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Phosphine by Bio-Corrosion of Phosphide-Rich Iron.

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Abstract

Phosphine is a toxic agent and part of the phosphorus cycle. A hitherto unknown formation mechanism for phosphine in the environment was investigated. When iron samples containing iron phosphide were incubated in corrosive aquatic media affected by microbial metabolites, phosphine was liberated and measured by gas chromatography. Iron liberates phosphine especially in anoxic aquatic media under the influence of sulfide and an acidic pH.

A phosphine-forming mechanism is suggested: Phosphate, an impurity of iron containing minerals, is reduced abiotically to iron phosphide. When iron is exposed to the environment (e.g. as outdoor equipment, scrap, contamination in iron milled food or as iron meteorites) and corrodes, the iron phosphide present in the iron is suspended in the medium and can hydrolyze to phosphine. Phosphine can accumulate to measurable quantities in anoxic microbial media, accelerating corrosion and preserving the phosphine formed from oxidation.

Keywords

phosphine, environment, corrosion, iron, iron-phosphide, microorganisms, fermentation, anoxic, anaerobe, sulfide, biogas, soil, sediment, manure, landfill, intestinal tract

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Introduction

Phosphine (PH₃), a very toxic agent, is both a raw material and a waste product of industry and food fumigation [1,2]. Recently phosphine was found to be part of the global phosphorus cycle in the environment, as well as in sediments, soils, the intestinal tracts of animals, faecal sludges, animal manure and landfills [3-7]. It was also discovered to be a trace component of the earth's atmosphere [8-9]. The common precept that phosphorus is present in the biosphere only as phosphate and that phosphine is not ubiquitously present on earth [2] has thus been overturned. Nevertheless there is still a lack of information concerning the formation mechanisms of phosphine in the environment.

The biotic reduction of phosphate to phosphine is the subject of controversial discussion [3-12]. Iverson [12] found a black precipitate containing iron phosphide on chips of iron after incubation in a microbial sulfate-reducing medium. Iverson postulated that this iron phosphide is a corrosion product of iron with phosphine formed after microbial phosphate reduction by *Desulfovibrio desulfuricans*.

We had doubts about Iverson's theory is stringent, because the elevated anoxic corrosion of iron is also possible in the absence of PH₃ when promoted by H₂S [13]), a product of sulfate reducing bacteria, or by other weak acid metabolites [14]. Additionally, technical iron regularly contains iron phosphide which can be

transformed to phosphine. It is known that when iron is dissolved in hot mineral acid, the iron phosphide contained is converted to phosphine [15].

The question arises of whether iron phosphide could also react with corrosive aquatic media affected by microbial fermentation (in sediments, intestinal tract, manure or sewage sludge) to phosphine in the environment. Previous experiments to identify the very slow reaction of iron phosphide with water failed due to the high detection limit of phosphine at that time [16]. Recently, we solved these problems of analyzing environmental phosphine traces by introducing adequate cryotrapping [8].

It is also known that matrices like soil and sludges after digestion in hot mineral acid liberate "matrix phosphine" [5, 7]. Its measurement is similar to the analysis of adsorbed phosphine residues in food after fumigation [17].

Experimental Section Our working hypothesis was that microorganisms can accelerate the corrosion of iron and the subsequent conversion of phosphide inclusions in iron to phosphine. Some heavy hydrolyzable phosphide inclusions may not be converted to phosphine and are precipitated as dispersed particles in several soils and sludges.

The guideline of our experiments is to incubate iron samples in corrosive microbial aquatic media and to measure the liberation of "free" phosphine in fermentation gas or of "matrix" phosphine from the medium after reaction with hot hydrochloric acid.

The trace analysis of phosphine was carried out by preconcentration and gas chromatography (25 m 0.53 mm 5 μ CP-Sil5CB GC column and HP NPD) [8].

The maximum potential of corroding iron to liberate phosphine (**Table 1, last row**) depending on its varying P content was measured by the rapid dissolution of the pure metal standard steel 165 H (BREITLÄNDER GmbH, Hamm, Germany) and pure iron 99.999% (Alfa/Johnson Matthey, no. 943001) in mineral acid. 5.6 mg of metal filings were dissolved in 3 ml anoxic 1N HCl (MERCK) at 100°C for 5 min. The dissolution gases were purged with helium, cryotrapped and analyzed by GC for phosphine. The quantity of matrix-bound phosphine in fermentation media was also measured by this procedure.

The abiotic reaction of iron filings in corrosive aquatic media affected by microbial fermentation was carried out in 50-ml disposable syringes in air or nitrogen. To ensure a cell- and enzyme-free reaction, a synthetic fermentation medium (representing conditions in sediments, intestinal tract, manure or sewage sludge) was mixed consisting of 5.6 mg iron filings (about 0.1 mm in diameter) in 2 ml buffer solution containing 0.1 mmol/l of each component (for composition, see **Table 1, col. 1**). After exposure for 2 days the quantity of phosphine liberated was measured by GC.

The reaction of iron chips (about 1 cm² area) in corrosive sulfate-reducing medium was carried out in 150-ml blood serum bottles containing 50 ml medium no. 63 (catalogue DSM, Braunschweig, Germany) at pH 7.6. After anoxic incubation with *Desulfovibrio vulgaris* at 30°C for 8 days the black precipitate covering the chips was removed by ultrasound. Free phosphine in the fermentation gas such as matrix phosphine in the medium and in the precipitate were measured as described above.

The reaction of iron filings (10 mg) in anoxic Schaedler Broth (DIFCO) inoculated with animal slurry (0.1% v/v) was carried out in 150-ml blood serum bottles containing 50 ml medium under nitrogen. After inoculation at 37°C the iron filings were magnetically extracted. Free phosphine in the fermentation gas such as matrix-phosphine in the medium were measured as described above. For abiotic control experiments the inoculated medium was autoclaved and the iron filings were sterilized by exposure to UV light for 2 hours.

Results and discussion

Filings of steel 165 H (rich in P) liberate up to about 9 mg/kg phosphine after a few days exposure in a cell- and enzyme-free synthetic fermentation medium (**Table 1, col. 2**). The quantity of phosphine rises in the presence of sulfide and an acidic pH, which are known to promote metal corrosion. Iron poor in P only liberates minor quantities of phosphine during dissolution in HCl and also during incubation in fermentation media. To test for the possible chemical reduction of phosphate to phosphine, phosphate was added to the reaction mixture. However, the phosphine yield remained unaffected.

What is the origin of this phosphine? Biotic phosphate reduction was discounted because of the absence of cells and their enzymes. No indications were not found of the in situ chemical reduction of phosphate to phosphine. As P-rich iron liberates phosphine, the phosphide in the iron used has to be considered as a source of phosphine.

Therefore, we postulate that the corrosion of iron and the subsequent hydrolysis of iron-borne phosphides are a possible source of phosphine emissions in the environment. Phosphine can accumulate to measurable

quantities in anoxic microbial media accelerating the corrosion and preserving the phosphine formed from oxidation.

High-P iron chips with an area of 1 cm² incubated in a corrosive sulfate reducing medium with *Desulfovibrio vulgaris* liberate 26 pg free phosphine (gas phase) (**Table 2, col. 7**). In the presence of bacterial H₂S, 13.9% (**col. 6**) of the mass of the chip was lost by corrosion. The black precipitate removed from the chip dissolved in HCl liberated up to 2,285 pg phosphine (**col. 9**). Pure iron with a low P level does not corrode and does not significantly produce phosphine. Because phosphate is present in the media, bacterial phosphate reduction to phosphine would not be limited. Hence, in the presence of sulfate-reducing bacteria the liberation of iron-born phosphine is accelerated.

Phosphine is liberated by high-P iron filings incubated in corrosive anoxic Schaedler Broth with 0.1% (v/v) of swine manure (**Table 3**, inoculated). Microbial growth was accompanied by a drop in pH from 7 to 5.5 and the production of H₂S. The comparably high phosphine values in control cultures (**Table 3**, sterile) are caused by the corrosive nature of the sterile Schaedler Broth. However, the phosphine concentrations in sterile media decreased as time progressed, whereas phosphine concentrations in incubated media rose even after microbial growth in Schaedler Broth had reached the stationary phase (after 1 day of incubation). Fermentation in Schaedler Broth may serve as a model for numerous natural and biotechnological processes where weak acid metabolites influence the corrosion of iron.

Conclusions

The corrosion of iron by microbial metabolites followed by the hydrolysis of iron-born phosphides may provide a convincing explanation for the occurrence of phosphine in the environment. A high P-content of iron corroded in an anoxic medium with low pH containing sulfide and other corrosive metabolites accelerate iron corrosion, phosphine liberation and preserve the phosphine formed from oxidation.

In detail we postulate the following multi stage mechanism of phosphine formation in the environment:

1. The chemical production of iron phosphide: The chemical conditions responsible for the reduction of phosphorus (P⁰) or phosphate (P⁺⁵) to phosphide (P⁻³) in iron (high temperatures, oxygen deprivation and an excess of reduction agents like iron) prevail in metallurgical iron melts and in liquid planetary cores. On a cosmic scale most P exists as phosphide because chemically reducing conditions are predominant [18]. Elemental metals present in the crust of the earth indicate strong reduction conditions also capable of reducing phosphate to phosphides [19] as part of minerals.

2. The distribution of iron (phosphide) in the biosphere: Anthropogenic iron containing iron phosphide comes into contact with the biosphere in the form of outdoor equipment (fermenters, basins, pipes) and waste (buried or sedimented scrap iron). Iron as a contaminant of iron milled food [20] enters the "inner biogas fermentor" intestinal tract. Cosmogenic iron phosphide (the mineral "Schreibersite") is part of cosmic falldown (iron meteorites and cosmic dust) [18].

3. The microbial corrosion of iron and conversion of iron phosphide **to phosphine and dispersed phosphides**: The phosphide contained in iron is suspended in the medium. The easily hydrolyzable phosphide particles react spontaneously to form "free" phosphine detectable in biogas [Lit free phosphine Devai, Glind 3,4,8]. Some long-term stable phosphide particles may further be distributed in the medium, become part of soils, sediments or sludges, and also constitute "matrix"-phosphine. Only when boiling these matrices in mineral acid do the stable phosphides form phosphine [5,17].

Is the proposed mechanism a microbial source of phosphine? Yes and no. A medium containing corrosive microbial metabolites may be an important factor elevating the corrosion of iron and the conversion of iron-born phosphide to phosphine. Philosophically this may be expressed as the "microbially mediated environmental chemical formation" [Lit Hanselmann 21] of phosphine. But this does not represent biochemical enzymatic phosphate reduction to phosphine. The question of whether if biogenic phosphate reduction to phosphine is possible cannot be answered at the present time.

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Table 1

Phosphine liberated by iron filings exposed for 2 days to cell- and enzyme-free corrosive media (synthetic fermentation media)

Column 1	2		3		4		5		6		7		8	
	Phosphine (ng P / kg Fe)													
	High-P iron, steel 165 H (0.089 % P)								Low-P iron (< 0.0001% P)					
					100 mMol PO ₄				100 mMol PO ₄					
Storage in complex putrefaction medium and its components (0.1 mmol / l)	Anoxic pH 4.66	Oxic pH 4.66	Anoxic pH 8	Oxic pH 8	Anoxic pH 8	Oxic pH 8	Anoxic pH 4.66	Anoxic pH 4.66	Anoxic pH 4.66	Anoxic pH 4.66	Anoxic pH 4.66	Anoxic pH 4.66	Anoxic pH 4.66	Anoxic pH 4.66
Oxalic acid, in buffer	31,400	15,000					31,900		60		46			
n-alkanic acids (C ₁ ...n-C ₄), in buffer	42,900	49,700					42,800		166		0			
Pure buffer (acetate or borate)	148,900	12,700	1,000	1,800			27,500		30		41			
Gallic acid, in buffer	178,800	30,500	3,000	400			45,400		194		90			
Na-sulfide solution, in buffer	6,674,300	450,700	124,300	9,300			9,302,900		2100		1,800			
Complex medium, in buffer	9,142,900	1,130,100					3,799,400		1700		3,200			
Maximum possible PH ₃ liberation (fast dissolution in hot 1N HCl)	53,260,000								17400					

Table 2

Phosphine liberated by iron chips incubated in a corrosive sulfate reducing medium with *Desulfovibrio vulgaris*

column 1	2	3	4	5	6	7	8	9	10
		Property, iron		Corrosion, iron		Mass of liberated phosphine			H ₂ S in gas (ppm)
		P-content of iron % P	Maximum possible PH ₃ - liberation ^a (pg P / mg Fe)	Mass of chip (1 cm ² area) (mg)	Mass loss (%)	In biogas (pg)	Matrix phosphine in medium (pg)	Matrix phosphine in precipitate (pg)	
		Without metal, sterile	average						
	1					1	48		0
	2					4	95		0
	3					0	15		0
	3					0	35		0
Pure iron, sterile	average	< 0.0001	17	412	0	0	100	3	0
	1			417	0	0	147	4	0
	2			421	0	0	115	3	0
	3			397	0	0	37	3	0
Pure iron, inoculated	average	< 0.0001	17	417	0	1	107	14	1,532
	1			412	0	0	260	13	1,030
	2			418	0	2	25	7	2,140
	3			422	0	0	35	22	1,425
Steel 165 H, sterile	average	0.089	53,260	34	3	12	46	262	0
	1			33	0	12	23	260	0
	2			35	2	10	90	236	0
	3			35	6	15	25	292	0
Steel 165 H, inoculated	average	0.089	53,260	34	14	26	18	2,285	1,930
	1			37	25	52	9	4,538	1,995
	2			33	11	19	36	1,957	1,410
	3			33	6	8	8	359	2,385

^aThe metal is rapidly dissolved in hot 1 N HCl to obtain a high yield of phosphine. The quantity of phosphine liberated is a measure of the quantity of hydrolyzable phosphides in the iron.

Table 3

Phosphine liberated by iron filings incubated in corrosive anaerobic Schaedler Broth with animal manure

Column 1	2	3	4	5	6	7	8	9	10	11
iron standard (10 mg filings)	P-content of metal (%)	Max. possible PH ₃ -liberation ^a (pg P / mg metal)	Free phosphine in biogas (pg)				Matrix phosphine in medium (pg)			
			Sterile		Inoculated		Sterile		Inoculated	
			1d	4d	1d	4d	1d	4d	1d	4d
Without metal			2	3	2	1	120	52	96	11
Steel 1.4301	n.d.	66	0	0	3	9	420	160	74	220
Steel 1.4571	n.d.	17	0	0	10	10	110	120	67	320
Steel 52.3	n.d.	1,928	3	8	1,800	1100	870	210	300	1,600
Steel 165	0.089	53,260	26	41	1,100	1400	1,500	18	3,900	15,200
Cast iron 665	1.11	111,250	280	140	610	520	12,400	15,900	5,600	16,000

^aThe metal is rapidly dissolved in hot 1 N HCl to obtain a high yield of phosphine. The quantity of phosphine liberated is a measure of the quantity of hydrolyzable phosphides in the iron.

Footnote to Table 2 and 3

a) The metal is rapidly dissolved in hot 1 N HCl to obtain a high yield of phosphine. The quantity of phosphine liberated is a measure of the quantity of hydrolyzable phosphides in the iron.