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Mechanism of aluminium-induced porphyrin synthesis in bacteria

Rivka Mamet*, Ram Scharf‡, Yoram Zimmels‡, Shlomo Kimchie‡ & Nili Schoenfeld*,†

**The Laboratory of Biochemical Pharmacology, Beilinson Medical Center, Petah Tikva, †The Department of Pathological Chemistry, Sackler Faculty of Medicine, University of Tel Aviv, Ramat Aviv and ‡The Department of Environmental Engineering, Technion-Israel Institute of Technology, Haifa, Israel*

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In previous studies, aluminium was found to retard bacterial growth and enhance porphyrin formation in *Arthrobacter aurescens* RS-2. The aim of this study was to establish the mechanism of action of aluminium which leads to increased porphyrin production. Cultures of *Arthrobacter aurescens* RS-2 were incubated in the absence and presence of 0.74 mM aluminium. After 6 and 24 h of incubation, various parameters of the haem biosynthetic pathway were determined. After 6 h of incubation with aluminium, the activities of the enzymes aminolevulinate synthase (ALAS), aminolevulinate dehydratase (ALAD), porphobilinogen deaminase (PBGD) and uroporphyrinogen decarboxylase (UROD) were increased by 120, 170, 190 and 203%, respectively, while that of ferrochelatase (FC) was found to be unchanged. However, after 24 h of incubation, no change in the activities of ALAS and ALAD was noted, while an about 2-fold increase in PBGD and UROD activities were observed. FC activity was decreased by 63%. It was concluded that aluminium exerts its effect by inducing the enzymes PBGD and UROD rather than by a direct or indirect effect on ALAS. Its effect on the final step in the haem biosynthetic pathway is discussed.

Keywords: aluminium, bacteria, porphobilinogen deaminase, porphyrin synthesis, uroporphyrinogen decarboxylase

Introduction

A toxic effect of aluminium was reported in a vast range of organisms, from bacteria to humans (Macdonald & Martin 1988, Wood & Cooper 1988, Rosseland *et al.* 1990). In fish exposed to relatively high levels of aluminium, severe haematological disturbances were noted (Witters *et al.* 1990). Aluminium-induced anaemia as well as increased erythrocyte protoporphyrin were reported in patients on chronic haemodialysis (McGonigle & Parson 1985, Rosenlof *et al.* 1990, Fontanellas *et al.* 1994). Various changes in the haem biosynthetic pathway described in uraemic patients were attributed to the elevated level of aluminium in plasma (Buchet *et al.* 1987, Bia *et al.* 1989). In a recent study it was shown that in a system of *Arthrobacter aurescens* RS-2, aluminium caused retardation in bacterial growth concomitantly with about 65% reduction in intracellular haem and a marked enhancement (5-fold) of porphyrin synthesis (Scharf *et al.* 1994). It was suggested that the toxic effects of aluminium towards bacteria might be connected to the interference of the metal with the haem biosynthetic pathway (Scharf *et al.* 1994). This work was carried out in order to further investigate the mechanism of action of aluminium which leads to impairment of porphyrin synthesis.

Address for correspondence: N. Schoenfeld, Laboratory of Biochemical Pharmacology, Beilinson Medical Center, 49 100 Petah Tikva, Israel. Tel: (+ 972) 3 9377710; Fax: (+ 972) 3 9219685.

Materials and methods

Materials

The radiochemicals [2,3-^{14}C]succinic acid (56 mCi mmol^{-1}) and $^{55}FeCl_2$ (0.8 mCi mol^{-1}) used for determining aminolevulinate synthase (ALAS) and ferrochelatase (FC) activity, respectively, were obtained from New England Nuclear (Boston, MA). Titrisol-aluminium standard, as well as solvents for HPLC, acetonitrile and methanol, were purchased from Merck (Darmstadt, Germany). Porphyrin acids marker kit and pentacarboxyl porphyrin I were obtained from Porphyrin Products (Logan, UT), sodium and mercury from Aldrich (Milwaukee, WI), and other chemicals used for the various enzymatic assays from Sigma

(St Louis, MO). All other chemicals used were of the highest purity available.

Bacterial culture conditions

A. aurescens RS-2, isolated and characterized as described previously (Scharf *et al.* 1993), was grown on artificial sea water-based medium, amended with Bacto-Tryptone, 5 g l^{-1}; Bacto-Yeast extract, 2.5 g l^{-1} and D-glucose, 1 g l^{-1}. Aluminium (as $AlCl_3$) was added from a 0.037 M stock solution (final concentration: 0.74 mM). The pH was adjusted to 7.0 with 5 N NaOH. When used, δ-aminolevulinic acid (ALA) was added to the medium at a final concentration of 1.2 mM. The pH of the medium was then readjusted to 7.0.

Determination of enzymes activities

Bacterial cells were harvested by centrifugation 6 and 24 h after seeding and suspended in 0.05 M Tris buffer, pH 7.4, containing 0.25 M sucrose. The suspensions were disrupted by sonic oscillation, 15 x 10 s cycles using a Branson sonic power sonifier S125. The sonicates were used for the various enzymatic assays.

ALAS (EC 2.3.1.37) and aminolevulinate dehydratase (ALAD) (EC 4.2.1.24) activities were determined according to Brooker *et al.* 1982) and Del C Battle *et al.* (1967), respectively.

Porphobilinogen deaminase (PBGD) (EC 4.3.1.8) activity was evaluated basically according to Magnussen *et al.* (1974). The quantification of the uroporphyrin was performed by a HPLC method (Schoenfeld & Mamet 1991, Lim & Peters 1984), as described previously.

The activity of uroporphyrinogen decarboxylase (UROD) (EC 4.1.1.37) was determined according to Luo & Lim (1991) with the following modifications: the substrate used was pentaporphyrinogen I, as described previously (McManus *et al.* 1988) and the termination of the reaction was carried out by adding DMSO and TCA to the incubation mixture (final concentrations 25 and 5%, respectively). The coproporphyrin formed was measured by a HPLC method (Lim & Peters 1984).

The method of Deybach *et al.* (1981) with slight modifications was employed for studying FC (EC 4.99.1.1).

Protein was determined according to Lowry *et al.* (1951).

Measurement of ALA, porphobilinogen (PBG) and porphyrins

Intracellular ALA and PBG were measured using the methods of Berko & Durko (1972) and Buttery & Stuart (1991), respectively. For analysis of porphyrins the medium was diluted 1:5 in 1N HCl and 100 μl was injected directly onto the system.

HPLC system

A HP 1090L solvent delivery system (Hewlett-Packard, Avondale, PA), equipped with a Rheodyne 7010 injector (Rheodyne, Cotati, CA) and a 100 μl external loop, was used. A type 73XX inlet filter was installed between the sample injector and the column. A HP reversed-phase column was used (100 mm x 4.6 mm I.D., HP Hypersil ODS, 5 μm) and the fluorescence was measured by a programmable fluorescence detector, HP 1046. The excitation wavelength was 404 nm and the emission wavelength was 615 nm. Quantification was performed by a HP-3393, a computing integrator. The separation procedure of Lim & Peters (1984) was employed.

Results and discussion

It is well accepted that coproporphyrinogen III decarboxylation by coproporphyrinogen III oxidase (CO) (EC 1.3.3.3) is a rate-limiting step in bacterial porphyrin biosynthesis (Javor & Febre 1992, Oelze 1992) and therefore amplification of the above-mentioned pathway, under various conditions, results in accumulation of coproporphyrin (Javor & Febre 1992, Avissar & Nadler 1978, Philipp-Dormston & Doss 1973). The same phenomenon was observed in *A. aurescens* RS-2 cultures, in which 5-fold elevation of coproporphyrin was recorded after treatment with 0.74 mM aluminium (Scharf *et al.* 1994). The activity of CO was undetectable in the above system; therefore, we could not rule out or confirm a direct inhibitory effect of aluminium on the enzyme's activity. However, indirectly, on the basis of circumstantial evidence, we came to the conclusion that aluminium does not reduce CO activity (Scharf *et al.* 1994). It may therefore be assumed that aluminium exerts its inducing effect by interfering with another stage in the pathway. The enhanced formation of porphyrins in the presence of aluminium could be the result of one or a combination of the two following possibilities: (1) Due to the reduction of intracellular haem induced by aluminium (Scharf *et al.* 1994), ALAS activity is increased as a result of the negative feedback mechanism exerted by haem on ALAS, leading to overproduction of the pathway products. (2) Aluminium activates directly an enzyme (or enzymes) of the pathway, between ALAS and CO, i.e. ALAD, PBGD and UROD.

In order to establish the mechanism of action of aluminium-induced coproporphyrin formation, its effect on the various stages of the haem biosynthetic pathway was examined.

The effect of aluminium on the initial part of the haem biosynthetic pathway

The first step of the haem biosynthetic pathway, i.e. formation of ALA catalysed by ALAS, is considered to be the rate limiting step and site of regulation by haem in mammals (May & Bawden 1989) and in various bacterial systems (Lascelles 1968, Tait 1973). In *A. aurescens* cells a 65% reduction in intracellular haem was observed, following addition of 0.74 mM aluminium to the culture medium (Scharf *et al.* 1994). If the control mechanism described above

existed in *A. aurescens*, increased ALAS activity as well as elevated ALA would have been expected to be measured, concomitantly with the decrease in the concentration of haem. However, the concentration of ALA in the cells grown in the presence of aluminium did not differ from that observed in control cultures (not shown) and a slight increase only (20%) was noted in ALAS activity 6 h after addition of aluminium to the medium (Table 1). No change was observed after 24 h incubation. It was therefore concluded that aluminium exerts its effect on porphyrin metabolism by affecting a further stage.

The effect of aluminium on the 'midstream part' of the pathway

We defined the midstream part of the pathway as the chain of reactions which lead to formation of coproporphyrin from ALA. In order to locate the site of action of aluminium it was added to cultures in which porphyrin synthesis was amplified due to the presence of 1.2 mM ALA, and the pattern of porphyrins formed and excreted to the medium was followed and compared to that observed in the absence of aluminium. The HPLC chromatograms of medium porphyrins demonstrated in Figure 1 show that after 6 h incubation while the concentration of coproporphyrin was similar in both treatments, uroporphyrin was observed only in the cells derived from cultures incubated in medium containing aluminium. Moreover, after 24 h in the presence of aluminium its effect on the concentration of highly carboxylated porphyrins was even more pronounced resulting in 5.6-, 5-, 2- and 3-fold increases in uroporphyrin, heptacarboxyporphyrin, hexacarboxyporphyrin and pentacarboxyporphyrin, respectively. Only a 50% increase

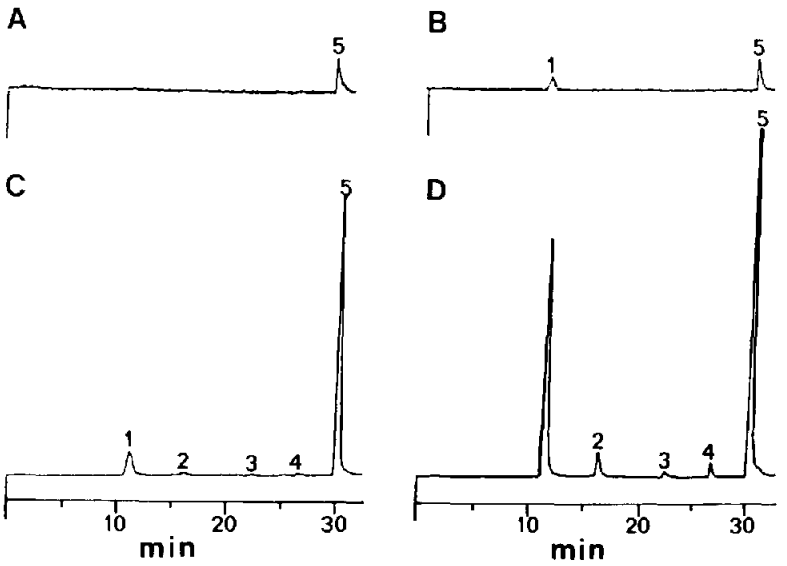

Figure 1. HPLC chromatograms of extracellular porphyrins of *A. aurescens* RS-2 cells grown in the presence of 1.2 mM ALA, in the absence (A and C) and presence (B and D) of aluminium, for 6 (A and B) and 24 h (C and D). The numbers above the peaks indicate: (1) uroporphyrin, (2) heptacarboxyporphyrin, (3) hexacarboxyporphyrin, (4) pentacarboxyporphyrin and (5) coproporphyrin.

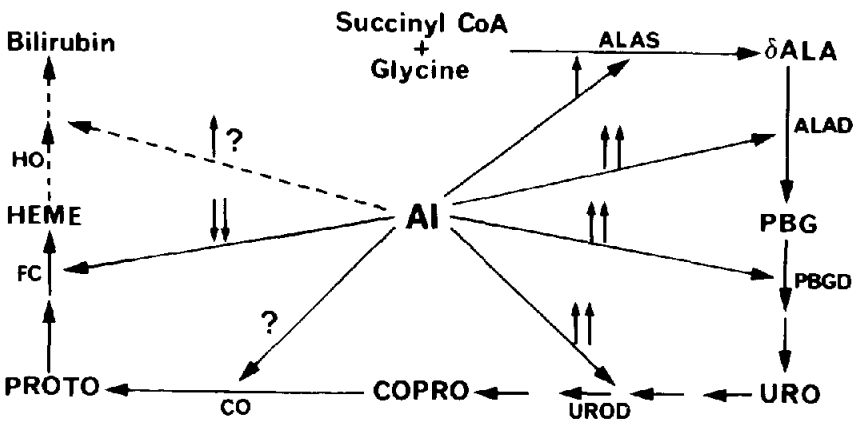

Figure 2. The proposed mechanism of action of aluminium which leads to amplification of porphyrin synthesis.

Table 1. The activity of enzymes of the haem biosynthetic pathway in bacteria incubated in medium containing aluminium

Enzyme	Basal activity (nmol product mg protein^{-1} h^{-1}) mean ± SD (n = 6)	Activity in the presence of aluminium (%)	
		6 h	24 h
ALAS	0.68 ± 0.08	120	100
ALAD	2.80 ± 0.3	170	100
PBGD	0.78 ± 0.06	270	310
UROD	0.36 ± 0.08	203	208
FC	0.15 ± 0.03	90	37

The activity of each enzyme measured in bacteria grown in a medium which did not contain aluminium was considered as 100%. The values obtained after 6 and 24 h incubation with aluminium (0.74 mM) were calculated accordingly.

in coproporphyrin, the predominating porphyrin, was observed.

In view of the above, it was suggested that aluminium induces both PBGD which catalyses the formation of uroporphyrin and UROD which is involved in the synthesis of coproporphyrin. As shown in Table 1 the induction effect of aluminium was noted 6 h after its addition to the medium. It should be pointed out that aluminium increased also the activity of ALAD (Table 1). An activation of ALAD by aluminium in an *in vivo* system in rats was previously described (Abdulla *et al.* 1979). In any case, since the basal activity of ALAD is much higher than the activities of the other enzymes of the pathway, an increase in this enzyme is not expected to affect the overall porphyrin synthesis. In agreement with the above finding, PBG, the product of ALAD, was similar in cells treated with aluminium when compared with control cells. Therefore, it may be concluded that the activations of PBGD and UROD play a crucial role in the amplification of porphyrin synthesis by aluminium.

The effect of aluminium on the final part of the haem biosynthetic pathway

A marked decrease (65%) in intracellular haem was observed already 3 h after addition of aluminium to the culture medium (Scharf *et al.* 1994). The aluminium-related haem deficiency could be explained by a previous inhibition of FC, the enzyme which catalyses haem formation. However, a 10% reduction in the enzyme's activity was measured only after 6 h of incubation in the presence of aluminium (Table 1), while a 65% decrease was noted 9 h following aluminium administration (not shown). Therefore it seems that the decrease in FC activity is not the only factor which determines the concentration of intracellular haem. Haem deficiency reported in aluminium-exposed bacteria might be a result of both reduction in its synthesis as well as an enhancement in its degradation. The latter could result from an inducing effect of aluminium on haem oxygenase [HO], the rate-limiting enzyme in haem catabolism (Chmielnicka *et al.* 1994).

Conclusion

The suggested mechanism of action of aluminium which leads to amplification of porphyrin synthesis is demonstrated in Figure 2. As shown in Figure 2, aluminium exerts its effect by activating the midstream enzymes of the haem biosynthetic pathway (ALAD, PBGD and UROD) and reducing the final part (FC). Coproporphyrin predominates due to the fact that CO is the rate-limiting enzyme in the above system. The profile of porphyrins obtained in other systems in the presence of elevated levels of aluminium may be different, in relation to the location of the rate limiting step.

References

Abdulla M, Svensson S, Haeger-Aronson B. 1979 Antagonistic effect of zinc and aluminum on lead inhibition of delta amino-levulinic-acid dehydratase EC-4.2.1.24. *Arch Environ Health* **34,** 464–469.

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

Berko G, Durko I. 1972 A new possibility for the demonstration of amino-laevulinic acid in urine on the basis of Mauzerall–Granick method. *Clin Chim Acta* **37,** 443–447.

Bia MJ, Cooper K, Schall S, *et al.* 1989 Aluminum induced anemia: pathogenesis and treatment in patients on chronic hemodialysis. *Kidney Int* **36,** 852–858.

Brooker JD, Srivastava G, May BK, Elliott WH. 1982 Radiochemical assay for δ-aminolevulinate synthase. *Enzyme* **28,** 109–119.

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

Buttery JE, Stuart S. 1991 Measurement of porphobilinogen in urine by a simple resin method with use of a surrogate standard. *Clin Chem* **37,** 2133–2136.

Chmielnicka J, Nasiadek M, Lewandowska-Zyndul E. 1994 The effect of aluminum chloride on some steps of heme biosynthesis in rats after oral exposure. *Biol Trace Element Res* **40,** 127–136.

Del C Battle AM, Ferramola AM, Grinstein M. 1967 Purification and general properties of delta-amino-levulinate dehydratase from cow liver. *Biochem J* **104,** 244–249.

Deybach JC, de Verneuil H, Nordmann Y. 1981 The inherited enzymatic defect in porphyria variegata. *Hum Genet* **58,** 425–428.

Fontanellas A, Coronel F, Santos JL, *et al.* 1994 Heme biosynthesis in uremic patients on CAPD or hemodialysis. *Kidney Int* **45,** 220–223.

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

Lascelles J. 1968 The regulation of haem and chlorophyll synthesis. *Biochem Soc Symp* **28,** 49–59.

Lim CK, Peters TJ. 1984 Urine and faecal porphyrin profiles by reverse-phase high performance liquid chromatography in the porphyrias. *Clin Chem Acta* **139,** 55–65.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJH. 1951 Protein measurement with folin-phenol reagent. *J Biol Chem* **193,** 265–275.

Luo G, Lim CK. 1991 Random decarboxylation of uroporphyrinogen III by human hepatic uroporphyrinogen decarboxylase. *J Chromatogr* **566,** 409–413.

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

Magnussen CR, Levine JB, Doherty JM, Cheesman JO, Tschudy DP. 1974 A red cell enzyme method for diagnosis of acute intermittent porphyria. *Blood* **44,** 857–868.

May BK, Bawden MJ. 1989 Control of heme biosynthesis in animals. *Sem Hematol* **26,** 150–156.

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

McManus J, Blake D, Ratnaike S. 1988 An assay of uroporphyrinogen decarboxylase in erythrocytes. *Clin Chem* **34,** 2355–2357.

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

Philipp-Dormston WK, Doss M. 1973 Comparison of porphyrin and heme biosynthesis in various heterotrophic bacteria. *Enzyme* **16,** 57–64.

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 effect of aluminum. *Environ Geochem Health* **12,** 17–27.

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

Scharf R, Mamet R, Zimmels Y, Kimchie S, Schoenfeld, N. 1994 Evidence for the interference of aluminum with bacterial porphyrin biosynthesis. *BioMetals* **7,** 135–141.

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

Tait GH. 1973 Control of aminolaevulinate synthase in *Micrococcus denitrificans*. *Enzyme* **16,** 21–27.

Witters HE, Van Puymbroeck S, Van Den Sande I, Vanderborght OLJ. 1990 Haematological disturbances and osmotic shifts in rainbow trout, *Onchorhynchus mykiss* [Walbaum] under acid and aluminum exposure. *J Comp Physiol B* **160,** 563–571.

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