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Endogenous Porphyrin Production in Bacteria by δ-Aminolaevulinic Acid and Subsequent Bacterial Photoeradication

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Abstract. In the present study we examined the effects of the accumulation of endogenous porphyrins on the microorganisms *Staphylococcus aureus* and *Escherichia coli*. δ-Aminolaevulinic acid (δ-ALA) 50 μg/ml can induce production of large amounts of porphyrins when incubated with the bacteria in the dark. This porphyrin production within the cells was examined directly by a fluorescence activated cell sorter (FACS) instrument and a significant increase in red fluorescence was observed. Incubation of δ-ALA with these microorganisms did not slow down their growth. δ-ALA or δ-ALA methyl ester induced accumulation of uroporphyrin in *S. aureus* cells and excretion of coproporphyrin into the growth medium. In *E. coli*, these inducers resulted in the accumulation of uroporphyrin and protoporphyrin within the cells and excretion of coproporphyrin and protoporphyrin from the cells. Photoinactivation of the porphyrin-loaded bacteria can be achieved by various light sources, depending on the dose. The most effective photokilling of *S. aureus* and of *E. coli* by its endogenic porphyrins was achieved by blue light (400–450 nm) at approximately 50 J/cm^2. With red light (600–700 nm), a 10-fold higher light dose was required to get a similar result for *S. aureus* killing. With a laser beam (632.8 nm), 50 J/cm^2 was necessary for 3 orders of decrease in the viability of *S. aureus*. With white light, 75 J/cm^2 was needed for 2–3 orders of decrease of *S. aureus* viability. With the last two light sources eradication of *E. coli* required at least 10 times higher doses of light. It seems that *S. aureus* is more susceptible than *E. coli* to photosensitisation when it is loaded with endogenous porphyrins. Ultrastructural alterations were observed in the bacteria incubated with δ-ALA in the dark and photosensitised by blue light (400–450 nm). Filamentous chromosomes were observed in *E. coli*, whereas honeycomb mesosomes were observed in *S. aureus*, with a destruction of the area around these mesosomes in the chromosomal area. The method described here is an additional approach to the photoinactivation of bacteria by porphyrins, with photoinactivation being accomplished by endogenous porphyrins.

Keywords: δ-Aminolaevulinic acid; *Escherichia coli*; Photoinactivation; Porpyrins; *Staphylococcus aureus*

INTRODUCTION

Photokilling of various Gram-positive bacteria has been demonstrated using photodynamic therapy (PDT) with exogeneous sensitisers [1,2]. Efficient exogenous photosensitisers are mainly porphyrins such as haematoporphyrin, haematoporphyrin derivative, deuteroporphyrin or aluminium phthalocyanine [1–5]. In contradistinction, Gram-negative bacteria are apparently not sensitive to the lethal action of PDT, since the porphyrins could not be transported into the cell due to the presence of the bacterial outer membrane, which serves as a permeation barrier for hydrophobic substances [1,6–9]. However, after enhancement of the permeabilisation of the outer membrane by exposure to polymyxin B nonapeptide (PMBN), which is a non-toxic disorganising agent, exogenous sensitisers were able to enter the bacterial cell membrane and illumination then killed the bacteria [9,10]. Cationic porphyrins have also been found to be efficient photosensitisers for inactivation of Gram-negative bacteria [11,12].

Since bacterial resistance to antibiotics is becoming an increasing problem, research is being directed towards PDT as an alternative approach for killing bacteria [13]. PDT can be used clinically in benign diseases and its uses are increasing. A new method of photosensitising cells is achieved by enhancing endogenous

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production of porphyrins after the addition of δ-aminolaevulinic acid (ALA), a naturally occurring metabolite in the synthesis of cellular haem. The addition of δ-ALA leads to an increase in synthesis of uroporphyrin, coporphyrin and protoporphyrin IX, the immediate precursor of haem [14].

Protoporphyrin produced in this way appears to be clinically useful in the photodynamic treatment of certain skin cancers. δ-ALA is a water-soluble, low-molecular-weight substance. Therefore, ALA may also enter the intracellular compartment of Gram-negative bacteria through hydrophilic pores in the outer membrane constituents of these bacteria [15].

The aim of this study was to determine the sensitivity of *Staphylococcus aureus* and *Escherichia coli* to photokilling by endogenously produced porphyrins. These two bacteria are representatives of Gram-positive and Gram-negative bacteria, respectively. It has been shown [16,17] that in most bacterial species an induction of porphyrin production occurs as a consequence of ALA addition to the culture media. Photodynamic therapy was undertaken by various light sources, including laser or intense light at various wavelengths.

MATERIALS AND METHODS

Bacterial Strains

Two bacterial strains were used in this study. *S. aureus* (strain 195), coagulase positive, DNAase positive belongs to phage type O [88/89/95]. The strain was resistant to methicillin, cephalothin, gentamicin, erythromycin, chloramphenicol and tetracycline. *E. coli* of serotype O_{111} B_4 was resistant to ampicillin, chloramphenicol, tetracycline and sulfadiazine. Both strains were recovered from clinical material at the Bacteriological Laboratory of Meir Hospital, Kfar-Saba, Israel.

Porphyrin Synthesis Induction

Overnight cultures of *S. aureus* or *E. coli* grown on brain heart infusion agar (Difco, Detroit, MI) were transferred into nutrient broth (Difco) at pH 6.5 to a final volume of 25 ml at an initial optical density of 0.1 at 660 nm and were allowed to grow at 37°C with aeration. δ-ALA or δ-ALA methyl ester (Sigma, USA) at a concentration of 50 μg/ml was added when the cultures reached an OD of 0.3 (at the beginning of the log phase). Bacterial growth was determined with a Novaspec, Biochrom LKB (Cambridge, UK) spectrophotometer by the increase in optical density at 660 nm as a function of time. Bacterial cultures grown under the same conditions but without any δ-ALA additions, served as controls.

Porphyrin Extraction and Identification

It was possible to detect the produced porphyrins by their extraction with 0.1 N NH_4OH : acetone (1 : 9, v/v) [18]. Intracellular porphyrins were extracted from the cell pellet after centrifugation. Extracellular porphyrins, which were excreted from the cells, were extracted from the growth medium supernatant after lyophilisation and extraction by the same procedure. Endogenous and excreted porphyrins were identified by HPLC using a C-18 column and a reversed phase system. Elution was performed on a gradient consisting of 10% acetonitrile in 1 M ammonium acetate, pH 5.1 (solvent A) and 10% acetonitrile in methanol (solvent B). The relative amounts were corrected to the same number of bacteria and calculated accordingly. HPLC measurements were performed at the Porphyrin Laboratory of the Rabin Medical Center, Beilinson Campus, Petach-Tikva, Israel.

Fluorescence Measurements

After extraction of the porphyrins, their fluorescence spectra were determined by a Perkin–Elmer model LS-50 spectrofluorometer interfaced to a data station 7500 computer. The excitation wavelength was 405 nm. Extractions from cultures without δ-ALA were used as controls.

Fluorescence Activated Cell Sorter (FACS)

Differences in the inner fluorescence of the bacteria during incubation with δ-ALA was detected with a FACS Calibre instrument (Becton-Dickinson). Bacteria were grown in culture medium with or without δ-ALA for the indicated times. Samples were taken at the beginning of the experiment and at the

indicated times. They were washed with 0.1 M phosphate-buffered saline (PBS), pH 6.9, and mixed by vortex until absolute separation of the cells, and applied to the FACS for fluorescence determination. Excitation of fluorescence was obtained by constant intensity of a laser beam at 410 nm and emission at 630 nm.

Illumination Methods

Illumination methods for photoinactivation of bacteria were as follows. White light was obtained by two tungsten lamps with an intensity of 140 W/m^2, placed at a distance of 30 cm and 45° above both sides of the test flasks. An intensed light system with a Universal ARC lamp (Ealing, MA, USA) was used and a red light filter at 600–700 nm was attached giving a light intensity of 80 W/m^2. An intensed light system with the same lamp and a blue light filter at 400–500 nm was attached giving an intensity of 80 W/m^2. A HeNe laser beam was used with an intensity of 15 W/m^2 at 632.8 nm.

Photoinactivation

Samples (2 ml) were transferred from culture media containing δ-ALA or controls without δ-ALA to small plates and were immediately illuminated under the various lights with gentle stirring. The light dose delivered and the wavelength varied according to the various illumination methods. Bacterial survival after photoinactivation was determined by plating. Viability was counted on agar plates from the proper dilutions and the survival rate was calculated according to the equation N/N_0=survival fraction, where N_0 is the number of colony-forming units (cfu)/ml at zero time and N is the number of cfu/ml in the samples which underwent illumination. The controls without δ-ALA were also illuminated and counted.

Preparation for Transmission Electron Microscopy

Photosensitiser-treated *S. aureus*, *E. coli* and untreated control cultures were: rinsed with saline; fixed in 2.5% glutaraldehyde/paraformaldehyde at room temperature for 1 h; washed with veronal acetate buffer; and fixed in 1% osmium tetroxide and uranyl acetate. The cells were dehydrated with ethanol and embedded in epon, thin-sectioned using an LKB ultratome III and examined with a Jeol 1200EX transmission electron microscope.

Data Calculation

All experiments were performed in triplicate. Each experimental treatment with the various light sources was repeated five times and the results presented are the means of all these experiments. Fluorescence measurements were also repeated four times.

The difference of error in the presented results fell within 10% and the curves were drawn as the tendency of the line.

RESULTS

Incubation of each of the tested bacterial species (*E. coli* or *S. aureus*) with δ-ALA (50 μg/ml) in the dark resulted in the following when bacteria were examined and separated by FACS. Figure 1 demonstrates a large increase in fluorescence in the cultures incubated with δ-ALA. In *E. coli* cultures incubated with δ-ALA, there was a moderate increase in the fluorescence of the treated cultures as compared with the untreated controls (Fig. 1c, d). A larger difference was observed in the δ-ALA-treated cultures of *S. aureus* (Fig. 1a, b). The results indicate more porphyrin synthesis in the δ-ALA incubated cultures. This porphyrin synthesis and accumulation is the result of δ-ALA induction. Observation of the pattern of porphyrin synthesis as a function of incubation time with δ-ALA for 18 h showed increased synthesis of porphyrins in the cells and concomitant excretion of porphyrins from the bacteria into the extracellular medium. Figure 2 shows the pattern of porphyrin production and excretion as a function of time. It was also found to be correlated to the growth curve of each bacterial species. The ratio was corrected and calculated for the same number of bacteria in the culture; the same relationship was also found for the extracellular porphyrins. A high ratio of porphyrin presence in the medium has also been shown in other bacterial species [16].

The emission peak of the *S. aureus* intracellular porphyrin was 622 nm and that of *E. coli* was 632 nm. A slight difference in the emission peaks of the extracellular excreted porphyrins was found: 617 nm for *S. aureus*

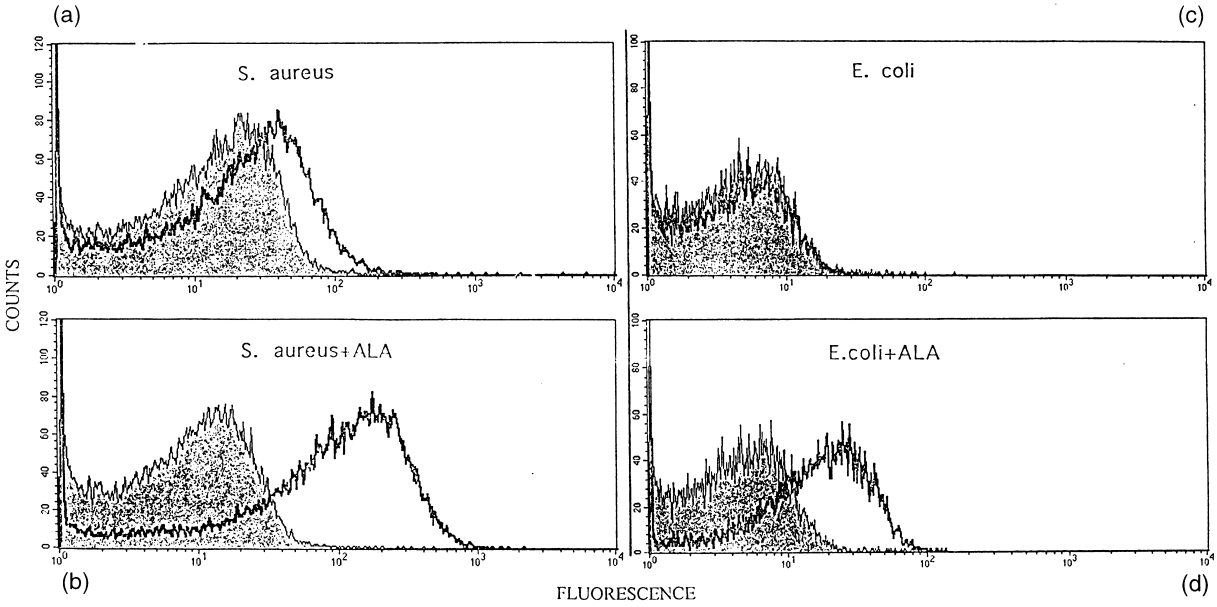

Fig. 1. Effect of incubation with (b, d) and without (a, c) δ-ALA on the intrinsic fluorescence of *E. coli* (c, d) or *S. aureus* (a, b) by flow cytometry. The shaded area: examination at zero time; bright area: examination after 18 h. (a, c) Untreated controls of bacteria which were grown at the same time.

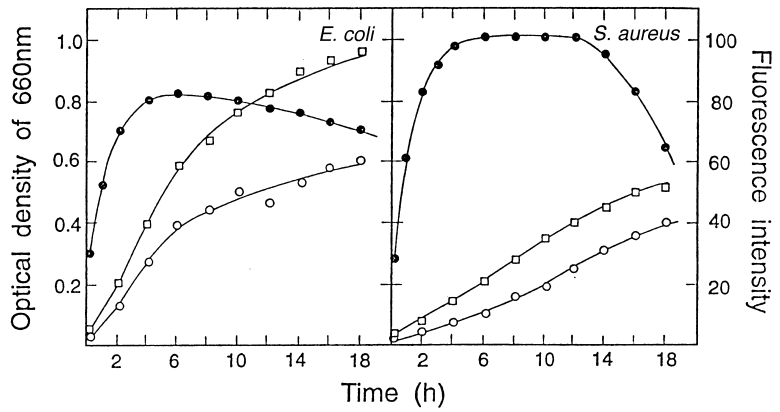

Fig. 2. Bacterial growth curve, porphyrin accumulation within the cells and excretion from the cells as a function of incubation time with δ-ALA. *S. aureus* and *E. coli* growth curves were determined by optical density at 660 nm (●). Corrected fluorescence emission intensities for intracellular produced porphyrins (○) and for porphyrins excreted to the medium (□). Excitation at 405 nm.

and 615 nm for *E. coli*. The porphyrins produced were also identified by HPLC and the amounts calculated (Table 1). The results clearly show that the major intracellular porphyrin is uroporphyrin, which comprised 65.3% in *E. coli* and 89% in *S. aureus*. In the presence of δ-ALA methyl ester as an inducer instead of δ-ALA, the amount of intracellular uroporphyrin was even higher and comprised 85.3% in *E. coli* and 93.7% in *S. aureus*. In contradistinction, the excreted porphyrin was mainly coproporphyrin: 57.2% by *E. coli* and 95% by *S. aureus*. After induction with δ-ALA methyl ester this was 61.1% by *E. coli* and 76.4% by *S. aureus*. Protoporphyrin was produced by *E. coli* at approximately 21.1%, whereas very low or no protoporphyrin was found in *S. aureus*. Induction by δ-ALA methyl ester demonstrates a lower amount of protoporphyrin within the bacterial cells of *E. coli* (only 5.5%). The other porphyrins consisted of 10.5% in *E. coli* and 8.4% in *S. aureus*. A similar pattern was observed when porphyrin synthesis induction was performed with δ-ALA methyl ester. These other porphyrins are also present in the extracellular excreted portion of porphyrins. According to the HPLC elution patterns, these other porphyrins consisted mainly of penta-, hexa- and hepta-carboxylicporphyrin.

Table 1. Intracellular production and extracellular excretion of different porphyrins upon induction by δ-ALA or δ-ALA methyl ester

	Induction by δ-ALA (%)				Induction by δ-ALA methyl ester (%)			
	Uro	Copro	Proto	Others	Uro	Copro	Proto	Others
Intracellular production								
E. coli	65.3	3.1	21.1	10.5	85.3	2.1	5.5	7.1
S. aureus	89.0	1.8	0.8	8.4	93.7	1.3	—	5.0
Extracellular excretion								
E. coli	2.0	57.2	22.7	18.1	—	61.1	27.7	11.2
S. aureus	0.4	95.0	—	4.6	13.8	76.4	—	9.8

Uro, uroporphyrin; Copro, coproporphyrin; Proto, protoporphyrin.

It was possible to inactivate cultures of *S. aureus* or *E. coli* grown with δ-ALA or δ-ALA methyl ester by photosensitisation. Photosensitisation of the bacteria was carried out under controlled illumination parameters of wavelength and light dose. The viability of *S. aureus* or *E. coli* cultures, after induction of porphyrin synthesis by δ-ALA or δ-ALA methyl ester, decreased very significantly but was dependent on the illumination method (Fig. 3). Illumination of both bacteria with blue light (Fig. 3a) after incubation with δ-ALA or δ-ALA methyl ester revealed that the viability of *S. aureus* incubated with δ-ALA or δ-ALA methyl ester is decreased at a light dose of approximately 50 J/cm^2 by 3–4 orders of magnitude. For *E. coli*, the same light dose of 50 J/cm^2 was needed for the same reduction of viability when prior to the illumination it was incubated with δ-ALA. When incubated with δ-ALA methyl ester the light dose required was much higher for the same effect (beyond the frame of the figure). With red light (Fig. 3b), a light dose of tenfold or higher than with the blue light was needed for 3–4 orders decrease of *S. aureus* viability or 1–2 orders decrease of the viability of *E. coli* (when incubated with δ-ALA methyl ester). In this case, 500 J/cm^2 was required for the decrease in viability of *S. aureus*. A very high light dose was needed for *E. coli* incubated with δ-ALA. The exact light dose has not been investigated and we did not obtain even 1 order of magnitude photokilling of *E. coli* even with 500 J/cm^2. Illumination with the laser beam revealed that 50 J/cm^2 are necessary for 3 orders of magnitude decrease in the viable count of *S. aureus*, whereas a very high dose of light will be needed for the same decrease for *E. coli*. With white light, 75 J/cm^2 were needed for 2 orders of magnitude decrease of *S. aureus*, whereas for *E. coli* it was again not possible to obtain a decrease of viability much beyond 1 order of magnitude.

Transmission electron microscope analysis of *E. coli* or *S. aureus* cells incubated with 50 μg/ml δ-ALA for 12 h and exposed to intense blue light (400–450 nm) revealed major ultrastructural alterations in the bacterial cell organisation. Bacterial cells incubated in the dark with δ-ALA exhibited a normal *E. coli* structure identical to the untreated cell controls (Figs 4a and b, respectively). However, the formation of distinguished fibrous structures located in the chromosomal region was demonstrated in photosensitised *E. coli* cells (Fig. 4c). *S. aureus* cells incubated with δ-ALA exhibited ultrastructural alterations (Fig. 5b) and were different from the untreated control cells (Fig. 5a). These induced *S. aureus* cells developed honeycomb-like mesosomes inside the cytoplasm near the septa areas of the dividing cells, even in the dark. Photosensitised *S. aureus* after induction exhibited a mesosomal structure in the middle of the cytoplasm (Fig. 5c). The majority of the observed mesosomes had a honeycomb structure which was connected to chromosomal fibres. Intracellular destruction was greater in the photoinactivated *S. aureus* cells than in the photoinactivated *E. coli* cells.

DISCUSSION

Photosensitisation of bacteria by endogenously produced porphyrins is of growing interest in the field of photodynamic therapy [16,17,19]. This approach is a new concept for the eradication of bacteria after establishing the photodynamic effects on Gram-positive and Gram-negative bacteria [1–3,6,9,11–13,20–22].

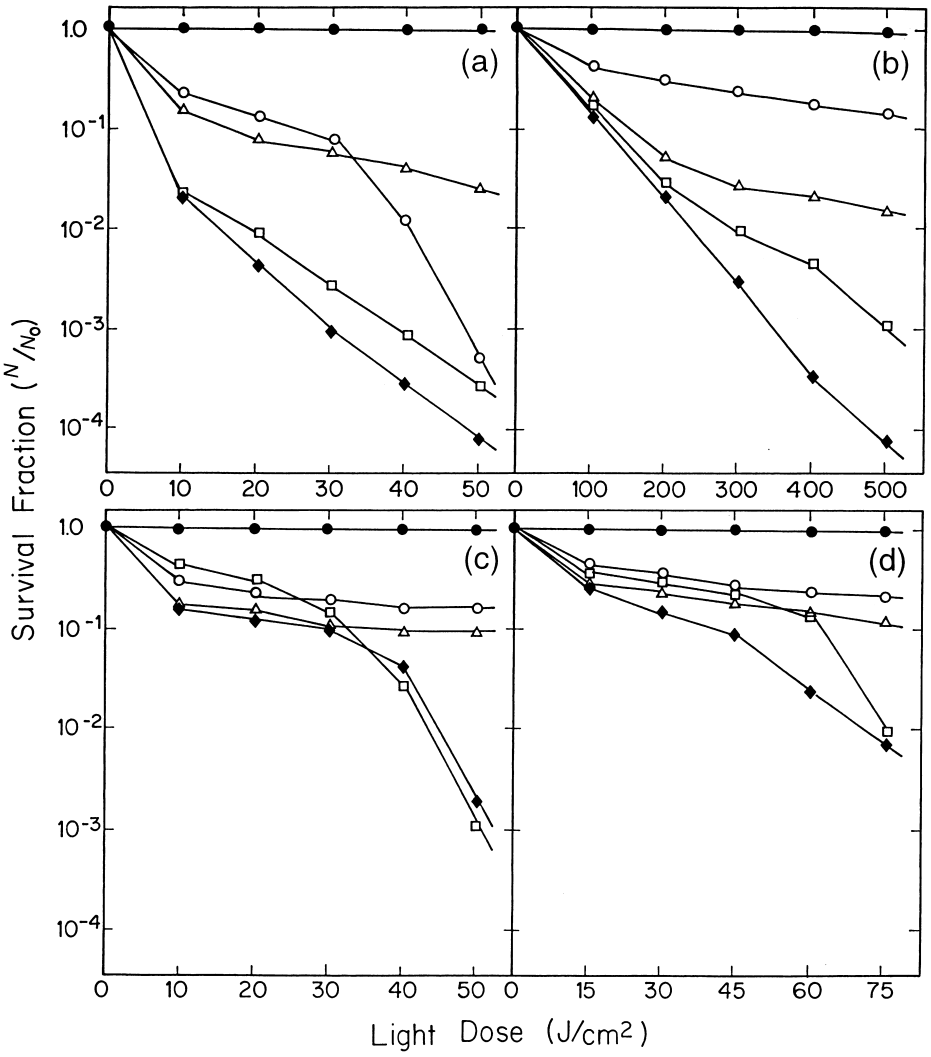

Fig. 3. Survival fractions of *E. coli* and *S. aureus* cultures grown with δ-ALA or δ-ALA methyl ester and illuminated with blue light (a) red light (b) laser beam (c) or white light (d). *E. coli* cultures were incubated with δ-ALA (○) or δ-ALA methyl ester (△). *S. aureus* cultures were incubated with δ-ALA (◆) or δ-ALA methyl ester (□). Control cultures were not incubated with δ-ALA or with δ-ALA methyl ester but were illuminated in the same way (●). Colony forming units were counted as a function of light dose (J/cm^2). Difference of error of the points shown fell within 10%.

Endogenous porphyrins can be produced in high quantities after incubation with δ-aminolaevulinic acid [14]. The latter compound is a precursor of the protoporphyrin synthesis pathway in the bacterial cells for the production of haemin, the iron-containing protoporphyrin [15]. It has previously been shown that induction of porphyrin synthesis is a consequence of the incubation of the cells with δ-ALA [16]. Enhanced porphyrin production has been observed in Gram-positive bacteria such as *S. aureus* or *B. megaterium* and in Gram-negative bacteria such as *E. coli*, *Aeromonas hydrophila*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii* or *Serratia marcescens*.

The phenomenon of porphyrin induction by δ-ALA has been demonstrated by fluorescence measurements of extracted porphyrins from the bacteria [16]. In the former study, the produced porphyrins were also excreted into the external medium. This may be a result of overproduction of the porphyrins and, as a consequence of their solubilised state, they may diffuse into the growth medium. The present study has shown that when high amounts of porphyrins are present in the cells, they can be detected by flow cytometry, demonstrating an enhancement in the intrinsic fluorescence intensity. This is direct proof of the intrinsic processes induced by the precursor of porphyrin synthesis, i.e. δ-ALA. The

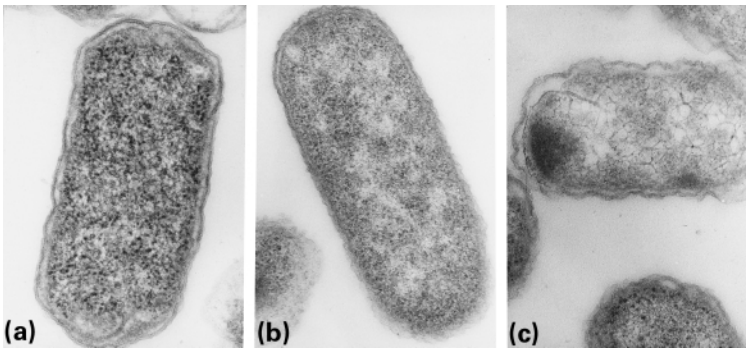

Fig. 4. Transmission electron microscopy of *E. coli* incubated in the dark with δ-ALA and illuminated with blue light (400–450 nm). (a) *E. coli* untreated control; (b) *E. coli* incubated with δ-ALA in the dark for 12 h; (c) *E. coli* incubated with δ-ALA in the dark for 12 h and illuminated. Magnification: ×60 000.

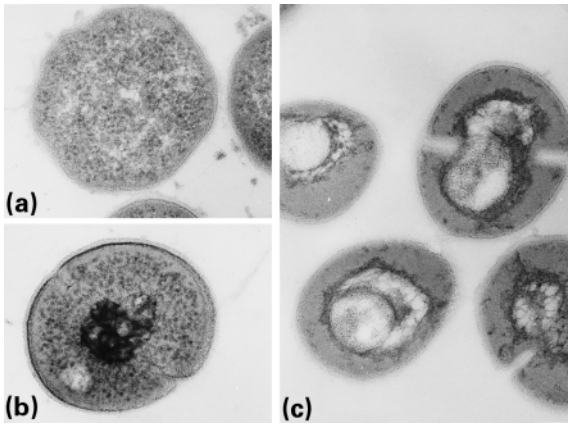

Fig. 5. Transmission electron microscopy of *S. aureus* incubated in the dark with δ-ALA and illuminated with blue light (400–450 nm). (a) *S. aureus* untreated control; (b) *S. aureus* incubated with δ-ALA in the dark for 12 h; (c) *S. aureus* incubated with δ-ALA in the dark for 12 h and illuminated. Magnification: ×60 000.

FACS results do not indicate which porphyrins are synthesised or are present at a specific time. Porphyrin content obtained after induction can be found and calculated by extracting the bacteria and the dried medium in which the bacteria were grown. HPLC analysis has revealed that the major porphyrin found in the cells of both bacteria is uroporphyrin. Protoporphyrin can be found in significant amounts only in *E. coli* and not in *S. aureus*-induced cultures. In *S. aureus*, the protoporphyrin is probably converted into haemin, the iron-containing protoporphyrin, which does not fluoresce. On the other hand, *S. aureus* and *E. coli* excrete large amounts of coproporphyrin during the process of porphyrin production and *E. coli* also excretes a small amount of protoporphyrin. The emission spectra of the extracellular porphyrin exhibit a peak at 617 nm for porphyrins excreted from *S. aureus* [16]. This peak can be related to coproporphyrin [17,23]. *E. coli* cultures excrete porphyrins with an emission peak at 615 nm, which can also be related to coproporphyrin with a shift, since it is in a mixture with other porphyrins. The intracellular porphyrins exhibit emission at 632 nm, which can be related to protoporphyrin. *S. aureus* exhibits a peak at 622 nm, which can be related to uroporphyrin, since protoporphyrin is not found in *S. aureus*. A similar but not identical pattern was obtained when porphyrin synthesis within the cells was induced by δ-ALA methyl ester. This ALA derivative is less hydrophilic and is thought to penetrate better into the bacterial cells [24,25].

Photoinactivation of both bacteria after incubation with δ-ALA or δ-ALA methyl ester and illumination by the various lights demonstrates effective killing of the bacteria. Viability of both bacteria decreased when the light dose increased. Viability decreased on illumination but demonstrated inactivation curves with double slopes or even triple slopes in some cases. One slope demonstrates a fast killing rate followed by a second slope where the viability decreases further at a slower rate. The third slope demonstrates again a fast killing rate. A two-slope killing rate has also been demonstrated for exogenously treated porphyrins [26]. The same phenomenon was found in this study. However, a difference was found between *S. aureus* and *E. coli*. In *S. aureus*, both inducing molecules (δ-ALA or δ-ALA methyl ester) resulted in a 3–4 orders of magnitude decrease with all light methods tested, whereas in *E. coli*, a second slope was obtained which decreased very slowly and did not reach sterility at light doses which killed *S. aureus* cells. The same phenomenon has been observed with *Haemophilus parainfluenzae* [17], another

Gram-negative bacterium, in which the killing efficiency was less than that found with exogenously treated porphyrins and light. Total eradication of *H. parainfluenzae* was also not achieved, only a decrease by 2–3 orders of magnitude. It has been suggested [17] that the limited extent of *E. coli* inactivation is a result of photobleaching of the produced endogenous porphyrins. Another suggestion is that this may be a reflection of a situation in *E. coli* cells in which the produced protoporphyrins form aggregates which may effectively shield against photoinactivation [27]. A difference in viability after photoinactivation was also found between the δ-ALA-induced *E. coli* cultures and the δ-ALA methyl ester-induced cultures. A better photokilling rate was always observed in the δ-ALA methyl ester-induced *E. coli* cells in the δ-ALA-induced cells. This may indicate a less aggregated state within the cells of the porphyrins produced by δ-ALA methyl ester. The different efficacies of photoinactivation by the different light sources correlate well with the extinction coefficient of the endogenous porphyrins produced. For this reason, blue light is more efficient in its photokilling effects.

Which porphyrin species is best photoreactivated and causes photoinactivation of the bacteria? In our experiments, we removed the excreted porphyrins and light illumination was carried out in the absence of extracellular porphyrins.

Photoinactivation of the bacteria by endogenous porphyrins can be caused by uroporphyrin and some other porphyrins in *S. aureus* cells. *E. coli* can be photoinactivated by uroporphyrin, protoporphyrin and the other porphyrins. Despite the fact that significant amounts of protoporphyrin were found in *E. coli*, these bacteria are less photoinactivated than *S. aureus* which do not exhibit the presence of this porphyrin. Protoporphyrin tends to aggregate more whereas uroporphyrin is soluble and its high content might cause the photoinactivation consequences. The amounts of this porphyrin are extremely high in *S. aureus* and lower in *E. coli*.

The ultrastructural changes observed after incubation of the two bacterial species with δ-ALA and illuminated with blue light are not different from the changes observed when these bacteria were treated with exogenous porphyrins and illuminated with visible light [1,2,8–10,20,21,28]. The incubation of *E. coli* cells with δ-ALA in the dark did not result in any significant alterations in bacterial cell structure but led to porphyrin synthesis. Only on exposure to light do the photodynamic processes begin and lead to structural alterations which consequently probably cause bacterial cell death. On the other hand, incubation of *S. aureus* cells with δ-ALA in the dark resulted in the formation of mesosomal structures, which are believed to be a consequence of the photosensitisation effect, when exogenic porphyrins were used on *S. aureus*. From this observation it can be concluded that mesosome formation did not necessarily lead to cell death, since incubation with ALA did not prevent any bacterial growth. From these results it can also be assumed that the presence of a photosensitiser within the cells is crucial for the photosensitisation process to take place. Until now there was only proof that the molecules must penetrate deep into the cytoplasmic membrane [9,21,27], but no proof has been presented of their obligatory presence in the cytoplasm.

The overall suggestion from this work is that by using δ-ALA or its derivative δ-ALA methyl ester or other similar and more hydrophobic derivatives, the porphyrin synthesis pathway can be induced and illumination can afford a good photodynamic effect on Gram-positive as well as Gram-negative bacteria.

CONCLUSIONS

1. δ-ALA or δ-ALA methyl ester enhances the production of endogenous porphyrins in Gram-positive as well as in Gram-negative bacteria.
2. The produced porphyrins are mainly uroporphyrin and a small amount of protoporphyrin in *E. coli* cells, whereas only uroporphyrin is found in *S. aureus*.
3. A large portion of the porphyrins produced are excreted into the growth medium. Excreted porphyrins consist of coproporphyrin as the major porphyrin in both types of bacteria, but in *E. coli*, a small amount of protoporphyrin can also be found in the growth medium.
4. Bacteria loaded with their own produced porphyrins can be killed by photosensitisation. *S. aureus*-loaded bacteria are more sensitive to photoinactivation than *E. coli*.
5. The best illumination methods by correlation of light dose needed for eradication are, in decreasing order, intensed blue light (400–450 nm), laser beam (632.8 nm) and

white light. Intensed red light (600–700 nm) requires very high light doses for total eradication of bacteria.

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