BioMetals 1994, 7, 135–141

Evidence for the interference of aluminum with bacterial porphyrin biosynthesis

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Received 18 May 1993; accepted for publication 6 November 1993

Aluminum (0.74 mM) was found to retard bacterial growth, and enhance porphyrin formation and excretion in *Arthrobacter aurescens* RS-2. Coproporphyrin III was shown to be the main porphyrin excreted by aluminum-exposed *A. aurescens* RS-2 cultures and by RS-2 cultures grown under anoxic conditions. Synthesis and excretion of porphyrins in *A. aurescens* RS-2 increased in a dose-dependent manner when the bacteria were exposed to increasing aluminum concentrations. Incubation of *A. aurescens* RS-2 with δ-aminolevulinic acid (δ-ALA, 1.2 mM) brought about the intense formation and excretion of porphyrins by the cells, in the presence or absence of aluminum. δ-ALA slightly enhanced the toxicity of aluminum towards RS-2 bacteria. Furthermore, the intracellular concentration of heme was reduced by 63.9 ± 8.67% in aluminum-exposed RS-2 bacteria when compared with control cultures. The results are discussed in light of the recent finding concerning aluminum toxicity and porphyrin biosynthesis in microorganisms.

Keywords: aluminum, *Arthrobacter aurescens*, coproporphyrin III, heme, δ-aminolevulinic acid

Introduction

Aluminum is a major constituent of most types of soils and sands. Most of the aluminum in nature exists in non-soluble forms, rendering it practically non-toxic. However, recent reports (Mulder *et al.* 1987, Driscoll & Schecher 1990, Neal *et al.* 1992) showed that aluminum might be mobilized (as its ionic and/or complexed forms) by natural (mainly nitrogen transformations) or man-made processes (industrial emissions and land management practises) from the soil solid phase and thus interact with microorganisms, plants and animals. Ample data has accumulated in recent years regarding the toxic effects of aluminum towards various organisms (Nyholm 1981, Thornton & Davey 1983, Macdonald & Martin 1988, Wood & Cooper 1988, Kok *et al.* 1990, Lindemann *et al.* 1990, Rosseland *et al.* 1990, Jackson-Moss & Duncan 1991). In microorganisms, aluminum was shown to impair microbial membrane fluidity (Vierstra & Haug 1978), interact with bacterial DNA (Johnson & Wood 1990), activate bacterial genes such as the *Escherichia coli fliC* gene (Guzzo *et al.* 1991), bring about mutations in bacteria as seen when *Rhizobium* spp. were exposed to aluminum and tested for enhanced rifampicin resistance (Octive *et al.* 1991), and even possibly act as an inducer of one of the *E. coli* SOS response genes, i.e. the ribonucleoside diphosphate reductase gene (Sitjes *et al.* 1992).

Following the isolation and characterization of *Arthrobacter aurescens* RS-2, which was found to excrete large amounts of coproporphyrin III in response to the presence of aluminum in its growth medium (Scharf *et al.* 1993), a study was initiated to examine interactions of aluminum with bacterial porphyrin biosynthesis. Porphyrins (the oxidized forms of porphyrinogens) are tetracyclic compounds (tetrapyrroles) which serve as metal chelating prosthetic groups in metallo-enyzmes, hemoproteins and cytochromes (Dolphin 1978), all having essential cellular functions. The impairment of porphyrin biosynthesis might be manifested in a wide variety of metabolic processes. The heavy metals silver, cobalt, copper, mercury and lead have all been described as effectors of porphyrin biosynthesis (Dailey 1982, Mori & Sano 1972, Ravishanker & Padmanaban 1985). Recently, aluminum was correlated with interferences in human porphyrin biosynthesis (McGonigle & Parsons 1985, Buchet *et al.* 1987, Bia *et al.* 1989, Rosenlof *et al.* 1990). This study

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presents strong evidence for the interference of aluminum with bacterial porphyrin biosynthesis.

Materials and methods

Bacterial strains and culture conditions

The isolation and characterization of *A. aurescens* RS-2 was described elsewhere (Scharf *et al.* 1993). Strain RS-2 was grown in artificial sea water (ASW, Scharf *et al.* 1993) based medium amended with ($g\,l^{-1}$): Bacto-Tryptone, 5; Bacto-Yeast extract, 2.5; D-Glucose, 1. Aluminum (as $AlCl_3$) was added from a 0.037 M ($1\,g\,l^{-1}$) stock solution before adjusting the medium's pH to 7.0 with 5 N NaOH and autoclaving for 15 min at 121 °C, $1.6\,kg\,cm^{-2}$. $AlCl_3$ stock solution was prepared from a Titrisol-aluminum standard (Merck, Darmstadt, Germany). When used, δ-aminolevulinic acid (δ-ALA; Sigma, St Louis, MO) was dissolved in double-distilled water, filter-sterilized (0.22 μm) and added to the sterile growth medium to a final concentration of 1.2 mM ($200\,mg\,l^{-1}$). After addition of δ-ALA, the pH of the growth medium was re-adjusted to 7.0 using a sterile NaOH solution.

Growth kinetics experiments were performed in RS-2 cultures grown in 100 ml of the above medium (with or without metal addition) in 500 ml flasks at 25 °C, 120 r.p.m. For growth under anoxic conditions, the medium volume was raised to 250 ml in 500 ml flasks. Bacterial growth was monitored through turbidity measurements at 575 nm.

Porphyrin analysis

Porphyrins were extracted from the culture supernatants after pelleting the bacteria at 15 000 × *g* for 5 min at room temperature. Three volumes of ethyl acetate:acetic acid (3:1) were added to the supernatants, the mixture shaken vigorously and the organic phase collected. Porphyrins were back-extracted into 1 volume of 3 M HCl. Extraction of intracellular porphyrins was carried out on cells pelleted (10 000 × *g*, 10 min, 4 °C) from 10 ml culture samples. The cells were washed once with ASW and re-pelleted as above. The cells were resuspended in ASW and 3 volumes of ethyl acetate:acetic acid (3:1) were added. The mixture was vortexed vigorously twice for 30 s and centrifuged at 1500 × *g* for 5 min in a table-top clinical centrifuge. The organic phase was collected for further extraction as described above for culture supernatants.

Porphyrin fluorescence was measured in the acidic phase using a Shimadzu RF-540 spectrofluorophotometer operated at 404 nm excitation and 595 nm emission wavelengths. Bacterial porphyrin concentrations were calculated from a coproporphyrin III (Porphyrin Products, Logan, UT)standard curve. The stock solutions of coproporphyrin III was prepared in dimethyl sulfoxide (DMSO), diluted in ASW to the required concentrations and extracted as described above for culture supernatants. By extracting the standard curve samples, no corrections due to yield-of-extraction were necessary when extracellular concentrations of porphyrins were calculated. However, when extracting intracellular porphyrins, a known amount of coproporphyrin III, prepared and diluted as above (internal standard), was added to a bacterial control sample (after the bacteria were washed and resuspended in ASW) in order to asses the yield of extraction from bacterial cells. Coproporphyrin III served as a standard for porphyrin accumulation since HPLC patterns have shown that it was the major porphyrin in aluminum-treated cultures (see Results). Porphyrin characterization was carried out by HPLC analysis (Lim & Peters 1984, Schoenfeld & Mamet 1991). A porphyrin acids marker kit and protoporphyrin IX (Porphyrin Products) were used as standards for porphyrin characterization in the HPLC analysis. For intracellular heme determination, bacteria grown as described above were harvested by centrifugation (15 000 × *g*, 5 min), washed once with ASW and pelleted as before. Heme determination was carried out by the method of Morrison (1967).

Metal analysis

The concentration of aluminum was verified routinely in every ASW–Al medium batch by flame atomic absorption spectrometry (Clesceri *et al.* 1989). Duplicates of 1.5 ml were centrifuged in polypropylene tubes (15 000 × *g*, 5 min). Then, 1 ml of the supernatant was transferred into a 10 ml volumetric flask and the volume was adjusted to 10 ml with 20 mM HNO_3. Aluminum levels in the samples were determined immediately after preparation using a Perkin-Elmer 460 flame atomic absorption spectrometer.

Results and discussion

Aluminum enhanced proporphyrin formation and excretion

A. aurescens RS-2 was isolated from sea water, as described previously (Scharf *et al.* 1993). Aluminum retarded bacterial growth (Figure 1) and enhanced the

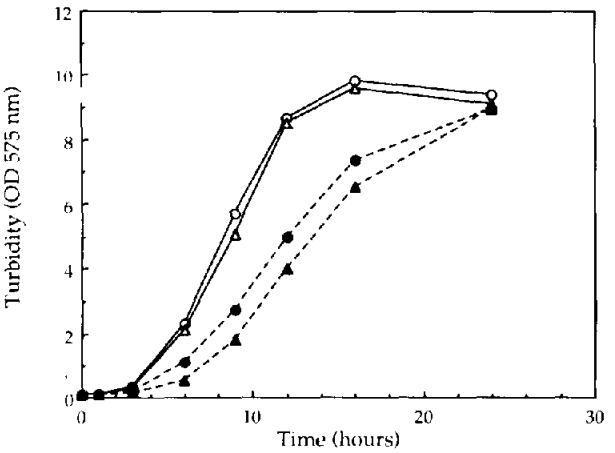

Figure 1. Growth kinetics of *A. aurescens* RS-2 in the presence or absence of aluminum (0.74 mM), with or without δ-ALA (1.2 mM). The results represent numerous (more than 10) independent experiments. ○, control; ●, aluminum; △, ALA; ▲, Al + ALA.

formation and excretion of coproporphyrin III in this bacterium (Figure 2B and Table 1). Cadmium, cobalt, copper and zinc (1 mM each), all having a negative effect on bacterial growth of *A. aurescens* RS-2, did not have a similar effect on coproporphyrin III production (Scharf *et al.* 1993).

More than a 5-fold increase in coproporphyrin III levels was detected in the medium of RS-2 cultures grown in the presence of 0.74 mM aluminum for 24 h, as compared with levels found in the medium of control cultures (Table 1). Most of the coproporphyrin III accumulated extracellulary and aluminum did not cause a parallel increase in intracellular coproporphyrin III levels (Table 1). Growth under oxygen limitation (referred to as *anoxic* conditions), which was previously reported to cause enhanced porphyrin excretion in other *Arthrobacter* spp. (Middleton & Gunner 1968) had similar effects on *A. aurescens* RS-2 (Figure 2C). Oxygen limited cells which accumulated extracellulary 12-fold more coproporphyrin III than control cells (after 24 h of growth), exhibited a 19-fold increase in intracellular coproporphyrin III levels (Table 1). Uroporphyrin, hepta-, hexa- and pentacarboxylic porphyrins, all being upstream precursors of coproporphyrin III (Figure 3), could be detected clearly only in supernatants of oxygen limited cultures (Figure 2C). Tricarboxylic porphyrins, which were implicated as the intermediate forms of the enzymatic oxidative decaboxylation of coproporphyrinogen III protoporphyrinogen IX by coproporphyrinogen III oxidase (Figure 3, Cavaleiro *et al.* 1973, Smith & Belcher 1974), were detected in elevated levels in supernatants of aluminum treated and anoxic cultures but not in control cultures (Figure 2). Protoporphyrin IX was almost undetected in any of the treatments (Figure 2).

Increasing the concentrations of aluminum to 0.74 mM resulted in a dose-dependent increase in the extracellular accumulation of porphyrins (Figure 4) even though the

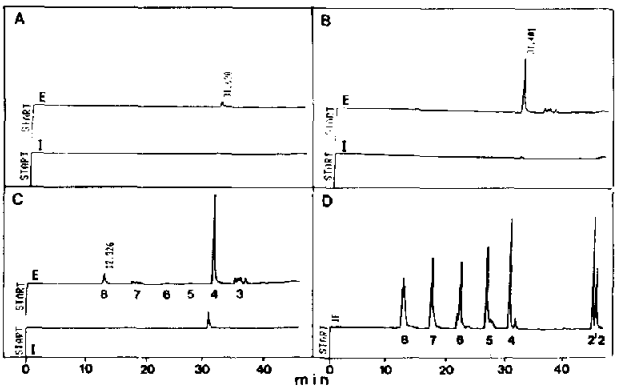

Figure 2. HPLC patterns of intra- (I) and extra- (E) cellular porphyrins in *A. aurescens* RS-2 cultures grown under the following conditions: (**A**) control, (**B**) aluminum (0.74 mM) and (**C**) anoxia. The separation of a porphyrin standard mix is shown in (**D**). The numbers under the peaks indicate carboxylic groups: 8, uroporphyrin; 7, heptacarboxylic porphyrin; 6, hexacarboxylic porphyrin; 5, pentacarboxylic porphyrin; 4, coproporphyrin III; 3, tricarboxylic porphyrins; 2', mesoporphyrin; 2, protoporphyrin.

Table 1. The concentration of porphyrins in cultures of *A. aurescens* RS-2 grown with aluminum or under anoxia

Treatment	Porphyrin concentration (pmol mg^{-1} protein)	
	cells	medium
Control	4.3	395.7
Aluminum	6.3	2216
Anoxia	82.8	4796.6

The results represent the mean of five independent experiments.

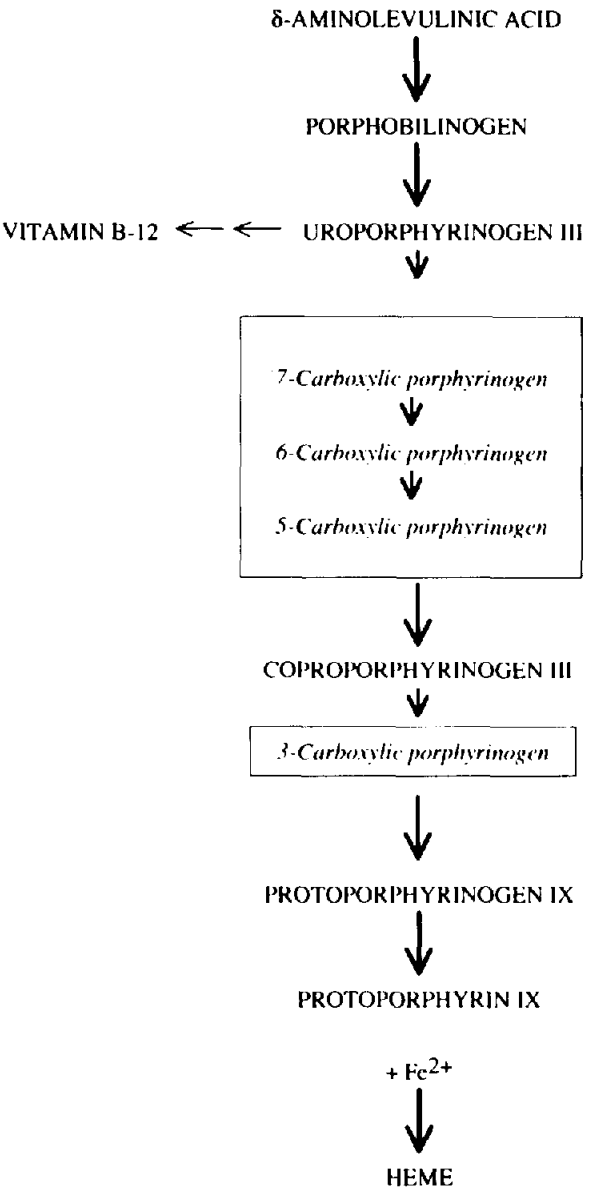

Figure 3. The biosynthetic pathway of porphyrins. The boxed-italicized porphyrins are enzymatic intermediates (adapted from Cavaleiro *et al.* 1973 and Biel 1992).

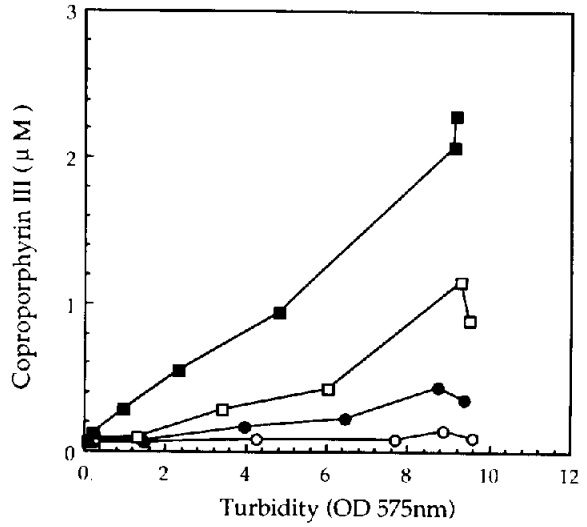

Figure 4. The aluminum dose-dependent accumulation of coproporphyrin III in supernatants of *A. aurescens* RS-2 cultures. The results represent the mean of three independent experiments. ○, control; ●, 0.074 mM Al; □, 0.37 mM Al; ■, 0.74 mM Al.

growth rates were negatively correlated with the concentration of aluminum (data not shown). Ploting the porphyrin concentrations, resulting from samples taken at various time intervals, against the corresponding culture turbidity values, as previously done by Biel (1992), permitted a comparison between porphyrin levels in cultures stressed with ascending aluminum concentrations, growing at descending growth rates. Enhanced porphyrin excretion in RS-2 cultures was affected by as little as 0.074 mM (2 mg l^{-1}) aluminum. A negative effect on bacterial growth with this concentration of aluminum was also detected (data not shown).

The enhanced formation and excretion of porphyrins by aluminum could be explained, in part, by the direct induction of an enzyme (or enzymes) in the heme biosynthetic pathway, prior to coproporphyrinogen III oxidase, by aluminum. Coproporphyrinogen III decarboxylation (protoporphyrinogen formation) was reported to be a rate-limiting step in bacterial porphyrin biosynthesis (Javor & Febre 1992, Oelze 1992) and the accumulation of coproporphyrin III was observed when the heme biosynthetic pathway was amplified (Avissar & Nadler 1978, Javor & Febre 1992). Hence, a possible amplification, by aluminum, of the biosynthetic pathway upstream from coproporphyrinogen III could result in coproporphyrin III accumulation. Our current results cannot completely rule out or substantiate this possibility and a study which examines the effect of aluminum on the activity of the 'upstream' enzymes of heme biosynthesis in *A. aurescens* RS-2 is currently being conducted.

The effect of δ-ALA on bacterial growth

δ-ALA is the first committed metabolite initiating the biosynthetic pathway towards coproporphyrinogen III and other tetrapyrrolic metabolites (Figure 3). Its multi-stage enzymatic formation via various pathways is regarded as one of the major control points in microbial porphyrin biosynthesis, upstream from coproporphyrinogen III (Avissar & Nadler 1978, Biel 1992). The addition of δ-ALA to growing cells usually results in the amplification of porphyrin biosynthesis. Since aluminum was suspected to affect bacterial porphyrin biosynthesis (and through this to affect bacterial growth), the growth of strain RS-2 in the presence of aluminum (0.74 mM) with exogenously added δ-ALA (1.2 mM) was studied in order to determine whether a possible amplification of the bacterial porphyrin biosynthetic pathway, induced by δ-ALA, could ameliorate aluminum toxicity. δ-ALA had almost no effect on the growth of control cultures whereas it had a slight inhibitory effect on the growth of RS-2 cells exposed to aluminum (Figure 1). However, the addition of δ-ALA resulted in the enhanced formation and excretion of all the precursors of heme, from uroporphyrin through protoporphyrin IX, including the 7-, 6-, 5-, 4- and 3-carboxylic intermediates (HPLC patterns not shown). A roughly 40-fold increase in the concentration of extracellular porphyrins was observed when RS-2 bacteria were incubated with δ-ALA, both in the presence or absence of aluminum. Thus, the cells responded positively, with regard to porphyrin biosynthesis, to δ-ALA addition. It is important to note that this was the only treatment where protoporphyrin IX was clearly detected by HPLC, though in very low levels, about 2% of the total porphyrins detected. This implied that the fact that protoporphyrin IX was not detected in the control or in other treatments, e.g. aluminum or anoxia, was due to its extremely low levels in the bacteria and not to the extraction method used.

The fact that δ-ALA did not ameliorate aluminum toxicity but rather enhanced it could be attributed to some yet unknown interaction of δ-ALA with aluminum which

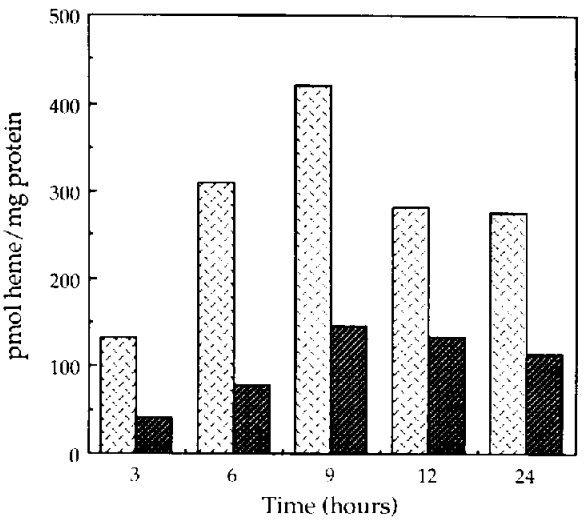

Figure 5. The intracellular heme levels of *A. aurescens* RS-2 during growth in the presence or absence of aluminum. The results represent the mean of three independent experiments. ⊡, control; ▩, aluminum.

might have enhanced the latter's toxicity (such as extracellular complexation/chelation of aluminum resulting in a more rapid influx of the metal). Furthermore, since porphyrins were shown to have a toxic sensitizing effect towards bacteria (Nir *et al.* 1991) such massive porphyrin formation might have acted intracellularly in concert with aluminum to produce a more pronounced inhibitory effect on bacterial growth.

The effect of aluminum on intracellular heme concentrations in A. aurescens *RS-2*

A. aurescens RS-2 was found to be an obligate aerobic bacterium, as slight changes in aeration conditions produced pronounced negative effects on bacterial growth (data not shown). Since *A. aurescens* RS-2 is an obligate aerobic bacterium, heme must play an important role in its energy metabolism. Growth of *A. aurescens* RS-2 in the presence of aluminum (0.74 mM) resulted in markedly decreased intracellular heme levels throughout all the bacterial growth phases as compared with the heme levels in the control cultures (Figure 5). On the average, a significant (t-test, $P < 0.001$) decrease of $63.9 \pm 8.67\%$ in intracellular heme contents was found in aluminum-stressed RS-2 cultures, as compared with the control cultures. Such low intracellular heme levels, mediated by aluminum, could at least partially explain the aluminum induced growth retardation observed in this aerobic bacterium.

The detection of elevated levels of coproporphyrin III in cultures grown in the presence of aluminum together with the aluminum-related heme deficiency observed in these cultures, raises the possibility that aluminum inhibits coproporphyrinogen III oxidase, the enzyme catalyzing the oxidative decarboxylation of coproporphyrinogen III to protoporphyrinogen IX, thus leading to heme deficiency and to the accumulation of this enzyme's substrate, in its oxidized form (coproporphyrin III). In spite of the logic of this possibility, it is probably not the case here since tricarboxylic porphyrins, whose formation and detection implies that coproporphyrinogen III oxidase activity is not impaired (Cavaleiro *et al.* 1973, Smith & Belcher 1974), were detected in aluminum-treated RS-2 cultures (Figure 2B). Furthermore, their detection in aluminum-treated cultures (as in the anoxic cultures; Figure 2C) might indicate that under these conditions this enzyme's activity is greater than in the control cultures. However, we were unable to detect any activity of coproporphyrinogen III oxidase activity in cell-free extracts of RS-2 bacteria grown either in the absence or presence of aluminum (data not shown). This was probably due to an extremely low activity of this enzyme in *A. aurescens* RS-2. Therefore, it was impossible to rule out or confirm an inhibitory effect of aluminum on this enzyme's activity. Similar findings, e.g. undetected coproporphyrinogen III oxidase activity, have already been reported in plants (Bevins *et al.* 1992), where metabolites downstream of coproporphyrinogen III (e.g. chlorophyll) were formed; hence, coproporphyrinogen III oxidase was present and active, but its activity *in vitro* was not detected.

A more likely explanation of the aluminum-related heme deficiency in *A. aurescens* RS-2 is the possibility that aluminum interferes with a downstream biosynthetic stage following coproporphyrinogen III decarboxylation, such as protoporphyrinogen IX oxidation or the insertion of iron into protoporphyrins IX, catalyzed by ferrochelatase (Figure 3), thus leading to heme deficiency. As might have taken place in anoxic RS-2 cultures, the aluminum-exposed bacteria might have amplified their porphyrin biosynthesis in order to overcome the deficiency in heme, brought about by aluminum. Similar findings were reported for mutants of *Saccharomyces cerevisiae*, defective in protoporphyrin IX or heme formation (Urban-Grimal & Labbe-Bois 1981) and for a *c*-type cytochrome-deficient *Rhodobacter capsulatus* mutant (Biel & Biel 1990). However, the amplification of porphyrin biosynthesis usually results in the accumulation of other upstream precursors such as uroporphyrin, which was almost undetected in aluminum-treated RS-2 cells, but could be clearly detected in RS-2 cultures grown under limited aeration (anoxia) and in cells incubated with δ-ALA. This finding might be connected to the fact that the increase in the accumulation of intra- or extracellular porphyrins was much lower in the presence of aluminum when compared with that under anoxic conditions or in the presence of δ-ALA, thus resulting in much lower levels of upstream precursors in aluminum-exposed cultures. Such amplification of porphyrin biosynthesis could have been mediated through the lower intracellular heme levels observed in aluminum-exposed bacteria since heme was reported to down-regulate various steps in microbial porphyrin biosynthesis (Burnham & Lascelles 1963).

Coproporphyrin III excretion and heme deficiency were reported in cases of aluminum impaired iron metabolism (Bia *et al.* 1989) and bacterial iron deficiency (Townsley & Neilands 1957). Furthermore, bacterial iron deficiency exacerbated aluminum toxicity in *E. coli* (Guida *et al.* 1991). Since iron and aluminum have similar effective ionic radii (Hughes & Poole 1989), the enzyme ferrochelatase, catalyzing iron insertion into protoporphyrin IX (Figure 3) might be a target to aluminum inhibition, directly or indirectly, through its iron requirements for proper function. Our recent findings show (R. Scharf, unpublished results) that *A. aurescens* RS-2 accumulated several-fold higher intracellular aluminum levels than other *Arthrobacter* spp., which were found to be more resistant towards aluminum and did not show enhanced porphyrin excretion in the presence of aluminum. Thus, it can be speculated that high intracellular aluminum levels in RS-2 might interfere with bacterial iron metabolism. The complexation chemistry of aluminum in solution under the conditions used in this work (e.g. the use of a complex organic medium with high ionic strength) did not permit us to study this hypothesis and further analysis is carried on in order to verify the connection between aluminum, iron and porphyrin metabolism in *A. aurescens* RS-2.

Acknowledgments

We are indebted to Dr R. Zelikson for her helpful comments and assistance in this work. The excellent technical assistance of Mrs R. Ravid and Mrs R. Mevasser is gratefully acknowledged.

References

Avissar YJ, Nadler KD. 1978 Stimulation of tetrapyrrole formation in *Rhizobium japonicum* by restricted aeration. *J Bacteriol* **135**, 782–789.

Bevins M, Yang CM, Markwell J. 1992 Characterization of chlorophyll-deficient mutant of sweetclover (*Melilotus alba*). *Plant Physiol Biochem* **30**, 327–331.

Bia MJ, Cooper K, Schnall S, Duffy T, Hendler E, Malluche H, Solomon L. 1989 Aluminum induced anemia: pathogenesis and treatment in patients on chronic hemodialysis. *Kidney Int* **36**, 852–858.

Biel AJ. 1992 Oxygen-regulated steps in the *Rhodobacter capsulatus* tetrapyrrole biosynthetic pathway. *J Bacteriol* **174**, 5272–5274.

Biel SW, Biel AJ. 1990 Isolation of a *Rhodobacter capsulatus* mutant that lacks *c*-type cytochromes and excretes porphyrins. *J Bacteriol* **172**, 1321–1326.

Buchet JP, Lauwerys R, Hassoun A, et al. 1987 Effect of aluminum on porphyrin metabolism in hemodialyzed patients. *Nephron* **46**, 360–363.

Burnham BF, Lascelles J. 1963 Control of porphyrin biosynthesis through a negative-feedback mechanism. *Biochem J* **87**, 462–472.

Cavaleiro JAS, Kenner GW, Smith KM. 1973 Biosynthetic intermediates between coproporphyrinogen-III and protoporphyrin IX. *JCS Chem Commun* 183–184.

Clesceri LS, Greenberg AE, Trussell RR, eds 1989 *Standard Methods for the Examination of Water and Wastewater*, 17th edn. Washington: American Public Health Association, **section 3111D**, pp. 3–25.

Dailey HA. 1982 Purification and characterization of membrane-bound ferrochelatase from *Rhodopseudomonas spheroides*. *J Biol Chem* **257**, 14714–14718.

Dolphin D. 1978 *The Porphyrins*. New York: Academic Press.

Driscoll CT, Schecher WD. 1990 The chemistry of aluminum in the environment. *Environ Geochem Health* **12**, 28–49.

Guida L, Saidi Z, Hughes MN, Poole RK. 1991 Aluminium toxicity and binding to *Escherichia coli*. *Arch Microbiol* **156**, 507–512.

Guzzo A, Diorio C, DuBow MS. 1991 Transcription of the *Echerichia coli fliC* gene is regulated by metal ions. *Appl Environ Microbiol* **57**, 2255–2259.

Hughes MN, Poole RK. 1989 *Metals and Microorganisms*. London: Chapman & Hall.

Jackson-Moss CA, Duncan JR. 1991 The effect of aluminim on anaerobic digestion. *Biotech Lett* **13**, 143–148.

Javor GT, Febre EF. 1992 Enzymatic basis of thiol-stimulated secretion of porphyrins by *Escherichia coli*. *J Bacteriol* **174**, 1072–1075.

Johnson AC, Wood M. 1990 DNA, a possible site of action of aluminum in *Rhizobia* spp. *Appl Environ Microbiol* **56**, 3629–3633.

Kok CJ, Meesters HWG, Kempers AJ. 1990 Decomposition rate, chemical composition and nutrient recycling of *Nymphaea alba L.* floating leaf blade detritus as influenced by pH, alkalinity and aluminum in laboratory experiments. *Aqua Bot* **37**, 215–227.

Lim CK, Peters TJ. 1984 Urine and faecal porphyrin profiles by reverse-phase high performance liquid chromatography in the porphyrias. *Clin Chem Acta* **139**, 55–63.

Lindemann J, Holtkamp E, Herrmann R. 1990 The impact of aluminum on green algae isolated from two hydrochemically different headwater streams, Bavaria, Germany. *Environ Pollut* **67**, 61–77.

Macdonald TL, Martin RB. 1988 Aluminum ion in biological systems. *Trend Biochem Sci* **13**, 15–19.

McGonigle RJ, Parsons V. 1985 Aluninium-induced anaemia in haemodialysis patients. *Nephron* **39**, 1–9.

Middleton KS, Gunner HB. 1968 Porphyrin production by *Arthrobacter globiformis*. *Can J Microbiol* **14**, 1095–1103.

Mori M, Sano S. 1972 Studies on the formation of protoporphyrin IX by anaerobic bacteria. *Biochem Biophys Acta* **264**, 252–262.

Morrison JR. 1967 Fluorometric micro-determination of heme protein. *Anal Chem* **37**, 1124–1126.

Mulder J, van Grinsven JJM, van Breemen. 1987 Impacts of acid atmospheric deposition on woodland soils in the Netherlands: III. Aluminum chemistry. *Soil Sci Soc Am J* **51**, 1640–1646.

Neal C, Reynolds B, Smith CJ, *et al.* 1992 The impact of conifer harvesting on stream water pH, alkalinity and aluminium concentrations for the british uplands: an example for an acidic and acid sensitive catchment in mid-Wales. *Sci Total Environ* **126**, 75–87.

Nir U, Ladan H, Malik Z, Nitzan Y. 1991 *In vivo* effects of porphyrins on bacterial DNA. *J Photochem Photobiol B: Biol* **11**, 295–306.

Nyholm E. 1981 Evidence of involvment of aluminum in causation of defective formation of eggshells and of impaired breeding in wildpasserine birds. *Environ Res* **26**, 363–371.

Octive JC, Wood M, Johnson AC. 1991 mutagenic effects of aluminium. *Mutat Res* **264**, 135–137.

Oelze J. 1992 Light and oxygen regulation of the synthesis of bacteriochlorophylls *a* and *c* in *Chloroflexus aurantiacus*. *J Bacteriol* **174**, 5021s–5026.

Ravishanker CH, Padmanaban G. 1985 Regulation of cytochrom P-450 gene expression. *J Biol Chem* **260**, 1588–1592.

Rosenlof K, Fyhrquist F, Tenhunen R. 1990 Erythropoietin, aluminium, and anaemia in patients on haemodialysis. *Lancet* **335**, 247–249.

Rosseland BO, Eldhuset TD, Staurnes M. 1990 Environmental effects of aluminum. *Environ Geochem Health* **12(1/2)**, 17–27.

Scharf R, Zimmels Y, Kimchie S. 1993 Metal induced extracellular protein excretion in *Arthrobacter aurescens*. *FEMS Microbiol Lett* **109**, 139–144.

Schoenfeld N, Mamet R. 1991 High-performance liquid chromatographic detection of pitfalls in porphobilinogen deaminase determination. *J Chromatogr* **570**, 51–64.

Sitjes J, Ysern P, Barbe J, Llagostera. 1992 Induction of ribonucleoside diphosphate reductase gene transcription by chemicals in *Escherichia coli*. *Mutagenesis* **7**, 47–49.

Smith SG, Belcher RV. 1974 Distribution of some intermediates of the haem biosynthesis. *Enzyme* **17**, 1–10.

Thornton FC, Davey CB. 1983 Acid tolerance of *Rhizobium trifolii* in culture media. *Soil Sci Soc Am J* **47**, 496–501.

Townsley PM, Neilands JB. The iron and porphyrin metabolism of *Micrococus lysodeikticus*. *J. Biol Chem* **224**, 695–705.

Urban-Grimal D, Labbe-Bois R. 1981 Genetic and biochemical characterization of mutants of *Saccharomyces cerevisiae* blocked in six different steps of heme biosynthesis. *Mol Gen Gen* **183**, 85–92.

Vierstra R, Haug A. 1978 The effect of Al^{3+} on the physical properties of membrane lipids in *Thermoplasma acidophilum*. *Biochem Biophys Res Commun* **84**, 138–143.

Wood M, Cooper JE. 1988 Acidity, aluminum and multiplication of *Rhizobium trifolii*: possible mechanisms of aluminum toxicity. *Soil Biol Biochem* **20**, 95–99.