

The First Cellular Bioenergetic Process: Primitive Generation of a Proton-Motive Force

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Summary. It is proposed that the energy-transducing system of the first cellular organism and its precursor was fueled by the oxidation of hydrogen sulfide and ferric sulfide to iron pyrites and two $[H^+]$ on the outside surface of a vesicle (the cell membrane), with the concomitant reduction of CO or CO_2 on the interior. The resulting proton gradient across the cell membrane provides a proton-motive force, so that a variety of kinds of work can be done. It is envisioned as providing a selective advantage for cells capable of harvesting this potential. The proposed reactants for these reactions are consistent with the predicted composition of the Earth's early environment. Modern-day homologs of the ancestral components of the energy-transducing system are thought to be membrane-associated ferredoxins for the extracellular redox reaction, carbon monoxide dehydrogenase for the carbon fixation reaction, and ATPase for the harvesting of the proton gradient. With a source of consumable energy, the cell could drive chemical reactions and transport events in such a way as to be exploited by Darwinian evolution.

Key words: Bioenergetics — Proton-motive force — Pyrite — Ferredoxin — ATPase

Introduction

Life as we know it, and even life as it can be imagined, has two key characteristics: information propagation and energy transduction. We may be closer

to understanding the nature of the information transfer of the first living entity now that we know that RNA can serve both as an information reservoir and as a catalyst for functions that can be subject to Darwinian selection.

Only with the Darwinian cyclical series of mutation–replication–selection can we have adaptive evolution that is capable of exploiting new domains, recruiting new resources, and increasing the efficiency of older processes. But selection for a favorable mutation requires that the gene products and function aid just the mutated allele and not the original form of the gene. Consequently, this kind of evolution began only when gene products were segregated: either within the confines of a lipid-enclosed cell (Koch 1985) or on a mineral surface as a result of ionic associations between gene products and the mineral support (Cairns-Smith 1982; Wächtershäuser 1988b).

We may be closer to understanding the original energy transduction system as well. Although it has been proposed that the initial mechanism was respiration, photosynthesis, or substrate level phosphorylation, a much simpler process is the direct functioning of a variant of the chemiosmotic process (Koch 1985). Koch (1985) proposed that a proton-motive force was used to favor endergonic processes and was generated in a way different than Mitchell's loop hypothesis (1966).

Although in most of today's organisms a proton-motive force is generated, as Mitchell suggested, by electron flow in the cytoplasmic membrane due to a respiratory chain, photosynthesis, or ATPase activity, these are not the only ways to generate a proton-motive force (or protonic potential) (Wood 1978; Odum and Peck 1981; Hooper and DiSpirito 1985). Mitchell's loop hypothesis (1966) (Fig. 1A)

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A.

External

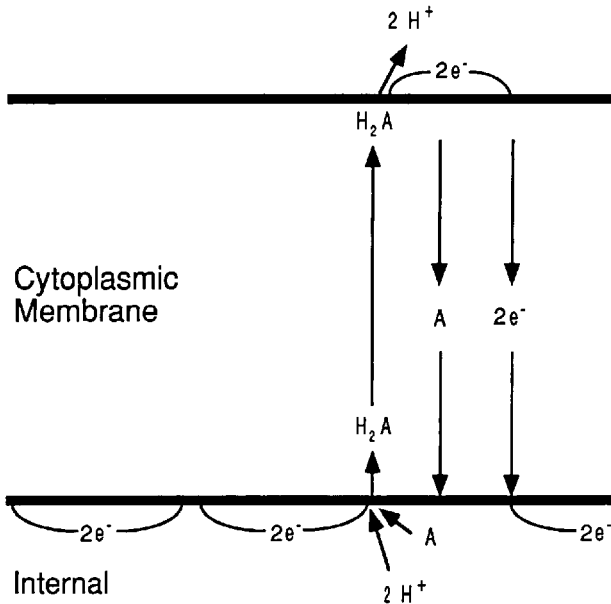


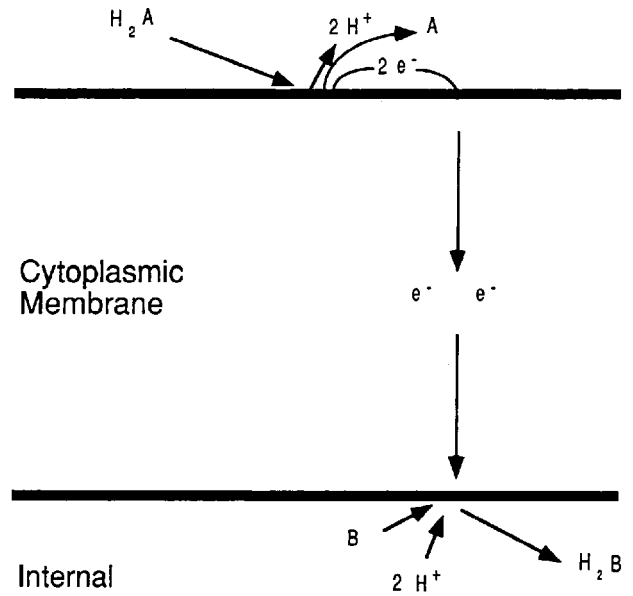
Fig. 1. Generation of proton-motive force. **A** Mitchell's loop hypothesis. Electrons are passed from carrier to carrier. At certain points, two electrons plus two protons from the cytosol react together to form molecular hydrogen or reduce an organic molecule. These products can permeate the membrane. At the outer surface, the reverse reaction takes place and the electrons return through the membrane mediated by metals (ferredoxins). The

is that electrons flow outward in the form of a reduced quinone carrying with them protons, and that the electrons without the protons then move inward by single electron transfers from metal center to metal center. This results in the accumulation of positive charge and protons near the external face and a negative charge and a high pH near the inside of the membrane. The loop hypothesis is still only a hypothesis, but it embodies most bioenergetic processes.

We propose that alternatively to the quinone function providing $[H^+]$ to the outer surface, originally reactants were present both inside and outside of the cell and that a chemical reduction took place catalyzed by a metal complex associated with the membrane. In several organisms today, e.g., *Desulfovibrio* sp. (see Odum and Peck 1981; Hooper and DiSpirito, 1985), proton-motive force is generated by two redox reactions, one occurring extracytoplasmically on or near the outer surface of the cell membrane and the other on or near the inner surface of the closed membrane. The two processes are coupled by electrons alone crossing the membrane. In these organisms usually a periplasmic or membrane-bound enzyme on the outer face and enzymes on the inner face are involved.

B.

External



electrons move to other carriers, the organic molecule returns to the cytosol. The net result is the development of a charge separation and a higher proton concentration outside than inside. **B** Formation of proton-motive force by extracytoplasmic reduction. In this case the reductant is available outside the membrane and the oxidant inside the membrane. A potential and a pH difference is simultaneously generated.

Thus we imagine that the first cellular life-form to transduce energy did so in a manner similar to that presented in Fig. 1B. This process was likely catalyzed by a metal-containing substance that was not originally proteinaceous. The electrons would be transmitted via lipid-soluble organometallic compounds that also may have served both to catalyze the external and internal reactions.

If electrons can pass through the membrane and protons or most charged species cannot, a reduction on one side of the cellular membrane and an oxidation on the other is all that is needed. This obviates the need for the movement of two $[H^+]$ and two electrons from inside to outside that in more modern organisms take place via molecular hydrogen, reduced quinones, etc. The net result can be an energy storage in the form of charge displacement and production of a proton gradient across the membrane. This proton-motive energy could then, as now, be mobilized for simple transport of substances and for driving reactions in an otherwise unfavorable direction. Particularly important in today's organism is the formation of ATP via a membrane-bound ATPase, but this is a sufficiently complex structure of many subunits that either an elementary version or an alternative mechanism

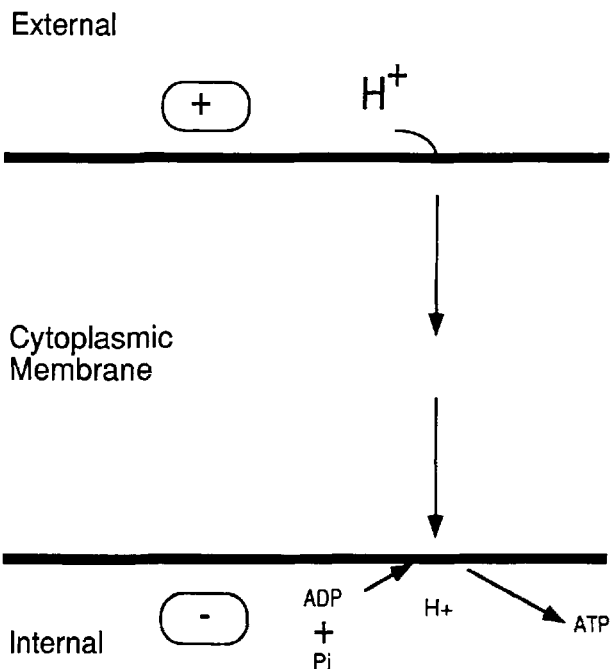


Fig. 2. Utilization of the proton-motive force. The charge difference (indicated by the boxes) and the hydrogen ion concentration (indicated by the size of the proton) drive the inward flow of protons. In modern organisms, the proton-motive force is used to force the synthesis of ATP. But this structure is complicated and sophisticated. A much simpler mechanism must have coupled the formation of ATP or some other energy-rich compound. One possibility has been given in Koch (1985). Other uses of the energy in modern organisms include symports and antiports. Presumably such processes are simpler and elementary prototypes can be imagined.

need have functioned in the first cell, although soon thereafter membrane-bound ATPase must have been developed (Fig. 2).

Below we consider available energy sources. We also explore the possible involvement of transition metals and iron-sulfur compounds ranging from totally inorganic complexes to prototypes of modern ferredoxins and CO reductases. We finally consider ATPases and other processes generating unstable phosphoryl compounds with the proton-motive force.

Exploitation of Free Energy Sources for Primitive Life Processes

But what reaction could have supplied electrons and what reaction could fruitfully utilize electrons in a way that creates free energy available for biological purposes? The framers of thermodynamics originally defined free energy as energy that can be trapped by humans for their own purposes; for this paper, read, 'first organisms' for 'humans.' The first cells needed to exploit an overall reaction that in terms of the ambient concentration of reactants and prod-

Table 1. Candidate half-cell reactions for early energy production: extracellular oxidations^a

Reactions	Pressure (atmospheres)	E' volts ^b		
		pH 0	pH 3.5	pH 7
$H^+ + e^- \rightarrow \frac{1}{2}H_2$	1 ^c	0.000	-0.207	-0.414
	10	-0.059	-0.266	-0.473
	38	-0.093	-0.300	-0.507
	174	-0.132	-0.340	-0.547
	319	-0.148	-0.355	-0.562
$\frac{1}{2}S^0 + H^+ + e^- \rightarrow \frac{1}{2}H_2S$		-0.144	-0.351	-0.588
$Fe^{3+} + e^- \rightarrow Fe^{2+}$		0.771	0.771	0.771
$\frac{1}{2}FeS_2 + 2H^+ + e^- \rightarrow \frac{1}{2}Fe^{2+} + H_2S$		-0.168	-0.582	-0.996
$\frac{1}{2}SO_4^{2-} + H^+ + e^- \rightarrow \frac{1}{2}SO_3^{2-} + \frac{1}{2}H_2O$		-0.108	-0.315	-0.522

^a Assumptions for Tables 1 and 2: Fe^{3+} , Fe^{2+} , SO_4^{2-} , SO_3^{2-} , NO_3^- , NO_2^- , CO_2 , CO , $HCOOH$, $HCHO$, and H_3COH are present at 1 M; H_2O at 55.5 M; FeS_2 and S^0 are pure solids; and the temperature was 25°C. Carbon at the oxidation level of formaldehyde is indicated by $HCHO$: this corresponds to $\frac{1}{6}$ of that of glucose. Values are computed from the free energies given in Weast (1986)

^b Voltages given are for the standard hydrogen reference electrode: pH 0; H_2 pressure, 1 atmosphere (Weast 1986)

^c H_2 pressures (in atmospheres) were chosen to agree with various hypothetical atmospheres of a primitive Earth considered by DesMarais and Chang (1983)

Table 2. Candidate half-cell reactions for early energy production: intracellular reductions^a

Reactions	E' volts ^b		
	pH 0	pH 3.5	pH 7
$\frac{1}{2}CO_2 + \frac{1}{2}H^+ + e^- \rightarrow \frac{1}{2}HCOO^-$	-0.181	-0.285	-0.388
$\frac{1}{2}CO_2 + H^+ + e^- \rightarrow \frac{1}{2}CO + \frac{1}{2}H_2O$	-0.150	-0.357	-0.564
$\frac{1}{2}SO_4^{2-} + H^+ + e^- \rightarrow \frac{1}{2}SO_3^{2-} + \frac{1}{2}H_2O$	-0.108	-0.315	-0.522
$\frac{1}{2}CO_2 + H^+ + e^- \rightarrow \frac{1}{2}HCOOH$	-0.071	-0.278	-0.485
$H^+ + e^- \rightarrow \frac{1}{2}H_2$	0.000	-0.207	-0.414
$\frac{1}{2}(HCHO) + H^+ + e^- \rightarrow \frac{1}{2}CH_3OH$	0.123	-0.084	-0.291
$\frac{1}{2}CO^+ + H^+ + e^- \rightarrow \frac{1}{2}(HCHO)$	0.165	-0.042	-0.249
$Fe^{3+} + e^- \rightarrow Fe^{2+}$	0.771	0.771	0.771
$\frac{1}{2}NO_3^- + H^+ + e^- \rightarrow \frac{1}{2}NO_2^- + \frac{1}{2}H_2O$	0.819	0.612	0.405

^a Assumptions: see Table 1

^b Free energies are from Weast (1986)

ucts and the standard free energy of the reaction is exergonic. In addition, for a model in which the coupling depends on charge separation across a membrane, the process needs to be decomposable into two reactions, one creating the elements of H_2 , i.e., two $[H^+]$ or two protons and two electrons, and one consuming them. Some electrochemical processes to be considered in accessing potential sources of reducing power for the outer face are listed in Table 1, and some electrochemical reactions as potential sinks for the reducing power on the inner face are listed in Table 2. The half-cell potentials are

presented in the standard chemical convention for a one-electron process. This means that an equation from each table must be subtracted to write a balanced equation for the entire process. The voltage that such a battery could produce is calculated by subtracting the corresponding half-cell potentials of Table 1 from Table 2. To consider the two-electron process, the chemical equation must be multiplied by two, the differences in the half-cell potentials when multiplied by 2×96.48 yields the free energy, $\Delta G'$ in kJ/mol and by 2×23.0614 to yield $\Delta G'$ in kcal/mol.

Potential Electron Sources in a Recently Cooled Planet

For a viable bioenergetic process the organism must exploit environmentally available resources. The reactants should be abundant and the products diffuse such that under ambient conditions the standard free energy of reaction is negative. In addition, some of the reactants must be capable of penetrating the membrane of the cell. In electrical terms, the donor should have a low potential and the acceptor a higher one.

In Table 1 we list some reactions yielding reducing power. Hydrogen is perhaps a viable possibility, particularly when present in high concentrations as suggested by some scenarios of atmospheric evolution (Chang et al. 1983). Because a proton is produced in the electrode reaction, the potential is more negative at higher pH values. H_2S and SO_3^{2-} are potentially good donors, but we find no speculation in the literature as to their abundance and supply. A ferrous-ferrocyanide couple has a potential that is much too high, but a combination of H_2S and Fe^{2+} has a very low potential. The reaction has a high pH dependence because two protons are produced. The reaction is pulled because of the insolubility of FeS_2 . This was pointed out by Wächtershäuser (1988a,b) and we will presume this reaction to be the source of the reducing power in much of the remainder of this paper.

Potential Oxidant as Sinks for Electrons in a Recently Cooled Planet

Some potential oxidizing agents are listed in Table 2. Some of these are straw men as the charged species such as sulfate, ferric, or nitrate ion could not easily get inside the cell, nor could their reduced products escape. Therefore the restricted list contains reactions involving the oxides of carbon. These will be discussed before returning to still other possibilities.

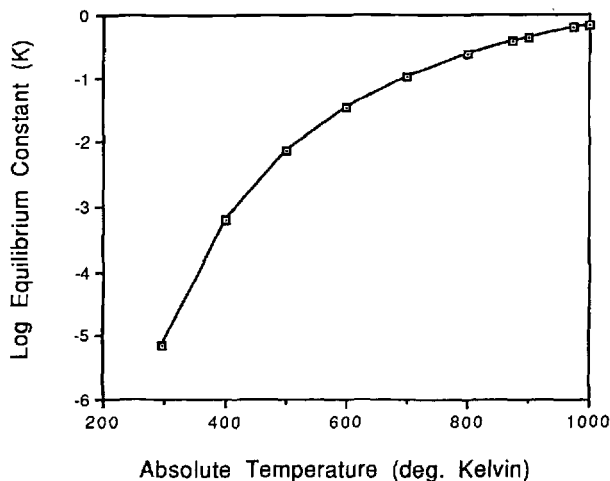
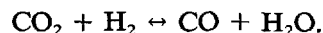


Fig. 3. Thermodynamics of the water-gas shift reaction.

There are numerous conjectures concerning the early environment on this planet (see Chang et al. 1983). There is also much conjecture about whether life of the kind we have on Earth originated here or elsewhere. But barring divine intervention, we can be sure that our type of life had to originate on a planet that had progressed to the point that it had liquid water, a nonoxidizing atmosphere, and a chemistry that could allow trapping of free energy, i.e., in the potential biosphere, it had not substantially reached chemical equilibrium in some important regard. It is not speculation that at this stage on this particular planet 3.8 Ga ago there was a great deal of CO_2 in the atmosphere. With slightly less confidence, we can believe that there would have been large amounts of both CO_2 and CO (Kasting 1989).

It is believed that at an even earlier stage of this planet, the atmosphere contained many atmospheres of gaseous water and CO_2 along with significant amounts of CO and H_2 (Chang et al. 1983). Certainly at high temperature the proportions would be largely governed by the water-gas shift (WGS) reaction:



The equilibrium constant for this reaction is shown as a function of temperature in Fig. 3. In many of the candidate carbon-rich atmospheres considered by Chang et al. (1983) the WGS reaction was assumed to come to equilibrium while the surface temperature was too high for liquid water to exist. As the temperature cooled, the WGS reaction will tend to be displaced to the left. Consequently, in principle, energy is available from the mixture equilibrated at the higher temperature after being chilled to a lower temperature into a nonequilibrium mixture. However, this change makes CO_2 a poorer sink

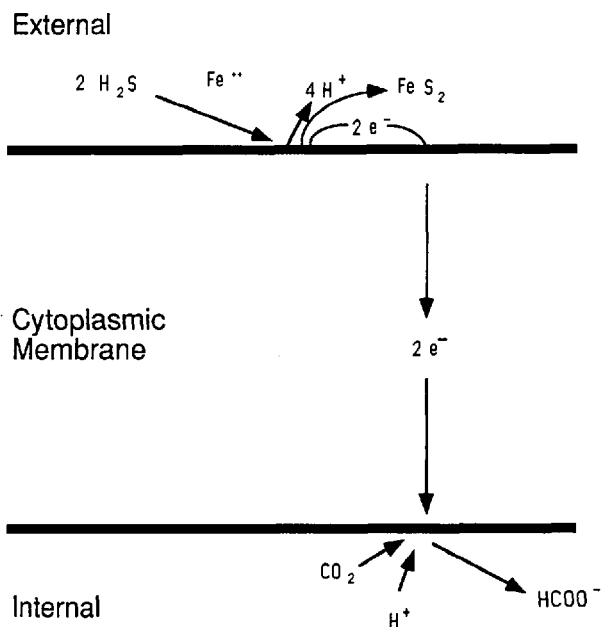


Fig. 4. A probable energy generation mechanism for early life. The extracytoplasmic reaction is the formation of iron pyrites from ferrous ion and hydrogen sulfide. The reaction on the cytoplasmic membrane is the reduction of carbon dioxide and the formation of formate ion.

for electrons. But it may have enriched the environment in CO that in turn can be an electron acceptor for the production of products even more reduced.

In assessing the possible reactions, it is necessary to take into account the concentration of reactants in the aqueous environment. Compared to the gaseous WGS reaction, condensation of water with lowering surface temperatures will favor the rightward reaction, whereas association with the aqueous phase of CO or CO₂ will shift the equilibrium to the left or right, respectively. In some thermodynamic calculations given below, we shall assume that the atmosphere was originally the source of reactants even though the primitive organism dwelt in an aqueous environment and that the aqueous phase had dissolved an equilibrium amount of CO and CO₂. It would be the concentrations in the gas phase that would buffer the environment and therefore determine the overall free energy of the process. Consequently, arguments based on the thermodynamics of the gaseous reactions are appropriate for at least the present purposes.

Other potential electron acceptors are few. We considered and rejected many of the redox reactions carried out by modern chemolithotrophs. To begin with, many of the substrates, which are now produced by other biological systems, would not have been available. Some may have been created due to geological processes such as oxidized sulfur and nitrogen of volcanic origin in which case, the distri-

bution of environments with both donors and acceptors in adequate concentrations may have been spotty. We also rejected the possibility of the reduction of phosphorus at +6 to some lower oxidation states.

Because biomass is poised more nearly to the level of formaldehyde than to any other oxidation state of carbon, reduction to this level is suggested. Carbon at this redox level could have arisen abiotically, or by reductions that generated a proton-motive force and produced formaldehyde or substances at its oxidation-reduction level as depicted in Fig. 4. We designate such material with the expression HCHO and have used $\frac{1}{6}$ of the molar properties of glucose in thermodynamic calculations (i.e., the ΔG for HCHO was taken to be 31.98 kJ/mol). For a living system, the availability of carbon at intermediate levels of reduction is important for making most cellular carbon compounds. Thus reducing CO has additional advantages that will not be considered further in this paper. The point of this paper is that an energy currency that can be spent on driving chemical reactions of a quite different nature was needed and that the proton-motive force was the most primitive system of biochemical 'barter.'

Free Energy for the Coupled Process

Because the reaction proposed by Wächtershäuser in which H₂S plus ferrous iron yields molecular hydrogen has a low half-cell potential, we use that reaction for more detailed calculations. It is more pH-dependent than the other reactions listed in Table 1 because it produces two protons as written. Consequently, it is a less favorable choice for more acidic environments. Figure 5 shows the energetics of coupling of the Wächtershäuser reaction with the reduction of carbon oxides in the acidic range as a function of pH for a number of processes that could serve as the coupled reaction consuming the reductant. In these calculations, we have taken the states of the reactants to be 1 M aqueous solutions for Fe²⁺, Fe³⁺, CO, CO₂, carbonic acid, formic acid, and H₂, and taken the standard state of water to be that of the pure liquid. Carbonic acid and formic acid correspond to the mixture of unionized and ionized species present at a specified pH. We have considered that the hydration of CO to formic acid may be slow enough so that CO₂ should be considered separately from formic acid. The free energy difference between these two assumptions is quite small; the $\Delta G'$ is 26.26 kJ/mol for HCHO and is 27 kJ/mol for gaseous HCHO.

The standard free energy values must be corrected for the ambient concentrations for the class

of mechanisms under consideration. Except when molecular hydrogen is the donor or is formed, the ambient partial pressure of hydrogen is irrelevant because the reactions neither produce nor consume protons and electrons.

The Action of Carbon Monoxide Dehydrogenase

A biological version of the WGS reaction is one component of the possible energy coupling process. It is, in fact, not the most favorable choice for the reduction reaction, yet we will outline its use because the concentration terms are highly favorable and we can imagine this process as taking place in a world without well-developed enzyme systems. Figure 4 shows an explicit model for the way that a proton-motive force would be generated from H_2S , Fe^{2+} , and CO_2 . Electrons generated from the external reaction are shunted through the primitive cell membrane and liberated protons appear outside the cell. On the inside of the membrane, protons are consumed during CO_2 reduction.

For carbon reduction, we imagine that a reaction functions akin to that carried out by the enzyme, carbon monoxide dehydrogenase. CO dehydrogenase serves in the reductive acetyl-CoA pathway for autotrophic CO_2 fixation in both eubacteria and archaeobacteria (Fuchs 1989). The enzyme reduces CO_2 to CO and also functions in an enzyme complex that can directly incorporate the carbon of CO into the carboxyl carbon of acetate. The modern-day version of the enzyme is membrane-linked and uses nickel as a cofactor. We imagine that a primitive version of the enzyme functioned without a protein component, and that protein involvement occurred during subsequent phases of evolution.

Development of Asymmetry

In modern-day organisms there is a biologically imposed asymmetry due to the asymmetric orientation of intrinsic membrane proteins and the secretion of periplasmic proteins through the cytoplasmic membrane. In the primitive system, stereospecific orientation was possible if certain charged reactants had access only from the outside of the cell. In this case, the inorganic analogue of carbon monoxide dehydrogenase might have been symmetrically located, but the system could still function because the substances producing the hydrogen equivalents would be restricted to the outside of the cell due to the slow or negligible rate of penetration of ferrous ion through the membrane. Any reduction on the outer face of the cell would represent lost energy, but some could still be trapped internally. Conse-

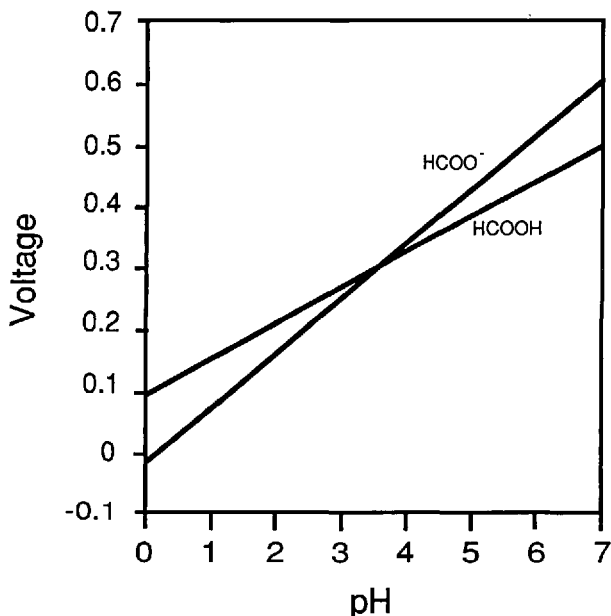


Fig. 5. The voltage produced by a proton-motive force generated by the scheme shown in Fig. 4. Each line corresponds to the reaction of HCOO^- and HCOOH in their standard state of 1 M. Because of dissociation, a solution 1 M in the sum of HCOO^- and HCOOH switches from the HCOOH curve at low pH to the HCOO^- curve at high pH.

quently, although the gaseous reactants and product could penetrate the membrane, a net proton-motive force could still develop on the outside of the cell positive with respect to the inside.

Iron-Sulfur Complexes

In membrane-enclosed cells, a pathway for electron flow across a lipid membrane is required. This transfer might originally have been made by iron-sulfur complexes; it is in almost all cases in modern organisms. It is known that mixtures of iron and sulfur compounds will form an iron-sulfur complex that has similarities to the reaction centers of today's iron-sulfur proteins (Berg and Holm 1982). The ubiquity of iron-sulfur proteins in virtually all modern-day electron transport chains argues for an early and pivotal role for the nonprotein iron-sulfur complex in energy transduction. These complexes may later have acquired a protein component and become the precursor of today's ferredoxins.

In the iron-sulfur proteins and their synthetic analogues, each iron atom is covalently linked to four sulfur atoms. A cluster may contain from one to four Fe atoms and a given protein may have several Fe-S clusters. The 4Fe-4S cluster is a cubane structure with the four Fe and four inorganic S atoms at alternating corners of a cube. In this structure, the Fe atoms are also combined with a sulfur compound protruding away from the cube. These can

be either inorganic, organic, or sulfurs of cysteine residues of specific proteins. Analogues of biological compounds are readily formed and can be engaged in oxidation–reduction processes as do naturally occurring iron–sulfur proteins. These complexes are too small, individually, to span a cell membrane or any imaginable type of bimolecular leaflet surrounding a primitive cell. However, a series of such complexes appears to span the membrane involved in photosynthesis (Evans 1982) or in oxidative phosphorylation (Ohnishi and Salerno 1982).

Consequently, it appears reasonable to suppose that certain compounds of iron, sulfur, and organically linked sulfur formed abiotically were sufficiently lipid soluble to become part of the early membranes and as a group function to catalyze electron movement. The redox potential for synthetic compounds spans a negative range (0 to -0.6 volt) of half-cell potentials that would be most useful for favoring the ur-biological processes proposed here.

Iron–sulfur compounds are ubiquitous in modern-day organisms, suggesting a major role for these compounds in cellular evolution. In terms of phylogeny, the best studied of the iron–sulfur protein are the ferredoxins, which are present in organisms from each of the three primary lines of evolutionary descent: the eukaryotes, the archaeobacteria, and the eubacteria. Ferredoxins are characterized in extant organisms by their ability to transfer electrons in redox reactions. Based on the accumulated sequences, the projected ancestral ferredoxin (George et al. 1985) is a simple protein notably deficient in amino acids thought to be evolutionary latecomers.

There are a variety of other iron–sulfur proteins that exist and are generally classified as part or all of an enzyme complex such as sulfite reductase, nitrate reductase, hydrogenase, and cytochrome oxidase. Thus the general picture emerges that the role of iron–sulfur complexes in electron conduction in membranes is important now and may have been important early in cellular evolution.

ATPase

Once the proposed reactions generated a proton gradient energy-consuming life could originate. There was an obvious selective advantage to organisms, in fact, an absolute necessity, of being capable of harvesting that electrochemical gradient and conserving the energy to do work of various kinds. In addition to powering active transport, extant organisms rely exclusively on ion-transporting ATPases to harvest proton gradients for the production of ATP.

Nucleotide sequences of the archaeobacterial and eukaryotic ATPases are 50% similar and obvious

homologs to the eubacterial ATPases (Gogarten et al. 1989). A phylogenetic tree derived from these data confirms the monophyletic divisions of the eukaryotes, eubacteria, and archaeobacteria established using RNAs (Woese 1987). Homologous characteristics found in all kingdoms of life must have been present in the universal ancestor and most likely in the evolutionary pathway leading to the last universal ancestor. Phylogenetic comparison identifies ferredoxins and ATPases as components of an early stage of cell metabolism, present at a time before the divergence of the last universal ancestor into the three kingdom lineages. Modern ATPases are very complex structures with many subunits. In the first living forms some simplified process must have served. One possibility is that the prototype ATPase functioned as a carrier mechanism (Koch 1985). The constraints are that there must be an abiotically formed molecule that is lipid soluble and can react to form a phosphate derivative that in spite of the additional charges when protonated is also lipid soluble. It is to be stressed that its lipid solubility must depend on binding a proton. These conditions are sufficient to provide a system that will actively move phosphate and create a high-energy phosphate bond.

Discussion

Wächtershäuser (1988a) made an important contribution in suggesting iron pyrites formation as a critical part of energy generation system in the first life. However, he imagined explicitly that instead of proton-motive force generation, 2H^+ and 2e^- were directly coupled to the reduction of CO_2 within a complex of the Fe^{2+} , H_2S , and CO_2 . Obviously the process had to be simple and arise naturally without the aid of Darwinian evolution, and in fact, was necessary to make Darwinian evolution possible. We incorporate his idea but modify it to serve as a source of proton-motive force, although we continue to consider other sources of proton-motive power. We do this by inserting our earlier idea (Koch 1985) of a separation in space of the two redox processes to generate a proton-motive force. We postulate that a closed membrane structure is important and that the pyrite-forming reaction or other sources of $2[\text{H}^+]$ and 2e^- take place outside the primitive cell and that the reduction of some abundant material, most probably an oxide of carbon, takes place on the inside of an electron-conducting proton-impermeable closed-membrane. We suggest that electrons are an intermediate, but that molecular hydrogen is not.

Obviously CO_2 reduction in methanogenesis and sulfate reduction as practiced by current organisms are evolutionarily advanced processes; they have

many stages, many cofactors, and many specialized roles for the membrane. We feel, however that they and almost every living organism, in part, do contain remnants of the most primitive energy-coupling process because all generate a proton-motive force by passage of electrons inward. We explore here the possibility that simple reduction of carbon dioxide and/or carbon monoxide are the logical candidates for the oxidant side of the primitive energy generation system even though no known extant organism transduces energy in just this way.

At the end of the Hadean period (3.9 Ga) beside the changes due to surface cooling, hydrogen would be escaping the Earth's gravitational pull. At some point it would no longer be a candidate for the process on the external face of cells. However, once the atmosphere cooled and liquid water became abundant, the reduction of CO₂ and CO with more powerful reductants than H₂ may have become possible in the aqueous environment. At neutral pH, much more energy is available with Fe²⁺ and H₂S as a reductant than with H₂ even at the very highest pressure imaginable on the planet earlier. The reason that the protocell could exploit this type of reaction is that the reduction of carbon oxides by hydrogen in the gas phase is not fast at the lower temperatures. In the aqueous phase, even the more exergonic reaction producing iron pyrites and H₂ is also slow without a catalyst. Thus, H₂S, ferrous ion, and CO₂ or CO could coexist without reaction at the ambient temperature less than 100°C in an aqueous environment in the absence of a catalyst.

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