrite can form only in one way : with elemental sulfur as electron acceptor (2).

$$FeS + S \longrightarrow FeS_2 \qquad\qquad (2)$$

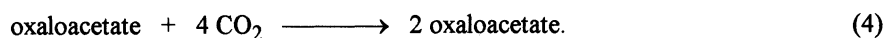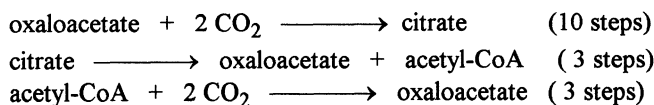Such a conflict between two theories can only be resolved by an experiment.

My theory is now being tested in collaboration with Karl-Otto Stetter in Regensburg. In our first experiment we discovered that the conventional doctrine of geochemical pyrite formation is wrong. We reacted iron sulfide with hydrogen sulfide in nearly neutral water under fastidiously anaerobic conditions (ref. 7). We found two products: pyrite and molecular hydrogen (3). This means that protons can serve as the electron acceptor for pyrite formation.

$$FeS + H_2S \longrightarrow FeS_2 + H_2 \qquad\qquad (3)$$
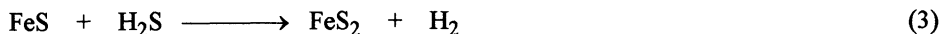
This experiment did two things. It posted a road sign to a vast uncharted territory of pyrite chemistry. And it offered an evolutionary explanation for the workings of the iron-sulfur clusters in hydrogenases. And as for my theory, it meant a chemical licence for evolutionary theorizing.

AUTOCATALYTIC CARBON FIXATION

With this chemical licence, let us now ask the next major question. What was the first autocatalytic carbon fixation cycle. Biochemistry gives us an answer. The reductive citrate cycle (4) is a carbon fixation cycle. And it is autocatalytic. It doubles the carbon dioxide acceptor with every turn.

$$oxaloacetate + 2\,CO_2 \longrightarrow citrate \qquad (10\ steps)$$
$$citrate \longrightarrow oxaloacetate + acetyl\text{-}CoA \quad (3\ steps)$$
$$acetyl\text{-}CoA + 2\,CO_2 \longrightarrow oxaloacetate \quad (3\ steps)$$

$$oxaloacetate + 4\,CO_2 \longrightarrow 2\ oxaloacetate. \qquad\qquad (4)$$

I propose that the origin of life coincides with the origin of an archaic version of this cycle, with FeS/H_2S as the sole reducing agent and without enzymes (ref. 8).
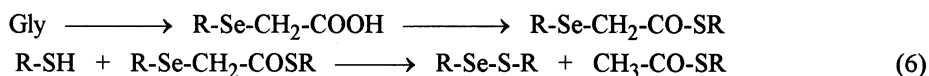
The most crucial step is obviously the step of reductive carbon fixation. Today this step is dependent on an iron-sulfur enzyme (5).
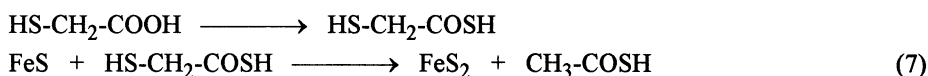
$$CH_3\text{-}COSR \; + \; CO_2 \; \xrightarrow[\text{[Fe/S]}]{\text{TPP}} \; CH_3\text{-}CO\text{-}COOH \qquad\qquad (5)$$
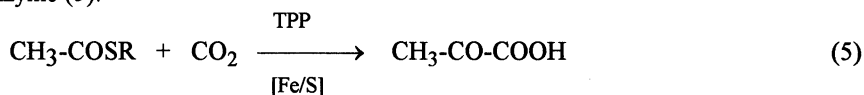
Can this reaction run without an enzyme and without TPP? Yes it can. Fifteen years ago this was shown for an iron-sulfur complex as catalyst and $Na_2S_2O_4$ as reducing agent (ref. 9). These are the facts.

Now, my speculation: The aqueous system FeS/H_2S will do three things for this reaction:

(1) It will provide the reducing power.
(2) It will form inorganic iron-sulfur clusters with catalytic functions like those of the iron sulfur enzymes.
(3) It will give rise to the required group activation, not as a thioester right away, but rather first as a thioacid. A mechanism for such a pyrite-pulled group activation is not immediately obvious. But it is suggested by biochemistry; specifically by the mechanism suggested for the formation of acetyl-CoA from glycine (ref. 10).

$$Gly \; \xrightarrow{\hspace{1cm}} \; R\text{-}Se\text{-}CH_2\text{-}COOH \; \xrightarrow{\hspace{1cm}} \; R\text{-}Se\text{-}CH_2\text{-}CO\text{-}SR$$
$$R\text{-}SH \; + \; R\text{-}Se\text{-}CH_2\text{-}COSR \; \xrightarrow{\hspace{1cm}} \; R\text{-}Se\text{-}S\text{-}R \; + \; CH_3\text{-}CO\text{-}SR \qquad\qquad (6)$$

If in this mechanism we replace Se by S, SR by SH and the organic sulfide-selenide by the disulfide dianion in pyrite we will arrive at a simple group activation reaction: The conversion of an α-mercapto acid into a thioacid (7) or, if H_2S is replaced by R-SH, into a thioester. I suggest this as the first form of group activation (ref. 4).

$$HS\text{-}CH_2\text{-}COOH \; \xrightarrow{\hspace{1cm}} \; HS\text{-}CH_2\text{-}COSH$$
$$FeS \; + \; HS\text{-}CH_2\text{-}COSH \; \xrightarrow{\hspace{1cm}} \; FeS_2 \; + \; CH_3\text{-}COSH \qquad\qquad (7)$$

This brings us to the next question. How do we obtain an α-mercapto acid. The answer is simple: By reacting an α-keto acid with hydrogen sulfide (8).

$$R\text{-}CO\text{-}COOH \; + \; 2\,H_2S \; \xrightarrow{\hspace{1cm}} \; R\text{-}C(SH)_2\text{-}COOH \; + H_2O \qquad\qquad (8)$$

The α-keto acid, of course, is the product of carbon fixation.

We have started to test this proposal and we have been able to show that aqueous FeS/H_2S reduces phenylpyruvate to phenylpropionate, oxaloacetate to succinate and mercaptoacetate to acetate (ref. 11).

So far we have considered the first carbon fixation process as a single cycle. However, in an enzyme-free metabolism there are no specific single reactions. There are only reaction classes. By applying this principle we can see at once that keto-glutarate will be reduced to activated glutarate which in term will undergo carbon fixation in a homo-citrate cycle. By extending this principle we obtain a whole series of concatenated homologous cycles. This network of cycles gives rise to longer and longer carbon chains and ultimately to long-chain dicarboxylic acid lipids. In todays metabolisms the higher homologous cycles have been abandoned. Only a portion of the homo-citrate cycle is maintained in a biosynthetic pathway to lysine. What could be the reason for this abandonment. The answer lies in the evolutionary changeover from an archaic pyrite-pulled metabolism to the extant metabolism. Under pyrite-forming conditions oxaloacetate is converted directly into succinate. With NADH as reducing agent this is not possible. Instead, we obtain malate. This is a ß-hydroxyacid and can easily undergo dehydration to fumarate which is hydrogenated to succinate. In the homo-citrate cycle keto-glutarate is converted under pyrite-forming conditions directly into glutarate. With NADH as reducing agent we obtain α-hydroxyglutarate. This is not a ß-hydroxy acid and therefore a simple water elimination is not possible. This means that with the abandonment of the pyrite-forming energy source for a lack of sufficient concentrations of ferrous ions and/or hydrogen sulfide the higher homologous citrate cycles where also abandoned.

The mechanism of the discovered pyrite-pulled reducing reactions is not known. It is here suggested that this mechanism is akin to the mechanism suggested by Arkowitz and Abeles for glycine reductase (ref. 10). This means that the primary reduction product is a carbanion:

$$\text{FeS} \;+\; {}^{-}\text{S}-\text{C}{\textstyle\lessgtr} \quad\longrightarrow\quad \text{FeS}_2 \;+\; {}^{-}|\text{C}{\textstyle\lessgtr} \tag{9}$$
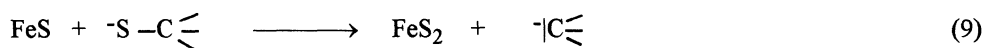
the carbanion may react subsequently by protonation. In competition to protonation it may undergo nucleophilic condensations. Carbon fixation is one such condensation. The Claissen-type condensation is another.

In summary, the chemical laws of oxidative pyrite formation are seen as giving rise to three important aspects of biochemistry:
(1) Providing the reducing equivalents for all biochemical reductions;
(2) Producing group activation, first by the formation of thioacids and later of thioesters; and
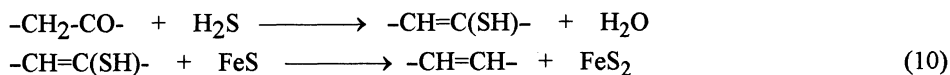(3) Producing carbanions for condensation reactions as the primary products of reduction.

THE EVOLUTION OF BIOCHEMICAL PATHWAYS

The constituents of the reductive citrate cycle are the starting points for all biosynthetic pathways: to the lipids, the sugars, the amino acids, the bases and all the coenzymes. All these pathways must have been thoroughly modified in the course of biochemical evolution. A reconstruction of the archaic pathways is one of the fundamental problems of biochemistry.

The oldest of these pathways are seen as dating back to a time when pyrite formation was the sole energy source for life; to a time when the process of evolution was not yet dependent on a genetic machinery with nucleic acids and coded proteins. This earliest mechanism of evolution is attributed to branch products of the reductive citrate cycle. Most of these branch products are a burden for the autocatalytic cycle. Occasionally, however a low probability branch product will show a dual catalytic feedback effect: into the productive autocatalytic cycle and into its own branch pathway. This means that such a catalyst will reproduce itself with a rate much higher than the rate of its spontaneous de novo formation. This is a memory effect which is essential for evolution. All extant coenzymes are of this nature and so are the nucleic acids. We will now take two of these pathways and attempt to reconstruct their archaic pre-enzymatic precursor pathway.

THE LIPID PATHWAYS

The accumulation of surface-bonded lipids renders the pyrite surface increasingly hydrophobic. This means that the competition between the protonation of carbanions and their condensation is shifted to favour carbon dioxide condensation and to favour also lipid formation by condensation. This means that it has a dual feedback. We will now discuss the early evolution of this pathway. We have experimentally shown that FeS/H$_2$S reduces carbonyl groups. This reaction is accompanied by an important side reaction which produces an olefin group (ref. 11)

$$
\begin{aligned}
-\text{CH}_2\text{-CO-} \;+\; \text{H}_2\text{S} &\longrightarrow -\text{CH}=\text{C(SH)-} \;+\; \text{H}_2\text{O} \\
-\text{CH}=\text{C(SH)-} \;+\; \text{FeS} &\longrightarrow -\text{CH}=\text{CH-} \;+\; \text{FeS}_2
\end{aligned} \tag{10}
$$

In the case of phenylpyruvate this reaction is a minor reaction. In case of acetaldehyde it is the major reaction. In the case of 2-ketoglutarate it gives rise to glutaconic acid (activated as thioacid or thioester).

$$\text{HOOC-CH}_2\text{-CH}_2\text{-CO-COOH} \;\longrightarrow\; \text{HOOC-CH}_2\text{-CH}=\text{CH-COOH} \tag{11}$$

Now, it is important to realize that glutaconic acid is the vinyloge of malonic acid By applying the class reaction principle we come to see that any pre-enzymatic or early enzymatic reaction system for the condensation of malonyl-thioester will automatically also give rise to the condensation of glutaconyl-thioester. A few such condensation steps, in conjunction with FeS/H$_2$S-driven reduction, will produce a fatty acid lipid. Since the glutaconyl-thioester is a direct branch product of the reductive citrate cycle the ATP-dependent formation of malonyl-thioester from acetyl-thioester is not required for the earliest fatty lipid biosynthesis.

Now we will consider another branch reaction of the reductive citrate cycle. Aconitic acid undergoes a facile decarboxylation, which in conjunction with a pyrite-pulled reducing reaction will give rise to methyl-succinic acid (its thioacid or thioester)

$$HOOC\text{-}CH=C(COOH)\text{-}CH_2\text{-}COOH \longrightarrow HOOC\text{-}CH_2\text{-}CH(CH_3)\text{-}COOH \qquad (12)$$
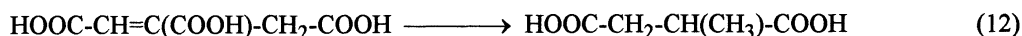
This in turn will give rise to a homologous reductive citrate cycle, producing 3-methyl-2-keto-glutaric acid and from there 3-methyl-glutaconic acid (its thio acid or thioester)

$$HOOC\text{-}CO\text{-}CH(CH_3)\text{-}CH_2\text{-}COOH \longrightarrow HOOC\text{-}CH=C(CH_3)\text{-}CH_2\text{-}COOH \qquad (13)$$

This branch product will also enter into the lipid condensation pathway giving rise to isoprenoid acid lipids. This is an important result. It shows that the fatty lipids of the bacterial and eucaryal organisms and the isoprenoid lipids of the archaeal organisms go back to a single archaic class of pathways producing both types of lipids as siblings.
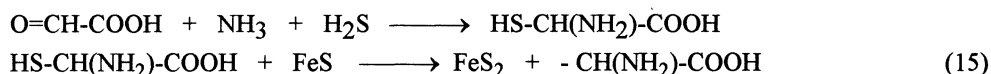
THE TETRAPYRROL PATHWAYS

Most of the branch products are nitrogen compounds (amino acids, bases, coenzymes). This raises the question of the origin of ammonia as the starting material of these pathways. We have solved this problem by demonstrating that FeS/H_2S is capable of reducing nitrate to ammonia (ref. 11). It is of course well known that nitrogen oxides and nitrate may be produced by discharges in an anoxic atmosphere of molecular nitrogen, water and carbon dioxide. We have also shown that FeS/H_2S reduces acetylene to a mixture of ethylene and ethane. This is a well known model reaction for nitrogen fixation. It is here proposed that the so produced ammonia units *in statu nascendi* will infiltrate the organic constituents of the pyrite-pulled surface metabolism. This will give rise to amino acids and to all nitrogen bases derived therefrom.

As an example we take a look at the origin of the tetrapyrrol pathways (ref.12). Heme, siroheme and the chlorophylls are products of oxidative pathways. Therefore they must be latecomers. Vitamin B12 is catalytic for a large class of reactions including methyl transfer reactions. Its own biosynthesis requires eight methylations. Therefore it is autocatalytic for its own biosynthesis.

There are today two pathways to δ-amino-levulinate, the starting material for all tetrapyrrols. One of them begins with glutamate. It involves many complex steps and a complex t-RNA carrier. Therefore, it must be a latecomer. The other consists of a condensation of succinyl-thioester with glycine in a single step.

$$HOOC\text{-}CH_2\text{-}CH_2\text{-}COSR + Gly \longrightarrow HOOC\text{-}CH_2\text{-}CH_2\text{-}CO\text{-}CH_2NH_2 \qquad (14)$$
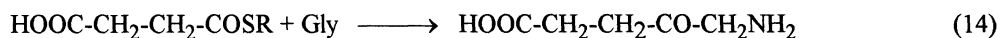
This biochemical reaction type is universal. It is used also for the biosynthesis of biotin and of the sphingolipids. Therefore, it is here proposed to be archaic. The carbanion for condensation is seen as resulting as the primary product in a pyrite-pulled reductive amination

$$O=CH\text{-}COOH + NH_3 + H_2S \longrightarrow HS\text{-}CH(NH_2)\text{-}COOH$$
$$HS\text{-}CH(NH_2)\text{-}COOH + FeS \longrightarrow FeS_2 + \text{ - }CH(NH_2)\text{-}COOH \qquad (15)$$

Now, we make a simple assumption. We assume that the earliest catalysts for this reaction are not selective. This means that alanine, obtained from pyruvate, will also enter the pathway. Now, if you replace glycine by alanine, there will be eight methyl groups introduced automatically into a tetrapyrrol ring. Exactly this number of methyl groups is introduced today by methylation with adenosylmethionine. Three are introduced exactly into the same methine position to be expected from the use of alanine as starting material. This shows how the streamlining of the metabolism (using Gly alone instead of Gly and Ala) is concommitant with a conversion of short archaic pathways into long derived pathways. Incidentally, the use of alanine would mean that some of the methyl groups must undergo a 1,5-migration, driven by conjugation energy. Exactly this type of a reaction has recently been discovered in the B12 biosynthesis (ref. 13) and previously for model compounds (ref. 14). In this fashion a cobyrinic acid of sorts is seen as the first functional precursor of vitamin B12. It is seen as sitting with its eight carboxylate groups on the pyrite surface like a spider. A covalently bonded axial ligand is not required. The axial ligand is provided by a sulfide group in

the pyrite surface. Much later in the course of enzymatization and abandonment of the pyrite base all the other modifications are "invented". The conversion of the carboxylate groups into amide groups assists the lifting off the pyrite surface. Pyrite anchoring is replaced first by covalent enzyme anchoring. Now a histidine group of the enzyme may provide the axial ligand. Much later covalent anchoring to an enzyme is replaced by non-covalent anchoring with a gain in versatility and controllability. Finally, an axial ligand is attached covalently to the tetrapyrrol, further increasing the functional and structural freedom of both the coenzyme and its enzymes.

This example shows how from the vantage point of a pyrite-pulled surface metabolism we may rationalize the evolution of even this most complex biosynthetic pathway. The overall process of early evolution is seen as a process of increasing emanzipation: a chemical emanzipation from the narrow chemical confines of pyrite formation; an organizational emanzipation from a surface-bonded two-dimensional metabolism; and a spatial emanzipation by the conquering of ever more remote and hostile spaces. This process has now been going on since some four billion years. And it is still going on. But at what price? At the price of unfathomable complications and ever more sophisticated controls.

REFERENCES

1. G.F. Joyce, *Nature* **334**, 564 (1988).
2. G. Wächtershäuser, *Syst. Appl. Microbiol.* **10**, 207-210 (1988).
3. G. Wächtershäuser, *Microbiol. Rev.* **52**, 452-484 (1988).
4. G. Wächtershäuser, *Progr. Biophys. molec. Biol.* **58**, 85-201 (1992).
5. G.A.M. King, *BioSystems* **15**, 89-97 (1992).
6. R.A. Berner, *Am. J. Sci.* **268**, 1-23 (1970).
7. E. Drobner, H. Huber, G. Wächtershäuser, D. Rose and K.O. Stetter, *Nature* **346**, 742-744 (1990).
8. G. Wächtershäuser, *Proc. natn. Acad. Sci. USA* **87**, 200-204 (1990).
9. T. Nakajima, Y. Yabushita and I. Tabushi, *Nature* **256**, 60-61 (1975).
10. R.A. Arkowitz and R.H. Abeles, *Biochemistry* **28**, 4639-4644 (1989).
11. E. Blöchl, M. Keller, G. Wächtershäuser and K.O. Stetter, *Proc. Natn. Acad. Sci. USA,* **89**, 8117-8120 (1992).
12. A. Eschenmoser, *Angew. Chem.* **100**, 5-40 (1988).
13. D. Thibaut, M. Couder, A. Famechon, L. Debussche, B. Cameron, J. Crouzet and F. Blanche, *J. Bact.* **174**, 1043-1049 (1992).
14. A.R. Battersby, M.G. Baker, H.A. Broadbent, C.J.R. Fookes and F.J. Leeper, *J. Chem. Soc., Perkin Trans.* **1**, 2027-2048 (1987).