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Crystal data of a new phosphate compound from microbial experiments on iron sulfide mineralization

ROLF O. HALLBERG

Department of Geology, University of Stockholm
Box 6801, S-113 86 Stockholm, Sweden

AND TOMMY WADSTEN

Arrhenius Laboratory
Department of Inorganic Chemistry, University of Stockholm
S-106 91 Stockholm, Sweden

Abstract

Samples from a continuous culture at 25°C and pH ≈ 8 of sulfate-reducing bacteria and subsamples heated in evacuated silica tubes at 120°C yielded a new phosphate compound. The chemical formula is estimated to be:

$$KNa_3(Fe_{1.5}Mg_{2.5})(PO_4)_3(OH)_3$$

The compound was formed as elongated, thin, transparent crystals together with various forms of iron sulfides. From electron and X-ray diffraction measurements the unit cell dimensions are: $a = 8.33$, $b = 12.86$, $c = 17.55$A, $\beta = 102.6°$, and $Z = 4$. The symmetry is monoclinic, with space group $P2_1/c$.

The continuous cultivation of bacteria was performed under chemical conditions as commensurate as possible with those in modern sediments. The compound may thus be important for phosphate fixation in reduced sediments in connection with authigenic iron sulfide mineralization.

Introduction

In modern sediments the formation of iron sulfides takes place in microniches between the solid particles. The hydrogen sulfide is produced by bacteria. In contrast to pure inorganic reactions between iron and hydrogen sulfide, the biosynthesis of metal sulfides is brought about with a moderate release of hydrogen sulfide. It is also possible that the biosynthesis involves processes on the surface of the bacteria that do not occur in entirely inorganic reactions. The bacteria may thus act as a template for the iron sulfide formation. In order to study the processes in the chemically-reduced sedimentary microniche, it was necessary to magnify it to a convenient size. This was done with an apparatus for continuous cultivation of sulfate-reducing bacteria, where the sulfides were formed under controlled conditions as commensurate as possible with those in modern sediments. The experiment and the procedure are described by Hallberg (1970, 1972). Part of the material from the continuous cultivation was transferred into silica tubes which were sealed and stored at 120°C. This was done in order to enhance the crystallinity of the black precipitate formed. When this material was examined under the electron microscope it was found that, apart from the sulfides, it also contained fine-grained aggregates of elongated laminar crystals with well-defined crystallographic habit (Fig. 1). This new compound is described in this paper.

Methods and materials

Two continuous cultivations were performed at pH values about 6 and 8. The new compound was only found in the material from the latter cultivation. Steady-state values of pH, pE and pS^{2-} in that cultivation were 7.9, −3.3, and 11.8, respectively. A constant temperature of 25°C was maintained in the reaction vessel during the cultivation period, which lasted for two months. In order to enhance the crystallinity and promote the chemical transformation of

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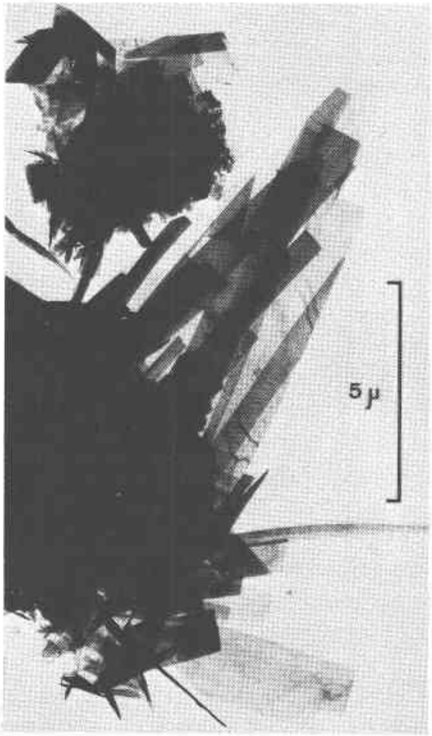

Fig. 1. Electron micrograph of an aggregate of the new compound. Subsample No 1.

the precipitate, samples were transferred from the cultivation vessel to evacuated sealed silica tubes which were subsequently heated at 120°C. Four such silica tubes were opened and examined after 8, 21, 27, and 70 months, respectively. These subsamples are numbered 1–4.

Cleaning procedure

The black precipitate from the continuous culture and from the silica tubes had been thoroughly freeze-dried. Under the microscope the transparent crystals were selected from the crude black polyphase product and transferred to a tube containing toluene. After vigorous shaking in different liquid media, foreign particles still adhered to the crystals. However, by a mild ultrasonic treatment in toluene it was possible to separate the new compound from contaminants. The crystals were subjected to ultrasound in a volume of 3 ml of toluene in a glass tube with an inner diameter of 10 mm. At a frequency of 400 kHz the ultrasonic cleaning lasted for 5–7 seconds. This procedure was repeated five times with decantation after each run. After the final treatment the solvent was allowed to evaporate, leaving the dry crystals. By a microscopic examination after the cleaning, which was performed at room temperature, no extra particles were seen and little reduction of crystal size was observed. The material is stable in air for several months at room temperature.

Table 1. Crystallographic data

Symmetry: monoclinic

Space group: $P2_1/c$

$\underline{a}$ = 8.328(3) Å
$\underline{b}$ = 12.86(3) Å
$\underline{c}$ = 17.55(2) Å
β = 102°.6(1)
V = 1835 Å^3

Diffraction techniques

Electron diffraction was obtained with a Philips EM 300 operating at 100 kV. The crystals were supported by a nitrocellulose film stabilized with a thin layer of Pd. Thallium chloride was used as external standard.

The X-ray powder pattern was obtained by the Guinier technique, with strictly monochromatized Cu$K\alpha_1$ radiation ($\lambda = 1.5404$A), using KCl ($a = 6.2390$A) as an internal standard. To avoid fluorescence due to iron content in the examined material, the film was protected by a thin Al foil. The photographically-recorded diffraction peaks were measured with an automatic film scanner (Abrahamsson, 1966). The cell parameters, refined by least-squares techniques, and peak intensities, are found in Tables 1 and 2, respectively. Single-crystal Weissenberg data

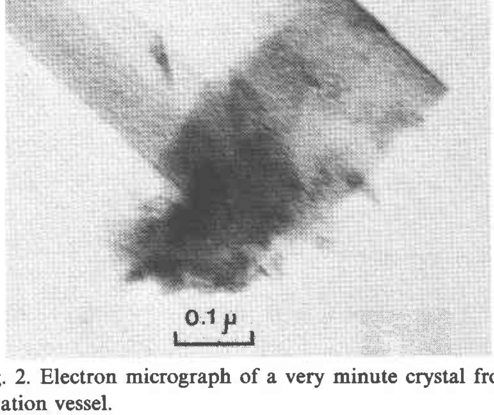

Fig. 2. Electron micrograph of a very minute crystal from the cultivation vessel.

were collected to index the power pattern and to determine the space-group symmetry (see Table 1).

Chemical analyses

A sample of the cleaned crystals (0.400 mg) wrapped in a small piece of Al foil (5.200 mg) was dissolved in 1 : 1 HCl. Concentrated HNO_3 was added, and the sample was gently heated until the yellowish vapors disappeared. It was then diluted to 50 g with distilled H_2O. Na, K, and Mg were analyzed by flame spectrophotometry, and Fe by atomic absorption on a Varian AA-6. Phosphate was analyzed according to Murphy and Riley's (1962) single-solution method.

Results

Diffraction and electron microscopy

Samples from the continuous culture did not give diffraction patterns which could be used for identification purposes. The lines were broad and diffuse. Very minute crystallites with lamellar structures were, however, observed under the electron microscope (Fig. 2). Occasionally these crystallites were thick enough to exhibit very distinct, but fragmentary, single-crystal diffraction patterns. The crystal data obtained with electron microscope and X-ray techniques from the heat-treated subsamples show good resemblance to the data from fragmentary crystals of the original culture.

The first subsample contained very thin and small crystals of the new compound. The crystals collect static electricity, are extremely thin, and were therefore not suitable for single-crystal X-ray techniques. The X-ray powder pattern of the black polyphase mixture, however, showed no anomalous line broadening. Except for six lines, all reflections can be identified as belonging to the iron sulfides pyrite, greigite, and mackinawite. The identification of the remaining reflections is doubtful, although three of them are similar to three strong reflections in Table 2. One line ($d = 3.34$A) can be identified as belonging to α-SiO_2.

Electron micrographs of the crystals show a banding because of lamellar growth structure (Fig. 3). In order to determine the crystallographic parameters of the laminar crystals, the electron diffraction technique was used. Crystals large enough to cover the selected area (1 μm) in the microscope and having a uniform thickness, indicated by an evenly distributed gray color, were chosen for study. Such crystals provide the most accurate intensity in distribution of the spots. The electron diffraction pattern shows two mirror planes (Fig. 4). The geometry and intensity symmetry suggest that the structure is either orthorhombic or tetragonal. The smallest parameters derived are $a = 8.35(2)$ and $b = 12.73(3)$A ($n = 10$ in both cases). The c axis could not be determined, even by using a universal stage in the electron microscope, because of the diffraction characteristics of thin plate-like crystals.

It was found that the thin crystals grow from the mother liquor in the sealed silica tubes at 120°C after heating for several months. In subsamples 3 and 4 the average size of the crystals in the longest growth direction equals 1–1.5 mm with a thickness of 0.01–0.02 mm. With the above procedure it became pos-

Table 2. Guinier powder pattern, Cu$K\alpha_1$ radiation ($\lambda = 1.5405$A), internally calibrated with KCl ($a = 6.2930$A)

d_{obs} Å	I_{obs}	h k l	d_{obs} Å	I_{obs}	h k l
10.22	100	0 1 1	2.549	69	1 4 $\bar{4}$
8.50	65	0 0 2	2.515	15	2 4 $\bar{2}$
6.805	4	1 1 $\bar{1}$	2.478	23	3 2 $\bar{3}$
6.011	5	1 1 1	2.404	6	1 5 1
5.878	10	1 1 $\bar{2}$	2.299	50	2 1 $\bar{7}$
5.020	3	1 2 $\bar{1}$	2.188	5	3 1 $\bar{5}$
4.181	5	1 0 $\bar{4}$	2.180	4	1 1 $\bar{7}$
4.054	9	1 2 $\bar{3}$	2.075	31	1 2 $\bar{8}$
3.843	5	0 3 $\bar{2}$	2.059	12	3 4 $\bar{3}$
3.608	15	1 3 $\bar{2}$	1.969	3	1 0 8
3.552	2	1 2 3	1.853	2	2 5 4
3.477	3	1 0 4	1.836	3	4 3 0
3.429	5	0 3 3	1.799	2	0 7 2
3.399	15	2 0 2	1.781	1	3 0 6
3.316	20	1 3 $\bar{3}$	1.684	7	2 2 8
3.018	7	1 3 3	1.643	4	5 0 $\bar{4}$
2.934	52	2 1 3	1.637	3	0 4 $\bar{9}$
2.803	31	2 3 $\bar{3}$	1.614	2	4 2 $\bar{8}$
2.672	8	2 0 $\bar{4}$	1.542	9	3 6 $\bar{6}$
2.625	4	3 1 $\bar{3}$	1.505	16	0 8 4

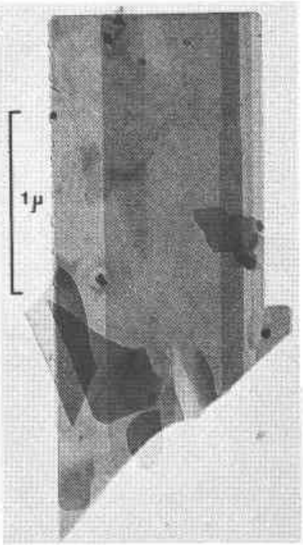

Fig. 3. Electron micrograph of a crystal showing distinct lamellar growth-structure.

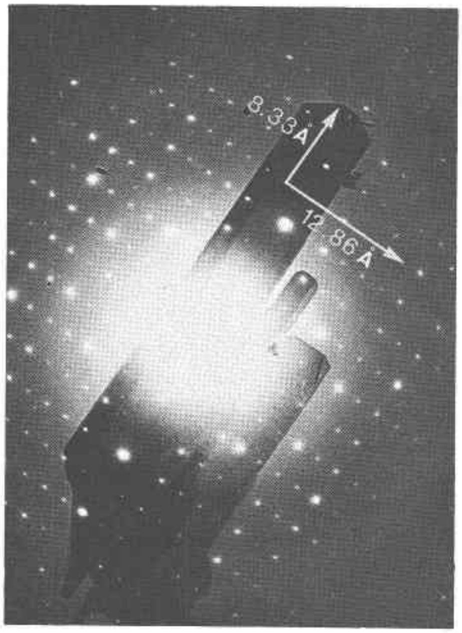

Fig. 4. Electron diffraction pattern exposed on its crystal image. The crystallographic *a* and *b* axis are indicated.

sible to use X-ray methods for further collection of diffraction information. An X-ray powder pattern (Table 2) was run in order to find the accurate lattice parameters and the characteristic X-ray data. The group of identification peaks was searched in 1978 with a computer program, Johnson–Vand X-ray powder diffraction search and match system (1969), but no corresponding material was found.

The electron diffraction data could not give complete information (*i.e.* the *c* axis and the true symmetry). However, by using single-crystal X-ray data from large crystals of subsamples 3 and 4, all necessary crystallographic data could be obtained (Table 1). During the work with single-crystal X-ray techniques pronounced twin-formation was observed. The marked tendency of twinning in the plate crystals can be referred to a mistake in the stacking of adjacent layers. This is illustrated in Figure 5.

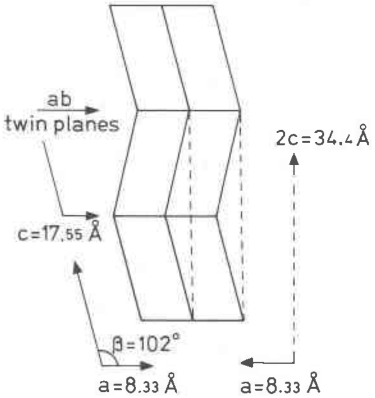

Fig. 5. The twin mechanism among the thin monoclinic crystals giving raise to a pseudo-orthorhombic symmetry with the *c* axis doubled. The *b* axis, 12.86A, is perpendicular to the projection.

Chemical composition

The chemical analyses are presented in Table 4. The chemical formula of the compound may accordingly be written as:

$$KNa_3(Fe_{1.5}Mg_{2.5})(PO_4)_3(OH)_3$$

Iron and magnesium are presumed to substitute for each other, and electroneutrality is satisfied by the addition of three hydroxyl groups. The compound probably does not contain any water molecules, as it is stable up to 350° ± 5°. At this temperature a decomposition takes place, the mechanism of which is

Table 3. X-ray powder patterns of some iron sulfides. The pattern of this work is obtained with a Guinier camera and internally calibrated with KCl (*a* = 6.2930A)

This work		Greigite ASTM 16-713		Mackinawite ASTM 15-37		Pyrite ASTM 6-0710	
d Å	I*	d Å	I	d Å	I	d Å	I
10.31	4						
8.62	3						
5.67	5	5.72	8				
5.03	2			5.03	100		
4.42**	0.5						
4.09**	0.5						
3.63	1						
3.48	4	3.50	30				
3.34***	1						
3.12	2					3.128	36
2.97	10	2.980	100	2.97	80		
		2.855	4				
2.709	8					2.709	84
				2.60	5		
2.471	3	2.470	55				
2.425	7					2.423	66
2.319	2			2.31	80		
		2.260	2				
2.211	6					2.2118	52
2.013	0.5	2.017	10				
1.915	5					1.9155	40
1.898	0.5	1.901	30				
1.839	1			1.838	40		
1.810	1			1.808	80		
1.744	5	1.746	75				
				1.725	40		
		1.671	1				
1.634	8					1.6332	100
1.565	2	1.563	4	1.562	30	1.564	14

* I = relative intensity by visual estimation of data from Debye-Scherrer camera where preferred orientation is supposed not to have taken place.

** Not identified.

***This reflection is more distinct than the other and identical with the strongest reflection for α-SiO_2 at 3.343 Å.

Table 4. Chemical analyses (weight percent)

K	6.63	
Na	11.71	
Mg	10.62	
Fe	14.25	
PO_4	46.33	(48.31)**
OH*	8.64	
Total	98.18	(100.16)**

* added to satisfy electroneutrality of the stoichiometric formula, see text.

**this value will correspond to the chemical formula, see text.

not yet understood. The discrepancy between the total percentage and 100% in the chemical analysis is caused by the analytical data for phosphate. The figure 46.33% is too low to match the chemical formula given above. It should be 48.31%, generating a total percentage of 100.16.

Physical and optical properties

The density can be estimated from the chemical formula (molecular weight = 589) and the volume of the unit cell (1835A^3). If the unit cell contains 4 formula units, then the density will be

$$D_x = (1.66 \times 4 \times 589)/1835 = 2.13 \text{ g/cm}^3$$

This was found to be in good accordance with tests in heavy liquids. Even with the difficulties in handling the very small quantities of crystals, and errors arising from surface tension, the density was observed to be of the order of 2–2.5.

The crystals are transparent with a very slight yellowish tint. The optical properties are somewhat uncertain because of the very small size and extreme thinness of the crystals. Under convergent polarized light the crystals exhibit a negative biaxial interference figure with a moderate axial angle. The birefringence is weak to intermediate.

Conclusions and implications

The new compound is stable under very reduced conditions and in air. It should be searched for in nature, as it may be an important mineral for phosphate fixation in sediments. It was formed only in the cultivations performed at pH $\approx$ 8 and may therefore serve as a paleo-pH indicator for sediment formation. The compound should be looked for in recent sulfidic sediments as well as in sedimentary sulfide ores, preferentially those containing mackinawite. The latter mineral has been found to be formed in continuous cultures preferentially at a pH > 7 (Hallberg, 1972). Suess (1979) has investigated mineral phases formed in recent anoxic sediments of the Baltic Sea. He has indicated the presence of two unknown phosphate compounds by a fractional leaching of the sediment with increasing H^+ ion concentration. Our new compound is stable to weak acids and may, therefore, be related to Suess's second phosphate compound.

A complete single-crystal structure determination and optical investigation is planned.

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