A Comprehensive Analysis of the Synergistic Properties Between the Cyanophyta, *Aphanizomenon flos-aquae* and Silica Hydride, a Silicate Mineral Supplement

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ABSTRACT

The foundation and core mechanism for the synergistic relationship between the cyanophyta *Aphanizomenon flos-aquae* and silica hydride, a silicate mineral supplement is investigated. Fundamental background, theory and hypothesis is covered for the *Aphanizomenon flos-aquae*, silica hydride, the importance of each and their inherent relationships. Particularly, the concept and action of biophotolysis and emulated dehydrogenase allow a synergistic symbiosis between the two compounds being studied. Six distinct chemical assays, investigating NADH reduction, ATP production, oxygenated hemoglobin production, cytochrome c and porphyrin reduction, oxidized cholesterol analysis and a plasma albumin workup were performed. The results of the assays are appended to this foundational and descriptive work. All of the quantitative indicate a clear and profound significance to the combination of *Aphanizomenon flos-aquae* and silica hydride.

INTRODUCTION

The metabolic nature of *Aphanizomenon flos-aquae* (AFA) and the resultant effect on the human body is quite profound. Naturally harvested AFA provides the essential and core components to provide pure energy for the body. This happens on many levels in the body's metabolic reactions. When utilized in a combinatorial fashion with a mechanistically related compound, silica hydride, a synergistic result occurs. Both of these compounds, AFA and silica hydride function by complimentary and symbiotic methods. To truly comprehend the magnitude of the symbiosis of AFA, silica hydride and the human metabolism, it is important to address the fundamental and core attributes and mechanisms of the compounds and the components that allow them to function.

Importance of hydrogen and the electron

In the plant and animal kingdoms, there is not a more important element than hydrogen [1]. It is the fundamental and primordial element that is responsible for the creation of all other elements. In plant and biological systems, most of the metabolic reactions take place through the use of, or transfer of molecular hydrogen. This use and metabolism of hydrogen produces the core energy utilized by the system. Particularly, the hydrogen metabolic processes produce reduced nicotinamide adenine dinucleotide (NADH) and adenosine 5'-triphosphate (ATP), among other important compounds [2,3].

With all reactions, the mechanism and overall pathway is mediated by the electron. An electron is the minute, negatively charged atomic particle that occupies shells that encompass the central, positively charged nucleus of an atom. The electrons are transferred from element to element to allow reactions to take place [4].

Introduction to the cyanophyta

Cyanophyta are an amazing genre. These algae genus are the only known eukaryotes with both oxygenic photosynthesis and a hydrogen metabolism [5]. Cutting edge research has presented physiological and genetic advancements, indicating a close connection between these metabolic pathways. Hydrogenases, which are enzymes that catalyze the simplest chemical reaction – the reversible reduction of atomic hydrogen to molecular hydrogen (H_2), were discovered in the 1930s [6]. Hydrogen metabolism is found in almost all taxonomic groups of prokaryotes [7], in some anaerobic microbial eukaryotes and in unicellular green algae [8]. It has been observed that many phyta can either oxidize molecular H_2 with a simultaneous CO_2 fixation (photoreduction) [9] or through illumination, produce hydrogen gas [10]. Because of these investigations, photobiological H_2 production by algae has been of great community and scientific interest.

There are numerous types hydrogenases, divided into three separate classes: [NiFe]-hydrogenases, [Fe]-hydrogenases and metal-free hydrogenases [11]. The [Fe]-hydrogenases are significantly differentiated from the others due to its distinct prosthetic group (H-cluster) in the center of the enzyme, which results in a 100-fold higher enzyme activity [12].

The vital role of [Fe]-hydrogenases in algae can be best determined by monitoring anaerobic, or fermentative metabolism. H_2 evolution in algae is identified as a consequence of anaerobiosis [13] or nutrient deprivation [14]. Although they carry out oxygenic, or aerobic photosynthesis, algae can encounter anaerobiosis in natural habitats: lake and sea sediments become anoxic due to incomplete water stirring or the resultant oxygen deprivation during phytal-algal blooms. Otherwise photosynthetic organisms, such as cyanophyta, survive under anaerobic conditions by switching their metabolism to fermentation. In the major pathway of fermentation, sugars are glycolytically degraded to pyruvate and metabolically converted to energy, yielding large quantities of ATP.

Fermentative pathways differ distinctly in light and darkness. In the dark, the responsible enzymes are produced constitutively [15], allowing a rapid response to environmental changes. The Calvin-Benson cycle, which acts as photosynthetic equivalent to the citric acid cycle in eukaryotes, fails to function as an electron sink and requires hydrogenase to facilitate the important role regulating photosynthetically generated electrons and reducing equivalents from fermentation, reoxidized them [16].

Background of Aphanizomenon flos-aquae

AFA, as harvested cyanophyta, is a tremendous resource of biologically essential compounds. AFA is rich in over 20 amino acids, 12 vitamins and 23 minerals, as demonstrated in **Table 1**.

Amino Acid	**Minerals**	**Vitamins**
Alanine	Boron	Ascorbic Acid
Arginine	Calcium	Biotin
Asparagine	Chloride	Choline
Aspartic Acid	Chromium	Cobalamin
Cystine	Cobalt	Folic Acid
Glutamic Acid	Copper	Niacin
Glutamine	Fluoride	Pantothenic Acid
Glycine	Germanium	Pro-Vitamin A Beta Carotene
Histidine	Iodine	Pyridoxine
Isoleucine	Iron	Riboflavin
Leucine	Magnesium	Thiamin
Lysine	Manganese	Vitamin E
Methionine	Molybdenum	
Phenylalanine	Nickel	
Proline	Phosphorus	
Serine	Potassium	
Threonine	Selenium	
Tryptophan	Silicon	
Tyrosine	Sodium	
Valine	Tin	
	Titanium	
	Vanadium	
	Zinc	

Table 1. A list of the main amino acids, minerals and vitamins contained in *Aphanizomenon flos-aquae*.

Recent publications on AFA indicate a significant effect in increasing immunological function, a significant source for polyunsaturated fatty acids, antimutagenic properties and producing increased circulation in cells [17]. Both *in vitro* and *in vivo* studies that have identified pharmacologically active compounds in many species of photosynthetic cellular compounds, including phycocyanin, chlorophyll, carotenoids and essential fatty acids, which offer profound biochemical reactions. Research has indicated that within 2 hours of consuming AFA, there was a 40% increase in the migration of circulating natural killer (NK) cells in healthy volunteers [18]. Natural killer cells scavenge virus-infected and cancerous cells, and function by inducing programmed apoptosis in the transformed cell.

AFA produces compounds having antibacterial [19] activity, and also antimutagenic activity in the Salmonella typhimurium test system [20]. Photosynthetic cellular microorganisms, including AFA, are also excellent sources of polyunsaturated fatty acids [21-23]. Rats on a high cholesterol diet fed the cyanophytan commune at 5% of their diet for 21 days had significantly depressed elevations of serum cholesterol [24].

Overall, the intake of AFA resulted in decreased blood cholesterol and triglycerides in rats. Thus, photosynthetic cellular microorganisms have a wide range of pharmacological effects *in vitro* and *in vivo* tests in various species, including humans.

Several studies have examined the use of whole cyanophyta in the context of the normal functioning immune response. One such study used oral doses of 1.5 grams of AFA on healthy human volunteers revealed it slightly decreases the phagocytic activity of polymorph nucleated cells *in vitro* [25]. This may indicate an anti-inflammatory, rather than anti-phagocytic effect on human neutrophils. Comparatively, in an investigation on the phagocytic function of feline bronchoalveolar macrophages *in vitro*, the percentage of cells that phagocytosed cells increased when they were exposed to a water-soluble extract of spirulina for two hours [26]. The number of particles ingested by the phagocytic macrophages did not change when compared to control cultures.

In a study designed to investigate the mechanism behind the stimulatory effect of the immune system by cyanophyta on the human monocyte/macrophage cell line THP-1, a crude extract of AFA was used to stimulate the cell line. The extract was half as potent as proteins in activating NF-kB, and the purified molecule is ten times more potent than said proteins. The molecule responsible for this activation has been identified as a novel polysaccharide [27].

Thus, multiple studies on whole AFA in humans, mice, rats, cats, and chickens have demonstrated an effect on phagocytosis, NK cell function, and inflammation.

Background of silica hydride

It is notable that with all of the intensive study on compounds ranging from vitamin c to hemoglobin and cytochrome *c*, that even the substrate proteins and catalysts unable to function pumping without the presence of organic hydrogen [1]. Silica hydride is a supplement based on the discovery of the primordial anti-oxidant known as active hydrogen.. Hydrogen and its associated electrons are the fundamental core components of most metabolic processes. Studies of mitochondrial cytochrome complexes have noted that the proton to electron ratios measured in the reaction kinetics were less than unity (<1:1), indicating the significance and relevance of the electron in the transport mechanisms [28].

The oxidative reduction potential, known as redox, is an exceptionally important control factor for the rate and direction of electron travel. Different biological compounds, such as proteins behave differently under specified redox conditions. Redox controls the geometry and coordination of the proteins, cytosolic gradients, localized ionic charge distribution, electron transfer and hydrogen bonding to other compounds [29]. It is hypothesized that the redox control and trigger for these reactions may not be entirely enzymatic, but rather redox controlled through electron rich ions. Previously, the biological transfer of an electron was described as that of a proton plus an electron that are simultaneously carried to the oxidized species. Current research postulates describe this hydride transfer and provides evidence that the physics is not that of a proton and electron, but rather a direct transfer of a hydrogen anion, known as a hydride ion [30]. Reactions ranging from electrolysis to the reduction of nicotinamide adenine dinucleotide to reactive oxygen species neutralization involve the hydride ion [31,32]. Shirahata, *et. al.* have contributed to research on hydrogen ions defining the factors of dissolved oxygen, dissolved hydrogen and redox potential as fundamental variables for the evaluation of the ions. These factors allow an accurate chemical description of the mechanism of the hydrogen ions. A more appropriate rhetoric to describe and characterize the hydrogen ion is that of “active hydrogen”, due, in part to its reactivity and ion activity in a solution.

The early studies on active hydrogen indicate its synthesis through electrolysis and creation of high dissolved hydrogen and negative redox potential which are the result of the production of atomic as well as molecular hydrogen and cathode of the electrode.

The creation of active hydrogen was, until recently, only able to be synthesized in an aqueous environment. A newly introduced solid compound, silica hydride, has been studied extensively for its unique properties to embed active hydrogen into a caged matrix of silica, bentonite, magnesium, carbonate and more [33]. The active hydrogen’s electron structure is not that of a traditional $1s^2$ orbital system, but rather that of a $1s1s'$ orbital structure where the $1s'$ orbital shell is orthogonal to the $1s$ shell and is more loosely held. In this activated state, the hydrogen atom is kinetically and energetically favored to donate its excess electron, reducing its symbiotic constituent, while becoming neutral atomic hydrogen itself [34]. A conceptual illustration of the mechanism of active hydrogen is shown in **Figure 1**:

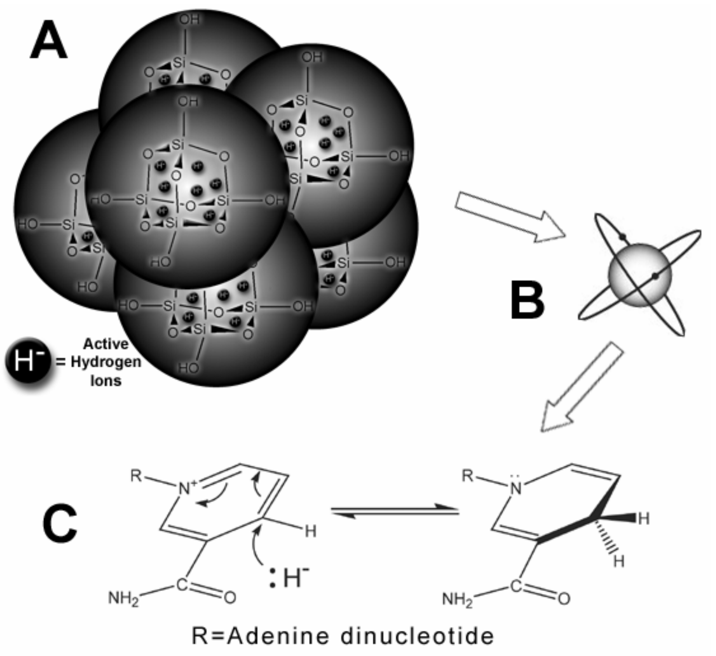

Figure 1. A conceptual representation of the electron donation by active hydrogen. Clusters of active hydrogen (A) release the tiny hydrogen atoms, readily able to donate (B) their excess electron for a direct hydride transfer from the anion to the (C) receptor portion of the nicotinamide compound. This mechanism directly moves the electron and hydrogen together, not as two separate reactions.

The silica hydride active hydrogen compound has demonstrated properties including reducing NAD^+ and neutralizing reactive oxygen species, including hydroxyl radicals, superoxide and singlet oxygen species, *in vitro* [35-37]. The active hydrogen of silica hydride has additionally increased metabolic energy production, doubling the ATP produced *in vitro*, as determined by capillary electrophoresis [38]. *In vivo* clinical studies have indicated abilities of the compound to increase cellular hydration as measured through phase and capacitance, non-segmental bioimpedance analysis [39].

It is important to note and understand that silica hydride and active hydrogen are not the same thing. Active hydrogen, the energetic ingredient in silica hydride, is the technology of harnessing negatively ionized hydrogen atoms. The hydrogen atom normally has one electron (negative charge) and one proton (positive charge). In the case active hydrogen, a special process is used in the manufacturing that allows for the creation of a negatively charged hydrogen ion (two electrons). The way that the electrons are oriented allows the extra electron to be readily donated in chemical and biochemical reactions.

Silica hydride, then, is a blend of active hydrogen ions that have been interstitially embedded into a matrix of Microcluster® Silica. This special form of silica that is approximately 50 angstroms (10^{-10} meter) in diameter. The specially formulated silica acts as an efficient carrier of the active hydrogen ions in the body.

In water, silica hydride creates a colloidal suspension and slowly releases the active hydrogen over time. The negatively charged hydrogen ions are full of energy and react by donating the extra electron to other compounds. This is known as reducing, and is part of the chemical process of redox. Redox chemistry has an integral part in an organism's biochemistry through the activation of enzymatic activity, protein production, cellular signaling and reaction rate control.

In the body, the released active hydrogen redox reactions work to control build up of free radicals and inhibit the cellular damage may occur. The way that the active hydrogen reacts with free radicals is also very similar to how internal energy is made in the mitochondrion of a cell. Further research has offered support and demonstration of the metabolic redox activity in the body, as investigated by the reduced levels of lactic acid production by the body after strenuous exercise [40]. The clinical studies performed indicate that study subjects that take silica hydride had increased cellular hydration and cellular membrane integrity, both signs of a metabolic mechanism of silica hydride.

Studying the combinatorial effects of Aphanizomenon flos-aquae and silica hydride

This work investigates the relationships between the combination of silica hydride and AFA. AFA contains a large portion of phycobiliproteins, which are the major photosynthetic accessory pigments in cyanophyta. They are in association with the outer surface of the photosynthetic lamellae, being constituents of the photosystem-II light harvesting apparatus [41]. Phycobiliproteins are brilliantly colored, water-soluble proteins, bearing covalently attached open chain tetrapyrroles. C-PC1 is the major component of the phycobiliprotein family. It is not only used as nutrient ingredients and natural dyes for food and cosmetics [42,43] but also used as potential therapeutic agent in oxidative stress-induced diseases [44-46], and as fluorescent markers in biomedical research [47]. Phycobiliproteins are soluble in water, therefore they can be easily isolated as protein pigment complexes.

AFA is additionally Chlorophyll (Chl) carrying and plays a pivotal role in aerobic photosynthetic organisms such as cyanophyta. Chl is a primary electron donor in the reaction centers of photosystem-I and photosystem-II (PSI and PSII, respectively) of the photosynthesis system. Moreover, Chl constitutes also the primary electron acceptor in a reaction center. A Chl derivative—pheophytin —is the primary electron acceptor in the reaction center for photosystem-II. Most Chls in both PSI and PSII, however, act as light-harvesting (antenna) pigments, supplying the reaction centers with sufficient excitation energy to maintain a high rate of photosynthesis.

Also AFA has a major constituent known as phycocyanin (Pc). Pc, when it was evaluated as an antioxidant *in vitro* was able to scavenge alkoxyl, hydroxyl and peroxyl radicals and to react with peroxinitrite and hypochlorous acid. Pc also inhibits microsomal lipid peroxidation by Fe $^{2+}$-ascorbic acid or the free radical initiator 2,2' azobis (2-amidinopropane) hydrochloride. Pc administered in a range of doses of 100–300 mg/kg 30 min before the agent which induces the injury. Pc exerted anti-inflammatory effects in a dose-dependent fashion in all of these. Thus, Pc reduced edema, histamine release, myeloperoxidase activity and the levels of prostaglandin and leukotriene in the inflamed tissues. These anti-inflammatory effects of Pc can be due to its scavenging properties towards oxygen reactive species and its inhibitory effects on cyclooxygenase 2 activity from mast cells. Taking into account that Pc is a major constituent of microphyta AFA (20% of algae dry weight), it might exert therapeutic effects when it is administered alone or included in the microalgae used as dietary supplement.

The combination of these factors, in conjunction with the mechanism of silica hydride and its electron-rich nature, the synergy of these two compounds have the potential to produce a phenomenon known as biophotolysis.

Since hydrogen has the highest gravimetric energy density of any known fuel and is compatible with electrochemical and combustion processes for energy conversion without producing carbon-based emissions that contribute to environmental pollution and climate change, hydrogen is the perfect biological energy source. Biological systems provide a wide range of approaches to generate hydrogen, and include direct biophotolysis, indirect biophotolysis, photo-fermentations, and dark-fermentation [48].

Photosynthetic production of hydrogen from water is a biological process that can convert sunlight into useful, stored chemical energy by either a direct or indirect biophotolysis, following the following general reaction for a direct conversion:

$$\mathbf{2\ H_2O + electron\ energy \rightarrow 2\ H_2 + O_2 + electron\ energy \rightarrow ATP}$$

AFA, under anaerobic conditions, can either use H_2 as an electron source in the CO_2-fixation process or evolve H_2. Hydrogen production by microalgae may take anywhere from several minutest o a few hours of anaerobic incubation in the dark to induce the synthesis and/or activation of enzymes involved in H_2 metabolism, including a reversible hydrogenase enzyme. The hydrogenase combines the hydrogen in the medium with electrons (generally donated by reduced ferredoxin) to form and release H_2. Thus, microalgae possess the genetic, enzymatic, metabolic, and electron-transport machinery to photoproduce H_2 gas. The synthesis of H_2 permits sustained electron flow through the electron-transport chain, which promotes synthesis of ATP [49]. The process of algal photosynthesis oxidizes H_2O and evolves O_2. Light energy absorbed by PSII generates electrons which are transferred to ferredoxin, using light energy absorbed by PSI. A reversible hydrogenase accepts electrons directly from the reduced ferredoxin to generate H_2 [50]. Because the hydrogenase enzyme responsible for evolution of molecular H_2 is highly sensitive to O_2, photosynthetic production of H_2 and O_2 must be temporally and/or spatially separated.

Cyanophyta can also synthesize and evolve H2 through photosynthesis via the following processes:

$$\mathbf{12\ H_2O + 6\ CO_2 + \text{electron energy} \rightarrow C_6H_{12}O_6 + 6\ O_2 + \text{electron energy} \rightarrow ATP}$$

$$\mathbf{C_6H_{12}O_6 + 12\ H_2O + \text{energy} \rightarrow 12\ H_2 + 6\ CO_2 + \text{electron energy} \rightarrow ATP}$$

AFA is part of a greater classification of cyanophyta, which are a large and diverse group of photoautotrophic microorganisms, which evolved and diversified early in Earth’s history [51]. Cyanophyta contain photosynthetic pigments, such as chl, carotenoids, and phycobiliproteins and can perform oxygenic photosynthesis. Species of cyanophyta possess several enzymes directly involved in hydrogen metabolism and synthesis of molecular H_2. [52].

Hydrogen production by cyanophyta has been studied for over three decades and has revealed that efficient photoconversion of H_2O to H_2 is influenced by many factors [53]. The structure and nature of the silica hydride provides the energy via the donation of the extra electron to autoimplement the biophotolysis process in AFA and produces a synergistic effect of energy production. The multifaceted nature of the hydride ion also mimics the hydrogenases normally required for the AFA to manufacture NADH and ATP as energy. Once oxidized and spent, these compounds are recycled by the electron donation in the silica hydride to reduce the NAD+ (oxidized) and ADP (oxidized) to the NADH and ATP reduced forms. This resurgence of the reduced metabolites can be used to further propagate the cyclic chain reaction to allow the AFA to produce even more energy, which again in turn is reduced by the silica hydride.

CONCLUSIONS

Biophotolysis reactions creating hydrogen and oxygen in solution in the AFA compound. Silica hydride reconstitutes this mechanism driving the reaction further. The combination of the two compounds, AFA and silica hydride create a synergistic and symbiotic relationship that results in the direct production of ATP and NAD through the mechanism. This energy production is a perfect example where the total energy produced is greater than the sum of the two individual components. Additional experimentation has clearly and statistically indicated a decrease in oxidized cholesterol of AFA/silica hydride treated aliquots, also inhibiting the oxidation and autooxidation of cholesterol. Increased plasma protein production (particularly serum albumin) and increased concentrations of reduced-porphyrin protein (cytochrome *c*) were additionally observed. See Appendix A-F for experimental details and results.

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