Ammonia from Iron(II) Reduction of Nitrite and the Strecker Synthesis: Do Iron(II) and Cyanide Interfere with Each Other?

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ABSTRACT.

The question of whether the production of ammonia, from the reduction of nitrite by iron(II), is compatible with its use in the Strecker synthesis of amino acids, or whether the iron and the cyanide needed for the Strecker synthesis interfere with each other, is addressed. Results show that the presence of iron(II) appears to have little, or no, affect on the Strecker synthesis. The presence of cyanide does interfere with reduction of nitrite, but the reduction proceeds at cyanide/iron ratios of less than 4:1. At ratios of about 2:1 and less there is only a small effect. The two reactions can be combined to proceed in each other's presence, forming glycine from nitrite, Fe^{+2}, formaldehyde, and cyanide.

INTRODUCTION.

Life is likely to have arisen from a number of separate processes, each of which is interlinked with the next (similar to natural systems today). Species from the environment, such as carbon dioxide and nitrogen, needed to be fixed into forms that were suitably reactive and reduced in order to take part in proto- biochemical reactions. Compounds also needed to be combined to form more complex species necessary for proto-cellular structure, information storage/transference, etc.. Finally, all this needed to be combined to form a "proto-metabolism" for the first organism.

To make the study of the origin of life tractable, these problems are often broken down into a series of steps, each leading to the next, which are then studied independently. However, the idea that, in general, reactions had to occur in the same place and under the same conditions is one that needs to be remembered (ie. the origin of life is a "one-pot synthesis"). This brings up questions... How will these reactions interact with each other? Do they interfere with each other? Can they occur under the same (prebiotically plausible) conditions?

The problem we address concerns the fixation, under a neutral atmosphere (CO_2, N_2), of nitrogen and carbon to compounds such as amino acids. In a strongly reducing atmosphere, compounds essential to the chemical evolution of life can be formed by reactions between HCN, NH_3, and carbonyl compounds produced in spark discharges, as well as by other Miller-Urey processes (Stribling et. al.; Schopf). However, geochemical evidence now seems to point to a non-reducing early atmosphere (Walker, 1985; Mattioli et. al.; Gregor et. al.; Kasting).

One important source of ammonia under such conditions is the reduction of nitrite and nitrate to ammonia by Fe^{+2}, equation 1 (Summers et. al.). An important use for this ammonia is as a starting material for the Strecker synthesis, equation 2 (March; Chang) Cyanide and formaldehyde can be produced by ultraviolet photolysis of gas mixtures having compositions within the limits proposed for a nonreducing primitive terrestrial atmosphere (Bar-Nun et. al.; Zahnle; Wen et. al.). Nitrite can be formed from NO produced by shock heating of a carbon dioxide/nitrogen atmosphere (Mancinelli et. al.).

$$6\ Fe^{+2} + 7\ H_2O + NO_2^- \rightarrow 3Fe_2O_3 + 11\ H^+ + NH_3 \qquad (1)$$

$$H_2CO + HCN + NH_3 \rightarrow CH_2(NH_2)(CO_2H) \qquad (2)$$

However, as discussed above, both reactions need to occur together. What effect will the presence of Fe(II) have on the Strecker synthesis? What effect will CN^- have on the reduction of NO_2^- to NH_3 by Fe(II)? Will complexation of the cyanide to the iron deactivate one or the other (or both)? Will both of the reactions proceed under the same conditions? Conversely, could a $Fe(CN)_n^{+(2-n)}$ type species provide some beneficial effect, either for the reduction of nitrite/nitrate or for the formation of amino acids?

RESULTS AND DISCUSSION.

When solutions of Fe^{+2} and CN^- are mixed, a red-orange $Fe_x(CN)_y$ precipitate (Sharpe, 1976) is formed. This brings up the question, does the formation of such a precipitate hinder reactions 1&2 by removing iron and cyanide from solution? The color of the precipitate changes somewhat when the pH of the suspension is adjusted. During the course of experiments, the color of the precipitate changes in color to a dark green, similar as seen in the oxidation of Fe^{+2} by nitrite (Summers, 1993). The color change occurs either with the addition of nitrite (in experiments where nitrite was used, see below) or more slowly over time when no nitrite is added. The color change is due to the oxidation of iron either by the nitrite or presumably by leakage of small amounts of air.

We will first examine the effect of cyanide of the reduction of nitrite by Fe(II), then the effect of Fe(II) on the Strecker synthesis, and finally we will consider the combination of the reactions to form glycine from nitrite, formaldehyde, and cyanide.

The effect of cyanide of the reduction of nitrite by Fe(II).

First we look at the extreme of cyanide complexation of iron as $Fe(CN)_6^{-4}$. When the reduction of nitrite is tried with a solution of ferrocyanide, instead of Fe^{+2}, no ammonia is observed (conditions were the same as in Table 2). Full coordination of the iron center by cyanide clearly deactivates it toward nitrite reduction.

Does complete coordination of the iron center deactivate it at all pH's or does protonation of CN^- free up enough coordination sites? In Table 1 the reduction of nitrite by $Fe(CN)_6^{-4}$ was studied as a function of the initial pH of the solution. (The pH rises during the course of the experiment, due to dissociation of small amounts of cyanide.) No ammonia is formed at any pH. Only when the pH is low enough to protonate all the free cyanide (near the pK_a of cyanide, 9.3), do we begin to see the consumption of nitrite, but no ammonia is detected. It would seem that, when the pH is low enough to produce a sufficient concentration of free sites on iron centers to reduce the nitrite, the pH is too low to form ammonia. In the low pH range the production of N_2O and N_2 are favored (Summers et al.).

Now we turn to conditions under which the amount of cyanide present is not sufficient to completely fill the six-fold coordination sphere of the iron and much of the iron and cyanide are present as a Fe_xCN_y precipitate (see above). Results with FeS have shown that the presence of Fe(II) in an insoluble form doesn't appear to have a major effect on nitrite reduction. Looking at the effect on ammonia formation of adding different amounts of CN^- to a solution of Fe^{+2} and nitrite (without active pH control), Table 2, we find that, at lower cyanide to iron ratios, ammonia is formed. Ammonia is formed when the cyanide/iron ratio is 2:1 or less, but doesn't form at ratios of 4:1 or higher.

The rate of reduction of nitrite to ammonia by iron is very sensitive to pH (Summers et al.), dropping dramatically below pH 7.5, and the reaction will lower the pH (see equation 1). The effect of pH can be eliminated by holding the pH constant with a pH monitor. In Table 3 we see the data from a series of experiments which were conducted at pH 8.0. Again, we can see that ammonia is formed when the cyanide to iron ratio is less than 4:1. Between 0:1 and 4:1 there is a decrease in the amount of ammonia formed. The amount of ammonia detected after 16 hours was that same as after 2 hours, indicating that all the nitrite was consumed after 2 hours. Thus the decrease in ammonia is due to a shift in the product distribution of the reaction away from ammonia and not due to only a portion of the nitrite reacting.

Why does the reaction stop when the cyanide/iron ratio is above 4:1? At that ratio we reach a stoichiometry of $Fe[Fe(CN)_6]_2^{-6}$. $Fe(CN)_6^{-4}$ species can coordinate to free Fe^{+2} through the nitrogen bases on the cyanides. If the $Fe(CN)_6^{-4}$ octahedron binds face on, it can occupy three coordination sites (see Figure 1). Thus, when the stoichiometry of $Fe[Fe(CN)_6]_x^{2-4x}$ reaches x=2 (a cyanide/iron ratio of 4) and above, it is possible to coordinate all the iron free sites, both on $Fe(CN)_6^{-4}$ and on Fe^{-2} species. Thus we only see ammonia production when the system has free iron for the nitrite, or other important species, to bind to the iron center and react.

Thus we can see that, if the pH is too low (as when free sites are created by cyanide protonation), no ammonia is formed, as is expected from the pH dependance of equation 1. If the pH is high enough, and the cyanide/iron ratio is below 4, the partial coordination and precipitation of Fe^{+2} by CN^- doesn't perturb free sites enough, or remove enough iron from solution, to prevent the reduction of nitrite to ammonia. However, it does shift the product distribution increasingly toward species such as N_2O and N_2. At higher iron/cyanide ratios, either $Fe(CN)_6^{-4}$ binding blocks all the free sites on the Fe^{+2} or the electronic configuration at the iron center is changed enough to make it unreactive.

Effect of Fe(II) on the Strecker synthesis.

The situation with respect to the Strecker synthesis is very different. In Table 4 we see that, if we run the Strecker synthesis, replacing free cyanide with an equal amount of cyanide bound up in ferrocyanide, similar amounts of glycine are produced. The formation of iminodiacetic acid, a side product to the reaction involving the double alkylation of the ammonia (see equation 3), is, if anything, reduced.

$$H_2CO + HCN + CH_2(NH_2)(CO_2H) \rightarrow HN(CH_2CO_2H)_2 \quad (3)$$

In Table 5 we see what happens to the products of the Strecker synthesis when the reaction is run in presence of increasing amounts of Fe^{+2}. While there is inherently more scatter in such experiments (the Strecker has more potential for side reactions than equation 1), there is no systematic change in the amount of glycine formed. This is not only true when the iron/cyanide ratio passes (1:1) (where each CN^- has it's own iron center to which to bind) but even up to values as high as 10:1. There also doesn't appear to be any change in the formation of the glycolic acid (another side product of the reaction involving attack by water on the reactive cyanohydrin intermediate instead of ammonia, see equation 4). The

$$H_2CO + HCN + H_2O \rightarrow CH_2(OH)(CO_2H) + NH_3 \quad (4)$$

formation of iminodiacetic acid, however, is decreased in the presence of added iron in all such runs, usually by about an order of magnitude. This is true for products obtained after 24 hours and after 48 hours. However, no decrease was seen under more dilute conditions (see below). It may be that, at higher iron concentrations, there is some coordination of the glycine formed, preventing if from reacting further to form iminodiacetic acid. However, a firm conclusion on this matter will have to await experiments where more systematic tests can be done (Such as using acetaldehyde as the starting aldehyde. Acetaldehyde has a more straightforward reaction in the Strecker synthesis than formaldehyde (Lerner et al. 1963)).

In Table 6 we see similar data for reactions run under more dilute conditions. Again we see no change in the formation of glycine. However we also don't see any change in the amounts of sideproducts formed. This is true for both glycolic acid and iminodiacetic acid.

Synthesis of glycine from nitrite with Fe^{+2}.

Lastly, we look at what happens if one puts all this together (equation 5). For example, in addition to the issues addressed above, does

$$H_2CO + HCN + 6\ Fe^{+2} + 7\ H_2O + NO_2^- \rightarrow 3Fe_2O_3 + 11\ H^+ + CH_2(NH_2)(CO_2H) \quad (5)$$

formaldehyde cause some unexpected complication in the nitrite reduction? Does nitrite interfere in some way in the Strecker synthesis?

We compared the formation of glycine using nitrite and Fe^{+2} as the source of ammonia with its formation using ammonia and with a control (containing neither nitrite/Fe^{+2} nor ammonia), Table 7. The production of glycine when iron and nitrite are used as an ammonia source, is not significantly different from the production of glycine from ammonia. In both cases the formation of glycine compares favorably (increased more than an order of magnitude) to cyanide as the only source. Using a FeS suspension as the source of Fe(II), instead of dissolved Fe^{+2}, similar results are obtained, though initially the amounts of glycine formed are lower. In both of these cases the production of iminodiacetic acid is similar or reduced, compared to the reaction using ammonia.

CONCLUSIONS.

The formation of ammonia by Fe^{+2} reduction of nitrite, can occur in the presence of cyanide, provided the cyanide/iron ratio is less than 4:1. Over ratios of 0:1 to 4:1 one sees a steady decrease in the product distribution away from ammonia. At a cyanide/iron ratio of 4:1 or greater, no ammonia is formed throughout the pH range studied.

Inhibition by cyanide would seem to provide little or no limitation on nitrite as a source of ammonia on the early earth. Since the most likely source of cyanide is in the atmosphere (Bar-Nun et. al.; Zahnle; Wen et. al.), the situation was one where cyanide was essentially being "titrated" into an iron rich ocean. The presence of the banded iron formations (Holland, 1973; Walker et. al. 1985; Derry et. al.; Holland, 1989) show that dissolved iron was present in excess quantities in the early ocean. This means that an excess of iron (which means a cyanide/iron ration of < 1:1) existed. Under such conditions, the change in the reduction of nitrite to ammonia is relatively small.

Conversely, the presence of iron in almost any quantity does not appear to interfere with the Strecker synthesis in any way. In fact, a decrease in side products, through complexation of glycine to Fe^{+2}, remains a possibility. It appears that the Strecker synthesis has little sensitivity toward whether the cyanide it uses is free or bound to a metal center.

Finally, it has been demonstrated that The Strecker synthesis and the iron (II) reduction of nitrite to ammonia can be combined to form glycine from nitrite, iron(II), cyanide, and formaldehyde.

EXPERIMENTAL.

All solutions were prepared using "Tech One" reverse osmosis purified water and were purged with nitrogen. In a typical nitrite reduction experiment, a $FeCl_2$ stock solution was added to a KCN solution (sometimes the KCN was added to a $FeCl_2$ solution with no change in results). The pH was then adjusted under nitrogen purge, and an appropriate amount of a nitrogen purged sodium nitrite solution was added to start the reaction. In experiments with pH control, a pH electrode was used with a pH monitor and a syringe pump to deliver nitrogen purged NaOH when the pH dropped below 7.8.

Ammonia analysis was either by colorimetric methods (Verdouw et. al) or by ion chromatography. Nitrite analysis was by ion chromatography. Ion chromatography was done on Dionex ion chromatographs: DX-100 for ammonium and 4500i for nitrite. Both instruments were run in suppressed conductivity mode for increased sensitivity. Iron and cyanide can form layered mineral precipitates into which ammonia can diffuse. Such minerals are broken up by strongly basic solutions, such as are used in the colorimetric ammonia analysis. Ion chromatography was only used for experiments with the soluble $K_4Fe(CN)_6$.

In a typical Strecker experiment, a solution of $FeCl_2$ and KCN was prepared as described above. The NH_4Cl stock solution (if any was used) was added before the pH was adjusted. Then an appropriate amount of a formaldehyde stock solution was added to start the reaction. In reactions where nitrite was used, it was added last. Aliquots were taken from these solutions, filtered to remove any particulate matter, and then applied to a cation exchange column (Dowex 50(H^+) cation exchange column initially regenerated with HCl). After desalting the amino and imino acids were analyzed as their trifluoroacetylisopropyl esters (Lerner et. al.) and glycolic acid was analyzed as its tert-butyldimethylsilyl derivative using GC-MS.

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Table 1. Effect of pH on the reduction of nitrite by $Fe(CN)_6^{-4}$.[1]

pH (Start)	pH(Final)	Nitrite (mM)[2]	Ammonia (mM)[2]
2.1	3.6	0.0018	0
2.3	7.7	0.0065	0
4.7	9.7	0.16	0
5.4	10.4	0.26	0
5.7	10.2	0.23	0
6.6	10.6	0.27	0
6.8	10.5	0.28	0
8.0	10.5	0.28	0
8.5	10.6	0.29	0
9.3	10.5	0.28	0

1) Also; Room Temperature, 17 mM $K_4Fe(CN)_6$.

2) Final concentration at the end of experiment, 24-48 hours. Initial NO_2^- concentration was 0.30 mM. Analysis was by Ion Chromatography (see experimental).

Table 2. Effect of Cyanide on the Reduction of Nitrite To Ammonia by Fe^{+2}.

	$[CN^-]/[Fe^{+2}]$	Ammonia (mM)
Fe^{+2}	0	0.009
Fe^{+2} + KCN (14 mM)	1.1	0.044
Fe^{+2} + KCN (26 mM)	2.1	0.011
Fe^{+2} + KCN (53 mM)	4.4	0[2]

1) Also Room Temperature, initial pH 8.0, 0.32 mM $NaNO_2$. All reactions ran 24 hrs. All entries labeled "KCN" also contained 12 mM $FeCl_2$.

2) Also no ammonia detected after 72 hours. Analysis was by colorimetric methods (see experimental).

Table 3. Effect of Cyanide on the Reduction of Nitrite To Ammonia by Fe^{+2}.

KCN [1]	$[CN^-]/[Fe^{+2}]$	Ammonia (mM)	Yield
0 mM	0	0.104	32
12 mM	1.0	0.091	28
26 mM	2.0	0.072	22
39 mM	3.2	0.049	15
53 mM	4.4	0	0

1) Also Room Temperature, pH 8.0, 0.32 mM $NaNO_2$, 12 mM $FeCl_2$. All reactions ran 2 hrs. Analysis after 24-48 hours gave similar results. Analysis was by colorimetric methods (see experimental).

Table 4. Strecker Synthesis of Amino Acids with Different Sources of Cyanide.

Conditions[1]	Product (mM)	
0.1 M KCN	glycine	0.57
	IDA[2]	0.30
0.017 M $K_4Fe(CN)_6$	glycine	0.43
	IDA	0.12

1) Also Room Temperature, 0.1 M H_2CO, NH_3 , initial pH 8.0. Reactions were allowed to proceed at least 6 days.
2) IDA = Iminodiacetic acid, $HN(CH_2CO_2H)_2$.

Table 5. Effect of Fe^{+2} on the Strecker Synthesis of Amino Acids.[1]

Fe^{+2} (M)	$[Fe^{+2}/CN^{-}]$	24 hours			48 hours		
		Glycine (μM)	IDA[2] (μM)	Glycolic Acid (μM)	Glycine (μM)	IDA[2] (μM)	Glycolic Acid (μM)
0	0	880	10.7	100	270	6.0	320
0.1	1	400	0.59	-	370	0.88	420
0.2	2	160	0.095	-	700	0.59	520
0.5	5	1400	0.66	140	950	0.11	360
1	10	560	4.62	710	790	0.88	240

1) Also Room Temperature, 0.1 M H_2CO, NH_3 , KCN. Initial pH 8.0.
2) IDA = Iminodiacetic acid, $HN(CH_2CO_2H)_2$.

Table 6. Effect of Fe^{+2} on the Strecker Synthesis of Amino Acids.[1]

Fe^{+2} (mM)	[Fe^{+2}/CN^-]	Glycine (μM)	IDA[2] (μM)	Glycolic Acid (μM)
0	0	4.6	0.15	5.9
1	1	7.4	0.76	12
2	2	4.0	0.051	3.8
4	4	3.8	0.14	3.8
6.7	6.7	23	N.D.[3]	3.6

1) Also Room Temperature, 0.001 M H_2CO, NH_3 , KCN. Initial pH 8.0. Reactions ran ~4 weeks.

2) IDA = Iminodiacetic acid, $HN(CH_2CO_2H)_2$.

3) N.D. = Not Detected.

Table 7. Strecker Synthesis of Amino Acids from Nitrite with Fe(II).

Conditions[1]	Product (mM)	24 hrs	144 hrs
-	glycine	0.006	0.015
	IDA[2]	0.011	0.056
0.1 M NH_3	glycine	0.20	0.57
	IDA	0.044	0.30
0.1 M $NaNO_2$, 0.1 M $FeCl_2$	glycine	0.092	0.62
	IDA	0.047	0.17
0.1 M $NaNO_2$, 1.3 g FeS	glycine	0.022	0.40
	IDA	0.054	0.081

1) Also Room Temperature, 0.1 M H_2CO, KCN , initial pH 8.0.
2) IDA = Iminodiacetic acid, $HN(CH_2CO_2H)_2$.

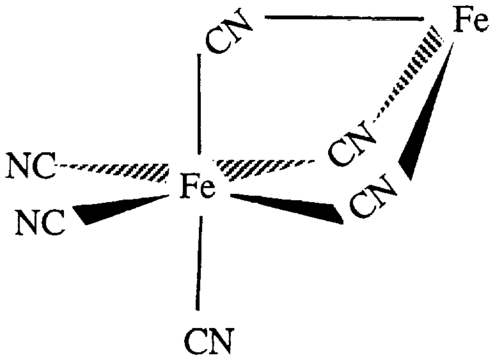

Figure 1. $Fe(CN)_6^{-4}$ coordination to free Fe^{+2}.