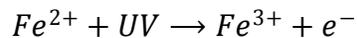


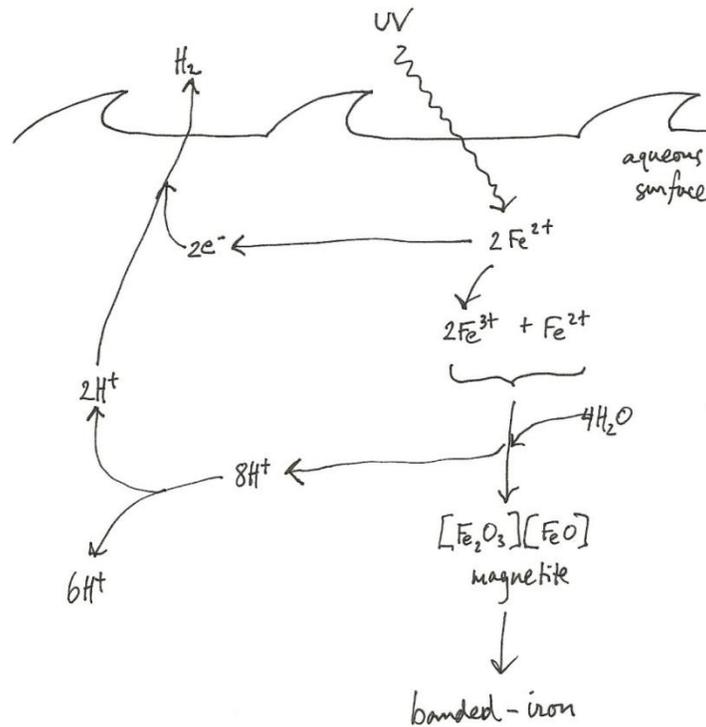
Harnessing Energy

Probably the most basic form of energy on the primitive Earth was heat. Those many forms of synthesis that involve dehydrations are inhibited by the abundance of water. Simple heat can remove water by evaporation, greatly reducing the inhibition. This process has the capacity to form polymers, as has been demonstrated with amino acids. Heat also can be transduced into chemical forms of energy as will be discussed below.

The UV radiation from the Sun has been available as a source of energy since the time the Earth formed over four billion years ago. Much of this radiation is around 400 nm in wavelength and has enough energy per photon to promote oxidation of iron



It has been known for a long time that H_2 is produced by irradiation of aqueous acid solutions of ferrous salts with UV (~250 nm). It is now known that this reaction occurs at neutral or even alkaline pH and does so more readily. Even up to 400 nm the quantum yield is 30%. This process very likely explains the banded-iron formations that date from 1.5 billion years ago back to 3.8 billion years ago. In these bands, iron is partly ferrous and partly ferric with the ferric portion ranging from 30% to 60% depending on the conditions of formation. These iron rich layers are interspersed with layers of shale and chert. The ferrous portion (FeO , wustite) and the ferric portion (Fe_2O_3 , hematite) co-precipitate as magnetite. During electron excitation, the iron combines with the oxygen atoms of water, releasing protons, some of which combine with the UV excited electrons, to form H_2 . The rest are available for reductions. These steps are depicted in the figure:



The generation of excited electrons provided the primitive Earth with reducing potential and the abiotic synthesis of reduced raw materials such as H_2 , CH_4 , NH_3 , HCN ,...

The redox potential [[lipids, membranes, and chemiosmosis](#)] for (Fe^{2+}/Fe^{3+}) at pH 7.0 is +772 millivolts, which is close to the +818 millivolts for ($H_2O/1/2O_2$) that is relevant for contemporary photosynthetic organisms that use visible sunlight. (Fe^{2+}/Fe^{3+}) with UV preceded ($H_2O/1/2O_2$) with visible light in the evolution of energy processing. Banded-iron formations are found world-wide and recurred for over 2 billion years. Only after oxygen producing bacteria became so plentiful that enough atmospheric oxygen was present to cause an ozone layer formation in the atmosphere did this UV driven process stop.

The concentration of inorganic phosphate in the oceans and in fresh water is very low, ~0.1 nanomolar, or $\sim 10^{-5}$ times that of living cells. This is due to the low solubility of calcium phosphate (the mineral apatite) at neutral pH. There has to be some way to understand the

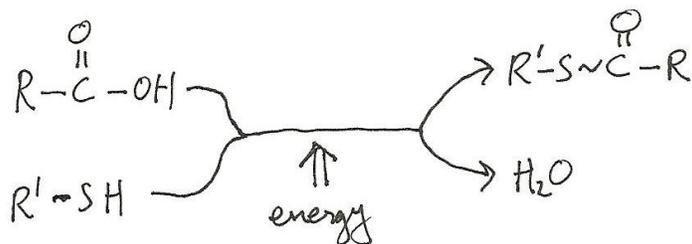
coupling of redox energy to phosphate energy given this scarcity of phosphate. A precursor to phosphate is needed that has the ability to add phosphate to the picture as energy processing evolves. One exists, the *thioester*. Many researchers have come to this conclusion, including Theodor Wieland, Feodor Lynen, Efram Racker, Lars Onsager, Christian De Duve, Fritz Lipmann and Gunter Wachtershauser. The natural energy sequence, before there was an enzyme based energy metabolism, was

redox
thioester
phosphate

The thioester has left its signature in contemporary metabolism in many places, e.g.: glycolysis, substrate level phosphorylation, the citric acid cycle [[energy metabolism](#)], in tyrocidine and gramicidin synthesis [[polymer biosynthesis](#)], in fatty acid synthesis, in the formation of nearly all acyl-ester bonds, in the formation of C-C bonds, in the coenzyme CoA and in many other rotary arm pantetheine enzymes.

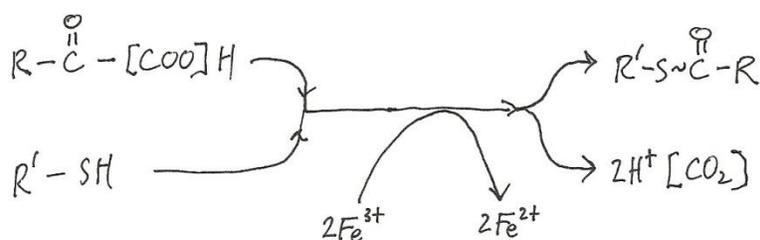
One can think of a stage of energy processing evolution before the advent of phosphate dependent processing that can be called the *Thioester World*, or better yet, the *Iron-Thioester World*. This World existed prior to the famous RNA World. In it several processes essential to the evolution of life could take place without contemporary enzymes. These include **reductions**, **multimerizations**, and **phosphorylations**. The generation of these thioesters can be coupled to the UV driven iron redox cycle, or to heat. Thus, the primitive Earth was a felicitous place for robust energy transductions prior to a genetic apparatus supporting protein biosynthesis.

With the input of energy, a thiol (-SH) and a carboxylic acid (-COOH) can combine to form a thioester and a molecule of water.



Thus, thioester formation is a *dehydration* and subject to the same inhibition any dehydration in aqueous environment faces. We will describe two ways energy can be harnessed to form thioesters.

Heat and low pH promote spontaneous formation of thioesters. Simply picture the figure above with energy replaced by heat and low pH. The thioester linkage formed is “energy rich” releasing between 7.5 and 8.5 kcal/mol upon hydrolysis. For example, this is enough energy to form pyrophosphate from two inorganic phosphates. There is also an oxidative pathway as is shown below.



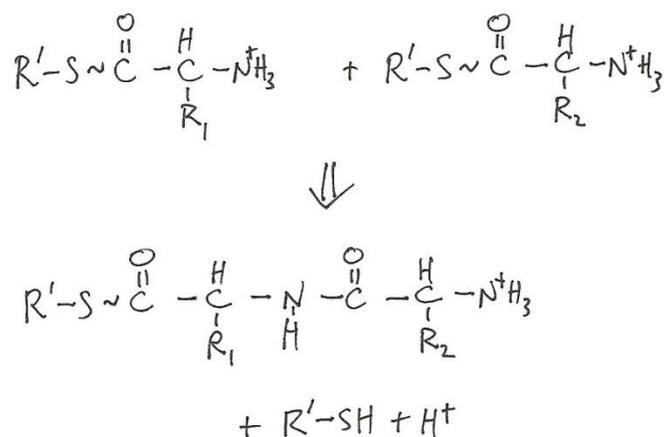
This process also shows how the ferric iron generated by UV can be recycled back into ferrous iron after promoting an oxidation. A special case of this oxidative mechanism has been demonstrated by Art Weber using glyceraldehyde and a thiol, at neutral pH and room temperature, with a good yield and reasonable rate. In the case above that includes the carboxyl group, we have a parallel to the oxidative decarboxylation of pyruvate to form acetyl-CoA that is the reaction connecting glycolysis to the citric acid cycle [[energy metabolism](#)]. In that case the oxidizer is NAD^+ , and the reduced by-product is $NADH$. The iron redox couple may be thought of as the precursor to the contemporary ($NADH/NAD^+$) redox couple. This modern conversion of pyruvate to acetyl-CoA may be a relic of the Iron-Thioester World when the iron redox couple was used instead of ($NADH/NAD^+$). On the other hand, the heat and low pH mechanism makes one think about hot sulfurous, acidic pools such as now found in Yellowstone Park, an active supervolcano. If such an environment also contained the mineral apatite, the acidity would help to dissolve phosphate for ultimate coupling into the thioester-energy world. Note that even though phosphate is scarce world-wide, in sites where apatite occurs it is plentiful. There is no reason to assume that phosphate distribution was spatially uniform.

When expressed in terms of “which came first, the chicken or the egg?” the problem of how energy processing developed appears to be an IC problem. Proteins and polynucleotides are used in contemporary organisms to generate chemical energy, and yet that same chemical energy

is required to make the proteins and polynucleotides. As long as the problem is viewed in this way, no resolution seems possible. Once it is realized that energy processing can develop to a sophisticated level without proteins and polynucleotides, the conundrum disappears. The emergence of proteins and polynucleotides took place in an Iron-Thioester World, a place where robust energy processing was driven by UV and heat, without the aid of modern enzymes.

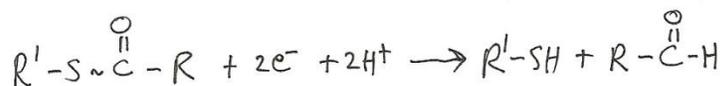
Multimerization is a term coined by Christian De Duve. In normal usage polymerization refers to making polymers from a single class of monomers, such as amino acids or mononucleotides or simple sugars. This idea can be generalized to allow for “polymers” made up of units from more than one class of monomers. These will be called *multimers*. An especially important group of oligomeric multimers is the group of contemporary coenzymes. These molecules contain sugars, phosphate, vitamins and other components, usually about half a dozen parts connected by dehydration linkages. The coenzymes may have been early multimers that reflect the entry of phosphate into the picture (many contain pyrophosphate). Thus coenzymes become a product of the earlier Iron-Thioester World rather than a product of the later RNA World as is often suggested. The (NADH/NAD⁺) redox couple is an example and may well have emerged during the Iron-Thioester World period when it would have replaced (Fe^{2+}/Fe^{3+}) as the primary excited electron carrier.

Of greater relevance for the present discussion is the possibility that thioesters could have driven the formation of multimers such as polypeptides. Amino acids activated as thioesters can spontaneously condense to form polypeptides. This has been demonstrated in the laboratory by T. Wieland with and without crude mineral catalysts. (Wieland did not generate the thioesters in a manner possible on the primitive Earth but rather used modern organic chemist techniques.) The mechanism is shown in the figure below.

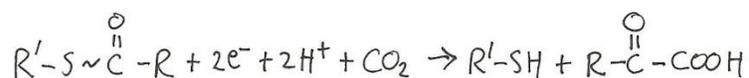


The R_1 and R_2 denote amino acid residues. Thus some small polypeptides could have already been present in the Iron-Thioester World without the presence of the modern protein biosynthesis machinery. If these polypeptides were of length 6-9 amino acids, then they could explain the finding of Edward Trifonov that certain sequences of 6-9 amino acids recur over and over again in modern proteins and may represent a modular structure for modern proteins. These short polypeptides can have rudimentary catalytic and structural properties, all before the advent of modern protein synthesis or the RNA World. Using heat, polymerizing amino acids yield *proteinoids* possessing catalytic and structural properties. These are easily 6-9 units long and longer. Therefore, the pre-RNA World was replete with energy, reducing potential, coenzymes and small polypeptides. The polypeptides bring many functional properties into the system.

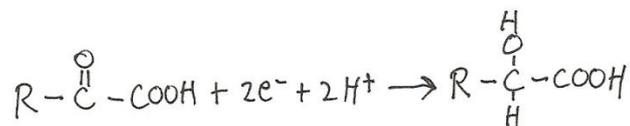
With the electrons and protons provided by the UV excitation of ferrous iron, numerous reductions are possible. A reduction of a thioester can produce an aldehyde (this amounts to the reduction of a carboxylic acid to its corresponding aldehyde via its thioester intermediate).



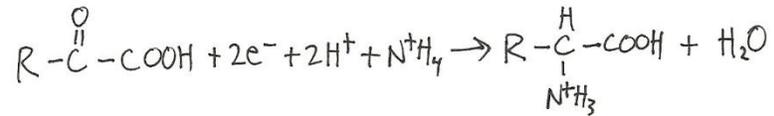
The reductive carboxylation of a thioester produces an α -keto acid.



Further reduction of the α -keto acid produces an α -hydroxyl acid.

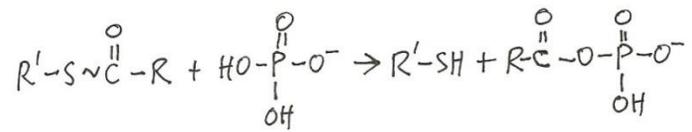


The reductive amination of an α -keto acid yields an α -amino acid.

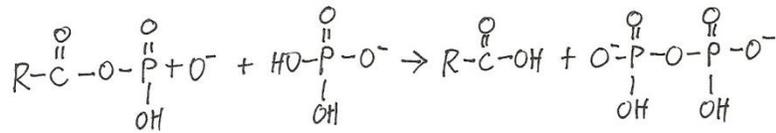


De Duve in particular has emphasized how these processes lead to an early metabolism before the advent of modern enzymes, and before the RNA World had emerged..

The incorporation of phosphate into this picture of thioester mediated processes is tantalizing. Art Weber has demonstrated in the laboratory the feasibility of the following two reactions. The first is the phosphorolysis of a thioester by inorganic phosphate to make an acyl-phosphate.



In modern metabolism acetyl-phosphate is made in this way from acetyl-CoA and phosphate. Thus the acyl-phosphate may be another relic of the Thioester World. The second is the formation of pyrophosphate from the energy rich acyl-phosphate.



Note that the input for these two combined reactions is a thioester and two phosphates. The output is a pyrophosphate, a thiol and a carboxylic acid. Remember that the formation of a thioester from a thiol and a carboxylic acid is a dehydration reaction that also releases a molecule of water as a byproduct. Similarly, synthesis of pyrophosphate from two phosphates also releases a molecule of water. That is why an activated pathway for synthesis is required, Here the water molecule is not manifestly evident. However, the thioester, generated by energy input earlier, has become a thiol and a carboxylic acid in the process and that is where the water molecule is hidden, the $-\text{OH}$ in the carboxylic acid and the $-\text{H}$ in the thiol.

The Iron-Thioester World becomes the *Iron-Thioester-Pyrophosphate World* once phosphate has been recruited by the Iron-Thioester World. All of this involves plausible, non-enzymatic chemistry. The stage is set for the development of more sophisticated polymerization mechanisms. The key insight is that the primitive Earth supported a robust primitive chemistry that produced reducing potential, thioesters and pyrophosphate. With simple small molecules, multimerizations could have given rise to many of the contemporary coenzymes. Since coenzymes are the centers of many catalytic functions, the Thioester World would have these additional catalytic elements to use for further development. Even small polypeptides are plausible products, with their catalytic and structural properties.

The monomers for polynucleotide synthesis are mononucleotides. Unlike amino acids that are simple small molecules, mononucleotides are small multimers made from ribose, nucleic acid bases and phosphate. These components are joined by dehydration linkages. Even one such molecule in a volume the size of a small bacterium is thermodynamically inhibited because of these linkages. However, in the Thioester World described above, plentiful energy of the right kind is available so that formation of mononucleotides is no longer difficult to imagine. The formation of ribose raises issues that will be addressed later when the formose reaction, with phosphate added, is discussed.