A Possible Prebiotic Formation of Ammonia from Dinitrogen on Iron Sulfide Surfaces**

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Dedicated to Professor
Wolfgang Beck and Professor Wolfgang Seidel

How the formation of ammonia from dinitrogen occurs within the theory of the chemautotrophic origin of life under reducing conditions is a hitherto unanswered question. It has been argued that the biochemical process of ammonia formation, from dinitrogen, by iron–sulfur enzymes may be traced back to a prebiotic "pyrite-pulled" nitrogen fixation.[1]

Nitrogen-fixing bacteria convert molecular nitrogen (N_2) into ammonia (NH_3) at a rate of about 1.7×10^8 tons per year.[2] This reaction is catalyzed by nitrogenase enzymes, which operate under rather mild conditions at atmospheric pressure (10^5 Pa) and ambient temperature (273–323 K). The details of this reaction are largely unknown and are currently being discussed.[3] Recently an improved high-resolution X-ray structure analysis of the nitrogenase FeMo protein from *Azotobacter vinelandii* was reported.[4] The FeMo protein contains the FeMo cofactor, which comprises two cubanelike building blocks [4Fe:3S] and [Mo:3Fe:3S] linked by sulfide ligands and clearly accommodating a light atom, possibly nitrogen, in an interstitial void. Thus, the ubiquitous and abundant elements Fe and S play an important role in nitrogen fixation. They also form the central components of the much-discussed theory of a chemoautotrophic origin of life in a primordial iron–sulfur world.[1,5]

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Herein we describe a method for the synthesis of NH_3 from N_2 using H_2S as a reductant and freshly precipitated iron sulfide as a mediator, which could have served as a primordial inorganic substitute for the enzyme nitrogenase. The reductant as well as the reaction conditions (atmospheric nitrogen pressure and temperatures of the order of 70–80°C) are rather mild and comparable to biological processes. The driving forces of the overall reaction are the oxidation of iron sulfide to iron disulfide and the formation of hydrogen from H_2S.[6]

Hazen et al. reported a nitrogen reduction at temperatures between 300 and 800°C and pressures of 0.1 to 0.4 GPa.[7] The molecular hydrogen necessary for this reduction was obtained from H_2O by using the magnetite/formic acid/metallic iron system as a reductant. Hazen et al. consider this a typical reaction, which could have taken place under conditions prevailing on the primordial earth.

In addition to the remarkable work done by biological scientists,[8] several models have been developed to study and understand how nitrogenase could work. These models are often based on low-molecular-weight compounds, such as metal complexes and metal hydroxide compounds. Important studies on the fixation of N_2 have been carried out by Shilov and Bazhenova:[9] Aqueous suspensions of Mo^{III}, Ti^{II}, V^{II}, Mn^{II}, and Ta^{III} hydroxides have been reported to reduce N_2 at pressures of approximately 10^7 Pa. Significant yields could be obtained from mixtures of V^{II} and Mg^{II} hydroxides. However, iron hydroxides did not reduce N_2 under the conditions chosen. The highest reaction rates were reported by Folkesson and Larsson for a catecholate V^{II} complex in methanol under a pressure of 1.5×10^6 Pa.[10]

Numerous experiments to reduce N_2 in nonaqueous environments have been made[11] since the pioneering work of Chatt et al.[12] A stepwise reduction of an end-on coordinated dinitrogen molecule via diazenido and hydrazido intermediates has been described.[13] Sellmann et al. synthesized a diiron diazene complex containing two pentadentate NS_4 ligands and characterized it by X-ray structure determination.[14–17] Diazene is considered to be an intermediate in the nitrogen-fixation process. This compound enabled Sellmann et al. to develop an alternative ammonia-formation model from N_2 and H_2 in the coordination sphere of metal–sulfur complexes.[15] Coucouvanis et al. reported structural analogues for the $MoFe_3S_3$ subunit in the nitrogenase cofactor and they discuss the accommodation and activation of N_2 by insertion into an Fe–Fe bond.[18]

Our approach uses the moderate reduction potential according to reaction (1) in the synthesis of ammonia by the following overall reaction (2; Scheme 1).[19] Thermodynamic calculations show that the redox system $FeS–H_2S/FeS_2$ is able to reduce dissolved molecular nitrogen to ammonia.[20]

$$FeS_{(s)} + H_2S_{(aq)} \longrightarrow FeS_{2(s)} + H_{2(g)} \qquad (1)$$

$$\Delta G° = -38.6\ \text{kJ/mol},\ E° = -600\text{mV},\ \text{pH} = 6.5$$

$$N_{2(g)} + 3\ FeS + 3\ H_2S_{(aq)} \longrightarrow 3\ FeS_{2(s)} + 2\ NH_{3(g)} \qquad (2)$$

Scheme 1. Synthesis of ammonia using iron sulfide and hydrogen sulfide.

To our knowledge, no successful attempts for the reduction of molecular nitrogen to ammonia in aqueous solutions at ambient pressure, with temperatures below 90°C, and in the presence of iron sulfide have been reported.[20]

The activation energy of molecular nitrogen is high. The activation barrier can be reduced if N_2 is bound on a catalytically active surface. Thus, specific surface features of fresh iron sulfide are crucial for the N_2-fixing behavior. Commercially purchased or aged iron sulfide did not show any N_2-fixing activity. The surface of freshly precipitated iron sulfide can be regarded as a library of different Fe–S clusters, some of which are seemingly well tailored for the fixation and reduction of N_2. It may be assumed that molecular nitrogen is chemisorbed at the surface and subsequently protonated in a stepwise manner under acidic conditions. To obtain a sufficient amount of ammonia for quantification the reaction was carried out on a large-scale (Figure 1).

An iron sulfide suspension was prepared by the reaction of $FeSO_4$ with Na_2S in water at room temperature under argon. Under the scanning electron microscope the iron sulfide obtained revealed a highly rugged surface. The pH value was lowered to 3–4 by adding diluted H_2SO_4, which resulted in production of H_2S. Subsequently, highly purified N_2 was bubbled through the suspension for a period of two weeks using a flow rate of $0.5\ L\,h^{-1}$.

To retain or decompose all the nitrogen-containing contaminations (e.g. NO, NO_2, N_2O, NH_3), the N_2 used was purified in a four-step procedure (Figure 1): 1) N_2-gaspurifier, 2) $KMnO_4/H_2SO_4$, 3) 10 M KOH-solution, and 4) 0.1 N H_2SO_4 solution. The ammonia formed was released by adding NaOH and then transferred to a diluted high-purity H_2SO_4 solution using a flow of argon ($5\ L\,h^{-1}$). The argon was purified prior to use by the same four-step procedure used for nitrogen. Ammonia was detected using a commercially available quantitative color test (MERCK). Ammonia formation proved to be reliably reproducible. The oxidation of sulfide to disulfide led to the formation of iron disulfide (pyrite) which was detected in the X-ray powder diffraction pattern.

Blank tests revealed that there was no appreciable ammonia formation without the presence of iron sulfide. Very low amounts of ammonia could be detected when using iron sulfide in an argon gas flow instead of a nitrogen gas flow. These amounts were significantly smaller ($< 10\%$) than in the reaction with N_2.

Final proof for ammonia formation from dinitrogen was obtained by carrying out the ammonia synthesis with ^{15}N labeled N_2, ($\delta^{15}N = 138‰$).[21] Isotope ratio mass spectrometry (IRMS) showed significant enrichment of ^{15}N in the synthesized $(NH_4)_2SO_4$.[22–24] An enrichment of up to 61‰ over the natural $^{15}N/^{14}N$ isotope ratio was detected. This result exceeds by a factor of 10 any possible enrichment from variation in the natural $^{15}N/^{14}N$ isotope distribution.

An ammonia yield of the order of 0.1% (3 mmol), based on 3 mol of iron sulfide, was found to be typical. To estimate the yield of ammonia relative to dinitrogen appears to be without merit, since for experimental reasons, the reaction with dinitrogen could not be carried out in a closed system. Studies upon reactivity changes of the system as a function of time are in progress. Considering the long reaction times, as

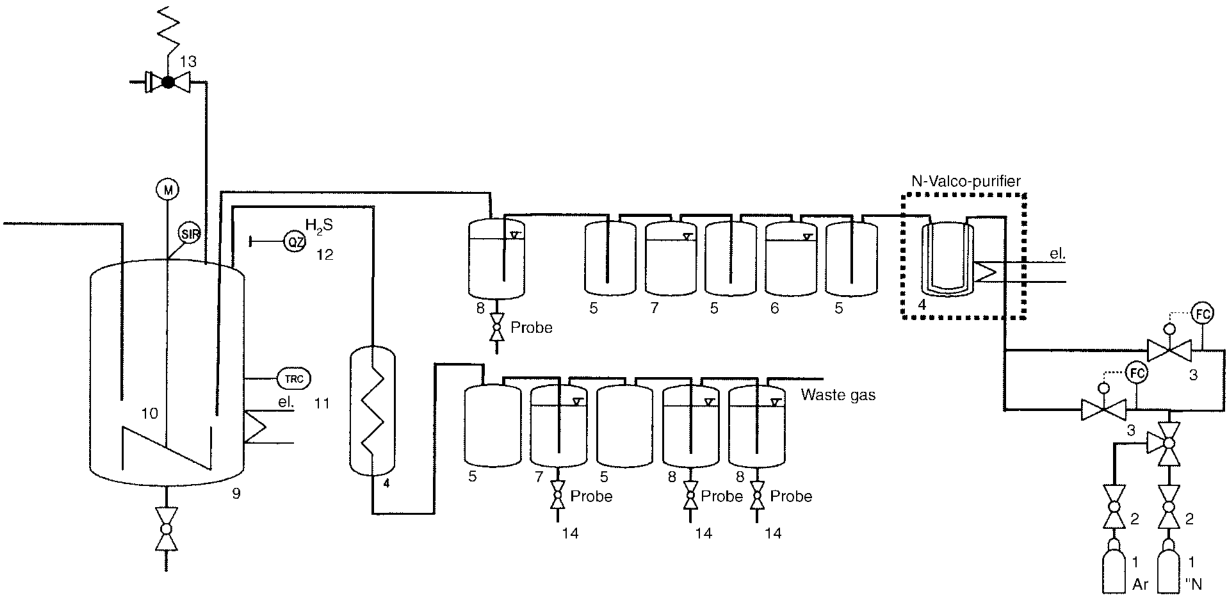

Figure 1. Flow scheme of pilot-plant size ammonia apparatus. 1) gas cylinders, 2) pressure control, 3) mass-flow controller, 4) N_2 gaspurifier (Valco Instr. Inc.), 5) security bottles, 6) oxidizing wash: acidic $KMnO_4$ solution, 7) alkaline wash: KOH, 8) acidic wash:0.001 N H_2SO_4, 9) 15-L reactor, 10) stirrer, 11) temperature control, 12) H_2S sensor, 13) safety valve, 14) NH_4^+ ion test.

well as the large amounts of iron sulfide and H_2S available on the primordial earth, a synthesis of ammonia similar to the one we describe herein could have contributed significantly to the ammonia budget on the early Earth. Hence, our synthesis of ammonia may serve as a model for a primordial nitrogen-fixing system and it conforms well with theories of chemo-autotrophic origin of life.

Experimental Section

Iron(II) sulfate heptahydrate (834.1 g, 3 mol) and sodium sulfide nonahydrate (720.6 g, 3 mol) each were dissolved in 3 L of O_2-free water at room temperature. O_2-free water was obtained by heating water to 80°C for 24 h while bubbling through high-purity argon. Both solutions were mixed at room temperature, followed by heating to 80°C. To remove all nitrogen-containing compounds, the pH of the black iron sulfide suspension was raised to 13 using NaOH (10 M) and then heated for eight days to 80°C while bubbling highly purified argon ($5\,L\,h^{-1}$) through the suspension. Subsequently, the pH value was lowered to 4 by mixing the suspension with diluted H_2SO_4. Highly purified molecular nitrogen was then bubbled through the suspension ($0.5\,L\,h^{-1}$) for seven days. The possible loss of H_2S was prevented by the steady formation of H_2S from FeS in the acidic solution, the pH of which was maintained between 3 and 4 over the entire reaction time. Finally, the pH value was raised to 13 with a solution of NaOH in water. Ammonia was released by passing argon through the suspension, and the ammonia was then captured in a solution of H_2SO_4 (0.001 N). The water was removed by evaporation. The resulting ammonium sulfate was directly suitable for the IRMS experiments, which were carried out on a Delta plus (XL) mass spectrometer (Finnigan MAT, Bremen). The powder-diffraction patterns were recorded using $Fe_{K\alpha}$ radiation to minimize the effects of X-ray fluorescence.

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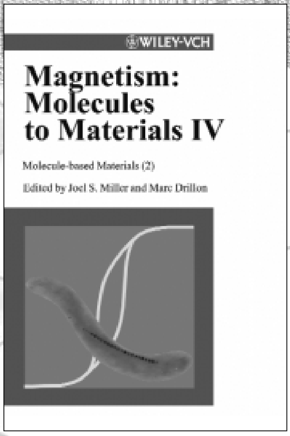

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