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Nova Biotechnol Chim (2021) 20(1): e880
DOI: 10.36547/nbc.808

Nova Biotechnologica *et* Chimica

Iron(II) reactions with glycine – suitable biomineralization model?

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Article info

Article history:
Received: 24th February 2021
Accepted: 21st April 2021

Keywords:
Iron biomineralization
Iron(III) oxide formation
Iron(II) salts

Abstract

The reactions of Mohr's salt with amino acid glycine in aqueous solution and aerobic conditions were studied and obtained samples consist of different compounds. The obtained products were characterized by elemental analysis, infrared spectroscopy and the iron determination also and exhibited very low organic matter content – the sum of N, C, H and S content less than 20 % and Fe content around 40 %. Prepared samples consist of iron(II) amino acid complex $[Fe(glyH)(SO_4)]_n$, known as iron food supplement (FeGS) as the only sulphur containing compound. Its content varies from 26 % up to 46 %. Ferrous glycinate was obtained in the samples collected initially after the reaction in very low content < 7 %. Iron(III) hydroxy-oxide FeO(OH) was obtained in the range 40 – 65 %. The FeO(OH) content increased with the decreasing FeGS content. Formation of inorganic FeO(OH) suggest the ongoing redox reaction under aerobic condition therefore, it is suggested as a biomineralization reaction model.

Introduction

The term biomineralization was introduced into chemistry three decades ago by Professor R.J.P. Williams group (Webb *et al.* 1986) on ferritin isolation and the wide current usage of this term was well explained in editorial (Bertazzo 2015) as "At the core of the biomineralization discipline is the inspiration for research drawn from diverse tissues presenting minerals synthesized by a living organism", e.g. magnetite production by magnetic microbes (Prozorov 2015), or silica formation in diatoms (Hildebrand and Lerch 2015), or coral biomineralization (Falini *et al.* 2015), or mineralization processes in the skeletons (Dean *et al.* 2015), and/or cardiovascular calcification (Hutcheson *et al.* 2015).

Possible explanation of the oxide formation by ferritin biodegradation together with the oxide observation in human spleen (Boča *et al.* 2013a; Kopáni *et al.* 2015) and observation of iron(III) oxide deposits in human *Basal Ganglia* (Boča *et al.* 2013b) are the inspiring points of our interests in this field. The understanding of chemical reactions occurring in the human brain is very important for health and treatment of some serious diseases. The iron oxide mixture formation in two steps reactions of ferrous salts with some amino acids in alkaline medium were explained by second step oxidation of iron(0) nanoparticles formed in anaerobic first step reaction (Klačanová *et al.* 2013; Kišš *et al.* 2017). The presented paper is dealing with analysis of the reaction products of selected amino acid

glycine with Mohr's salt under different aerobic reaction conditions.

Experimental

Mohr's salt (ammonium iron(II) sulfate hexahydrate) $(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$ analytical grade, was bought from Aldrich and used without further purification. L-glycine (analytical grade, Centralchem) was used as received.

Samples were prepared by reaction of glycine and Mohr's salt aqueous solutions. Glycine (100 mmol, 7.507 g) dissolved in warm water was added under stirring to Mohr's salt (50 mmol, 19.607 g) dissolved in warm water and the reaction mixture was stirred for 30/60/120 min with the temperature kept at 60 °C. The reaction mixture was then cooled to room temperature and the formation of precipitate was observed. The time of aging is listed in Table 1. The solid precipitate was isolated by filtration under reduced pressure. The products were dried at laboratory temperature. The filtrates were left for further product formation.

Elemental analyses were carried out on a CHNSO FlashEA™ 1112 Automatic Elemental Analyzer. Inductively coupled plasma atomic emission spectrometer, model 5100 ICP-OES (Agilent, USA) was used for iron content determination. Infrared spectra (4,000 – 400 cm^{-1}) were measured using the NICOLET 5700 FT-IR spectrophotometer at room temperature using the ATR technique.

Table 1. Reaction conditions of samples prepared.

Sample	Glycine[mmol] / Water [cm^3]	Mohr's salt [mmol] / Water [cm^3]	Reaction time [min]	Filtered off after [h]
KM 04.2.2	20 / 50	10 / 20	30	24
KM 04.3.2	20 / 50	10 / 20	30	24
KM 13.1.3	100 / 250	50 / 100	30	48
KM 14.1.2	100 / 250	50 / 100	60	24
KM 14.1.3	100 / 250	50 / 100	60	48
KM 15.1.1	100 / 250	50 / 100	120	0
KM 15.1.2	100 / 250	50 / 100	120	24
KM 15.1.3	100 / 250	50 / 100	120	48

Results and Discussion

The experiments were realized according to the procedure published (Ghasemi *et al*. 2012) as the procedure for preparation of Iron-Amino Acid chelates $[Fe(Gly)_2]$ as growth stimulator used in nutrient solution culture. The initial light green colour of Mohr's salt solution has rapidly changed to darker brown-green colour due to iron(II) glycine complex formation and proceeding reactions were recognized by further colour changes to brown shades and usually some brown-coloured solid products formation were observed. The obtained brownish products were analyzed by elemental analysis and results are shown in Table 2. It should be stressed that all the obtained samples show in addition to the rather high sulphur content very "low organic matter content" (Table 2). The data show nitrogen-to-carbon stoichiometric ratio $\nu(N) : \nu(C) \approx 1 : 2$ that corresponds to the ratio of the amino acid used in the reaction – glycine. Moreover the stoichiometric ratio $\nu(N) : \nu(S)$ is roughly close to 1 : 1 which means that the representation of amino acid and sulphur in the obtained samples is approximately 1 : 1 which is more similar to therapeutic "Ferrous Sulfate-Glycine Complex" (Rummel 1959) than to "Iron-Amino Acid Chelate" (Ghasemi *et al*. 2012). Infrared spectra of all samples are similar, and the Fig. 1 gives as an example two of them together with the spectrum of glycine, the amino acid used. All spectra show the broad bands presence in the region 3,300 – 3,500 cm^{-1} that could be assigned to O–H vibrations probably of uncoordinated water

molecules present in samples. Absence of O–H vibration in the spectrum of glycine is logically explained by existence of "zwitter ion" structure $^{+}NH_3CH_2COO^{-}$ in the solid state (Drebushchak *et l.* 2002). The region from 3,000 to 3,300 cm^{-1} can be assigned to the N–H vibrations of the amino group. This confirms the presence of amino acid in the samples. Difference in the spectra of glycine and samples is a region around and below 3,000 cm^{-1} that could be assigned to the hydrogen-bonding present in the samples which differ from that one in pure glycine spectrum. The presence of glycine in samples could be confirmed by bands in the region 1,700 – 1,300 cm^{-1} – the COO vibrations of the carboxyl group. Very strong and rather broad bands in the region 1,100 – 1,000 cm^{-1} could be assigned to the S–O vibration of sulphate group.

Table 2. Elemental analysis and calculated stoichiometric ratios of selected samples.

Sample	Elemental analysis / Stoichiometric ratio				
	N [%] / *ν*(N)	C [%] / *ν*(C)	H [%] / *ν*(H)	S [%] / *ν*(S)	Sum[%] NCHS
KM 04.2.2	3.26 / 1.19	4.68 / 2.00	2.80 / 14.2	6.25 / 1	17.0
KM 04.3.2	-*	4.54 / 2.11	2.63 / 14.5	5.74 / 1	12.9
KM 13.1.3	2.94 / 1.22	5.61 / 2.72	2.66 / 15.4	5.51 / 1	16.7
KM 14.1.2	2.80 / 1.11	4.14 / 1.92	2.64 / 14.6	5.76 / 1	15.3
KM 14.1.3	3.08 / 1.12	4.63 / 1.96	2.74 / 13.8	6.29 / 1	16.7
KM 15.1.1	-*	2.86 / 2.08	1.85 / 16.0	3.67 / 1	8.40
KM 15.1.2	3.01 / 1.17	4.41 / 2.00	2.71 / 14.7	5.88 / 1	16.0
KM 15.1.3	3.44 / 1.19	4.95 / 2.00	2.79 / 13.4	6.62 / 1	17.8

* Low nitrogen content evaluated as zero by running computer programme.

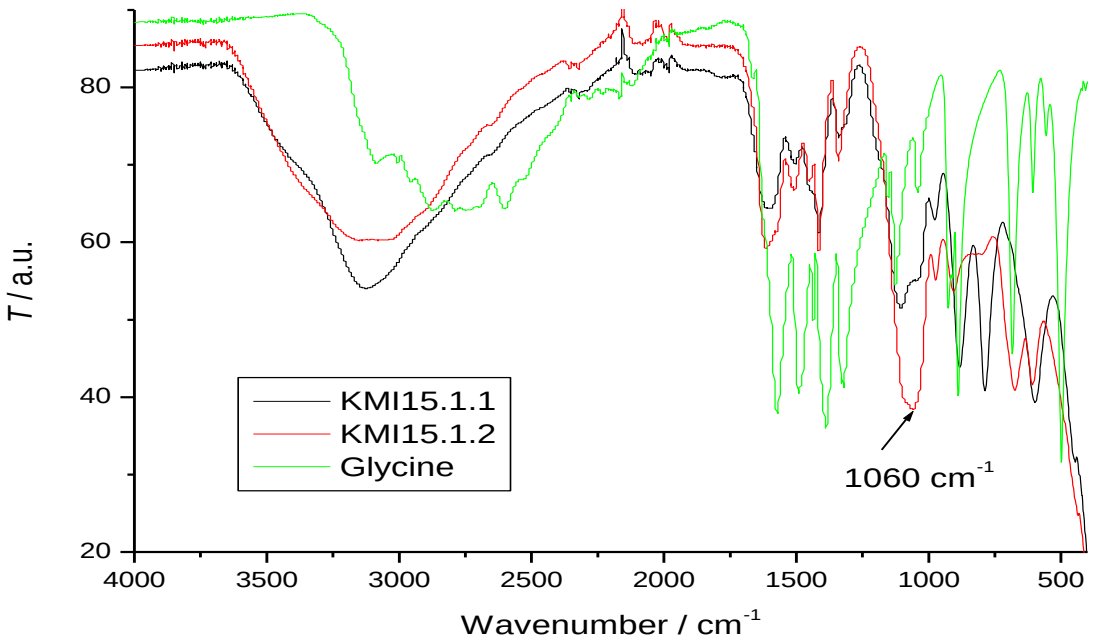
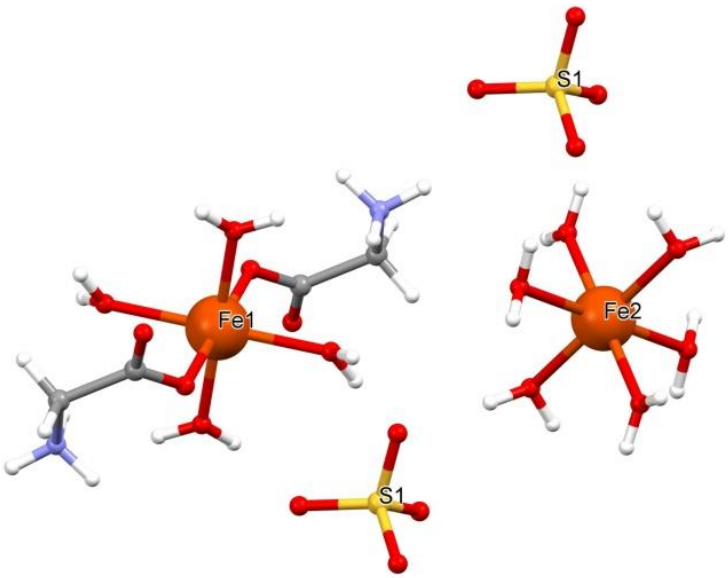


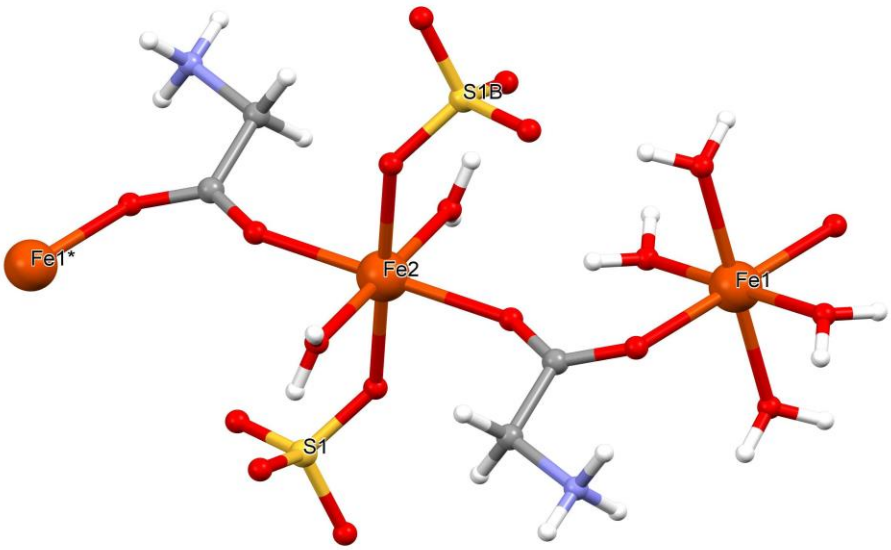
Fig. 1. Comparison of the infrared spectra of the samples prepared along with the infrared spectrum of the amino acid glycine used.

Elemental analysis, along with the infrared spectroscopy, suggested that products contained glycine and the sulphate group. A search through the Cambridge Crystallographic Database has shown that there are only two ferrous glycine complexes with a solved structure. The first complex is $[Fe(glyH)_2(H_2O)_4][Fe(H_2O)_6](SO_4)_2$, where glyH = glycine, (Fig. 2 at the top) containing two different iron(II) cations together with two sulphate anions (Ougey *et al*. 2013). The second complex found is polymeric *catena*-$[\{Fe(H_2O)_4\}(\mu\text{-}glyH)\{Fe(H_2O)_2(SO_4)_2\}(\mu\text{-}glyH)]_n$ (Fig. 2 at the bottom) (Ougey *et al*. 2013). Both complexes show common stoichiometric formula $\{Fe_2(glyH)_2(SO_4)_2(H_2O)_x\}$ where x = 10 or 6.

Just recently the third polymeric complex structure



$[Fe(glyH)_2(H_2O)_4][Fe(H_2O)_6](SO_4)_2$



catena-$[\{Fe(H_2O)_4\}(\mu\text{-}glyH)\{Fe(H_2O)_2(SO_4)_2\}(\mu\text{-}glyH)]_n$

Fig. 2. Structures of the hexa-aqua-iron(II)-bis(ammonioacetato)-tetra-aqua-iron(II) sulfate complex (GLYCFE01 above), and the *catena*-[bis(μ_2-ammonioacetato)-hexa-aqua-bis(sulfato)-di-iron(II)] complex (UDOPIO below) (Ougey *et al*. 2013).

Ferrous Glycine Sulfate (FeGS) was published (Dinnebier *et al*. 2016) as structure of dietary supplement $[Fe(glyH)(SO_4)]_n$ (Fig. 3). There are two conclusions drawn out of all three structures mentioned above. At first, all three complexes exhibit the same stoichiometry ν(Fe) : ν(glyH) : $\nu(SO_4)$ = 1 : 1 : 1 and they differ only by the decreasing water to iron(II) stoichiometry. Moreover, the FeGS food supplement is prepared by the reaction of glycine with iron(II) sulphate, and it should be taken as one of the principal components in our products formation.

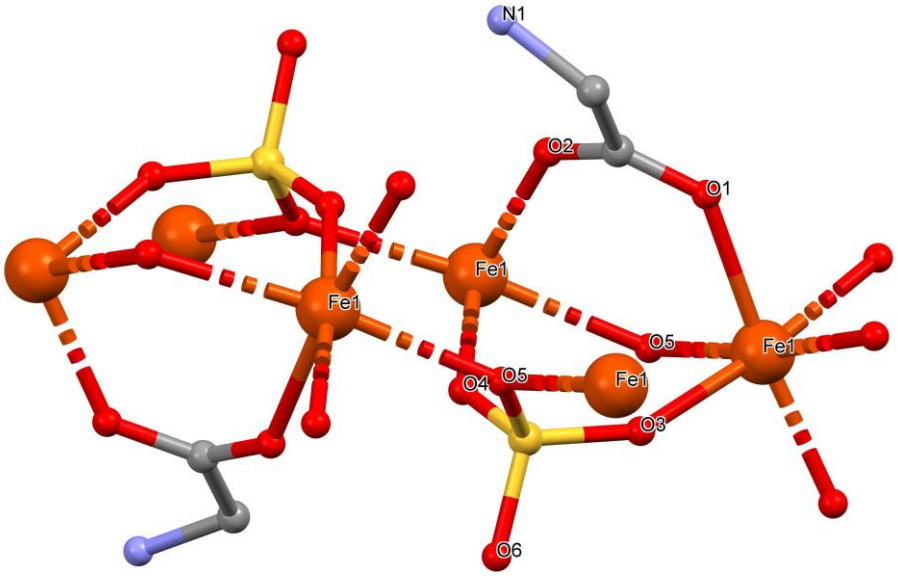


Fig. 3. Structure of the complex Ferrous Glycine Sulfate $[Fe(glyH)(SO_4)]_n$ (ANIVOK) (Dinnebier *et al*. 2016).

In particular conclusion of the qualitative analysis above, it could be stressed that studied reactions gave, due to their complexities, the reaction products composed of at least three iron containing components. The Ferrous Glycine Sulfate FeGS, or its "hydrated forms" are surprisingly at the top to be accepted due to the elemental analysis and sulphate spectral evidence. The Ferrous Glycinate $Fe(Gly)_2$, listed also as Fe-Glycine Chelate (Ghasemi *et al*. 2012), could be partly accepted as iron and glycine containing minor component in addition to the major FeGS one. The minority of the $Fe(Gly)_2$ in our products (despite the suggestion (Ghasemi *et al*. 2012) is based on the elemental analysis results - only few samples gave the ν(C) : ν(S) ratio higher than 2 : 1. Moreover, the formation of the $Fe(Gly)_2$ in the reaction $Fe(SO_4)$ with glycine could be to greater extent seen after addition of more NaOH (Ashmead 1980). Finally, the third completely inorganic component FeOOH should be included into the list, and that allows to analyze the composition of obtained products.

Two products KM 13.1.3 and KM 15.1.1 have been recently analyzed for iron content (Table 3) and that together with sulphur and carbon allowed us to calculate all three iron compounds content. The calculation is based on assumptions that the FeGS is only component with sulphur content (3rd column in Table 3) and the total carbon content comprise of the glycine in the FeGS and glycinate anion presence in $Fe(Gly)_2$ (4th column in Table 3). The total iron content could be found only if samples contained calculated amount of inorganic iron oxide formally as $FeOOH \approx ½(Fe_2O_3 \cdot H_2O)$. The obtained results are interesting for the possibility to explain consistently on the base of the elemental analysis the composition of prepared samples, e.g., KM 13.1.3 contains 84.6 % of iron containing compounds and similarly the content of these compounds in KM 15.1.1 is 91.6 %. The calculation based on these data has shown that hydrogen content in mentioned components is far less than hydrogen content determined in elemental analysis (Table 2). It shows together with the infrared spectra (presence of the broad absorption

bands between 3,500 and 3,300 cm^{-1} in all samples) that water content should be included into the composition of the prepared samples. For these two samples evaluated, water could be up to 10 % or 5 % in KM 13.1.3 or KM 15.1.1, respectively.

The results shown above allowed us to do some approximations for those samples where iron could not be determined because of the insufficient number of samples. The results (Table 4) show that all samples exhibit over 40 % of FeGS content and practically all samples have shown carbon content approximately equal to glycine content in the FeGS. The estimated FeOOH content could be in the range 40 – 45 % that gives the total iron content in range 35.9 – 38.2 % and finally, the water content from 10.5 to 12.5 %.

Table 3. Elemental analysis and calculated Iron compound content.

Sample	Elemental analysis / Iron compound content			
	Fe [%]	S% / FeGS [%]	C% / $Fe(Gly)_2$ [%]	FeOOH [%]
KM 13.1.3	35.9	5.51 / 39.0	5.61 / 6.28	39.3
KM 15.1.1	47.3	3.67 / 26.0	2.86 / 0.46	65.1

Table 4. Elemental analysis and calculated/estimated Iron compound content.

Sample	Elemental analysis / Stoichiometric ratio			
	S% / FeGS [%]	C% / $Fe(Gly)_2$ [%]	$FeOOH_{est}$ [%]	Fe_{calc} [%]
KM 04.2.2	6.25 / 44.3	4.68 / -	~40	35.9
KM 04.3.2	5.74 / 40.7	4.54 / 1.00	~44	37.8
KM 14.1.2	5.76 / 40.8	4.14 / -	~45	38.2
KM 14.1.3	6.29 / 44.6	4.63 / -	~40	36.0
KM 15.1.2	5.88 / 41.6	4.41 / 0.02	~42	36.5
KM 15.1.3	6.62 / 46.9	4.95 / -	~40	36.5

The estimated data all together gives us the chance come to conclusion concerning the reaction procedure under study. First of all, data are in good agreement with the conclusion the Mohr's salt in solution primarily gives "Ferrous Sulfate-Glycine Complex" containing Fe : GlyH ratio 1 : 1. The excess of glycine in all experiments (Fe : GlyH ratio 1 : 2 was used) apparently allows the $Fe(Gly)_2$ formation, but probably the formed product undergoes some further reactions in solution and only a small part of this substance is included into the final solid product. This rather complicated solid phase *vs* solution equilibria could be to some extent demonstrated on KM 15 samples that were from the reaction mixture filtered off at different times. The initially (just after cooling down the reaction mixture) separated part of product KM 15.1.1 contains the lowest 26.0 % FeGS content and the highest 65.1 % content of FeOOH. The increasing content of FeGS and simultaneously decreasing content of FeOOH in samples KM 15.1.2 (separated after 24 h) and KM 15.1.3 (filtered off after 48 h) could be taken as data supporting the idea of these reactions between the forming solid phase and solution. At this state of experiments one cannot give any proof which of two glycine containing components undergo the biomineralization procedure leading to the formation of iron(III) product – FeOOH, but it is clear that this study brings some suitable results.

Conclusions

In conclusion, we can say that the formation of the products FeGS and $Fe(Gly)_2$ containing glycine in the form of a molecule or chelating anion together with iron(II) was proved. It was also shown that some of the products undergo the decomposition together with oxidation iron(II) to iron(III) reactions that altogether could be called as biomineralization reactions and they probably lead to iron(III) oxide formation.

Acknowledgement

This research was funded by Agency APVV (APVV-19-0087) and UCM grant FPPV-35-2021. Furthermore, we would like to thank Assoc. prof. M. Horník, PhD. for his kind and great support of this work by guaranteeing the iron content analysis of samples at Geoanalytical laboratories of State Geological Institute of Dionýz Štúr in Spišská Nová Ves.

Conflict of Interest

The authors declare that they have no conflict of interest.

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