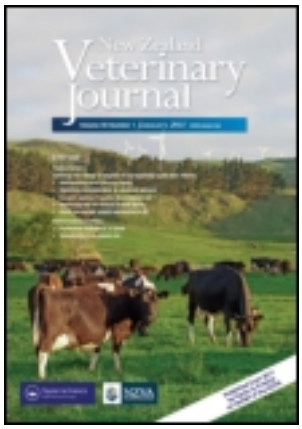

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Phylloerythrin: Mechanisms for cellular uptake and location, photosensitisation and spectroscopic evaluation

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Scientific Article

Phylloerythrin: Mechanisms for cellular uptake and location, photosensitisation and spectroscopic evaluation

E Scheie*§, A Flåøyen*†, J Moan‡ and K Berg‡

Abstract

AIM: To elucidate the photobiological behaviour of phylloerythrin by studying the cellular uptake and intracellular localisation pattern of phylloerythrin and its spectral properties in Chinese hamster lung fibroblast cells (V79).

METHODS: Phylloerythrin was diluted in dimethylsulfoxide (DMSO). Fluorescence emission and excitation spectra were measured using a luminescence spectrometer equipped with a red-sensitive photomultiplier. V79 cells were cultured in monolayers and labelled with 0.25 µg/ml phylloerythrin for uptake, cell survival and intracellular localisation studies. For cell survival and intracellular localisation studies, cells were subsequently exposed to blue light at a fluence rate of 9.0 mW/cm^2.

RESULTS: The fluorescence excitation spectrum of phylloerythrin in DMSO was characterised by a Soret band exhibiting a maximum peak at 418 nm. The fluorescence emission spectrum had peaks at 643 and 706 nm. The corresponding spectra in cells were red-shifted to 422, 650 and 712 nm, respectively. The cellular uptake of phylloerythrin was complete after about 10 h of incubation. The uptake together with the activation energy and analysis of cells incubated with phylloerythrin at 37°C and 0°C using fluorescence microscopy indicated that the dye is taken up into cells via a diffusion-mediated pathway. Measurements of subcellular marker enzymes were performed immediately after light exposure of phylloerythrin-treated cells. The mitochondrial marker enzyme, cytochrome-*c* oxidase, and the marker enzyme for the Golgi apparatus, UDP galactosyl transferase, but not those for lysosomes, β-N-acetyl-D-glucosaminidase (β-AGA), and endoplasmic reticulum, NADPH cytochrome-*c* reductase, were inactivated upon photodynamic treatment.

CONCLUSION: These results indicate that phylloerythrin is located mainly in the Golgi apparatus and mitochondria of V79 fibroblasts cells.

KEY WORDS: *Golgi, mitochondria, fibroblast, phylloerythrin, photosensitiser, hepatogenous photosensitisation.*

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Introduction

Hepatogenous photosensitisation occurs in ruminants when a toxin, normally produced by a higher plant, a fungus or a cyanobacterium (alga), causes liver injury or dysfunction, resulting in retention of the photosensitising agent, phylloerythrin (Clare 1952; Kellerman et al 1988; Flåøyen and Frøslie 1997). Phylloerythrin (Figure 1) is a metabolic product of chlorophyll, produced in ruminants by rumen microorganisms (Rimington and Quin 1933; Quin et al 1935). Normally, phylloerythrin is conjugated by the liver and excreted into bile. However, liver injury or dysfunction may lead to depression or cessation of hepatic elimination of phylloerythrin. In such cases phylloerythrin may enter the blood stream and reach skin cells and accumulate.

An acute inflammatory response of the skin can be induced when phylloerythrin is excited by sunlight. Excited phylloerythrin molecules react with oxygen, resulting in singlet oxygen ($^1O_2$) and possibly other reactive oxygen species being formed. $^1O_2$ is believed to be the main cytotoxic product formed during photochemical treatment of cells (Weishaupt et al 1976; Moan and Sommer 1985). $^1O_2$ is short-lived in cells and has a diffusion length of 10–20 nm (Moan and Berg 1991). Thus, the primary cytotoxic effect of a photosensitiser after exposure to light occurs close to the site of $^1O_2$ formation.

The efficiency of a photosensitiser in sensitising cells to photoinactivation depends on its ability to be taken up by the cells as well as its photochemical properties. Several factors may influence the rate of uptake, such as the molecular structure of the photosensitiser (Woodburn et al 1991; Boyle and Dolphin 1996), time after administration (Peng et al 1990; Peng and Moan 1995), cell line, and incubation conditions (Hanania and Malik 1992). Incubation conditions, such as the temperature of the medium surrounding the cells during light exposure, have a major affect on the rate of cellular uptake of photosensitisers and the sensitivity to light (Moan et al 1981; Rud et al 2000).

A	Absorbance
β-AGA	β-N-acetyl-D-glucosaminidase
CCD	Cooled charge-coupled device
DMSO	Dimethylsulfoxide
FCS	Foetal calf serum
MEM	Minimal essential medium
NADPH	Nicotinamide adenine dinucleotide phosphate
$^1O_2$	Singlet oxygen
PBS	Phosphate-buffered saline
UDP	Uridine diphospho
V79	Chinese hamster lung fibroblast cell line

The rate of cellular uptake of a xenobioticum is also highly dependent upon its lipophilic properties. In cells in vitro, lipophilic dyes generally localise in membrane structures including plasma, mitochondrial, endoplasmic reticulum, and nuclear membranes, while hydrophilic dyes seem to accumulate in lysosomes (Moan et al 1989). Some photosensitisers seem to be less phototoxic when located in the plasma membrane than in other cellular compartments (Moan et al 1984). There is also some evidence for a lower quantum yield of cell inactivation for lysosomally located dyes than for non-lysosomally located ones (Moan et al 1992). Dyes that locate in mitochondria are highly efficient at sensitising cells to photoinactivation (Woodburn et al 1992; Morgan and Oseroff 2001).

To elucidate the photobiological behaviour of phylloerythrin in skin cells we studied the spectral properties and the intracellular localisation pattern of phylloerythrin in Chinese hamster fibroblasts.

Materials and Methods

Cell line

V79 Chinese hamster lung fibroblasts were cultured in monolayers at 37°C in an atmosphere of 100% humidity and 5% CO_2 added to air. Cells were grown in Eagle's minimal essential medium (MEM) with Hanks' salts (Gibco, Paisley, UK) containing 10% foetal calf serum (FCS), 100 U/ml penicillin and 100 µg/ml streptomycin. The cells displayed a doubling time of 10 h and were kept in exponential growth by subculturing twice weekly.

Chemicals

Phylloerythrin was provided by Porphyrin Products (Logan, USA). The dye was dissolved in DMSO (Sigma, St Louis, USA) to a concentration of 1 mg/ml as a stock solution and stored at −80°C. Nocodazol, cytochrome *c*, the reduced form of β-nicotinamide adenine dinucleotide phosphate (β-NADPH), and p-nitrophenyl-N-acetyl-β-D-glucosaminide were purchased from Sigma (St Louis, USA). Uridine diphospho-D-[6-^{3}H] galactose was purchased from Amersham Pharmacia Biotech (Freiburg, Germany).

Fluorescence and absorption of phylloerythrin in DMSO

Phylloerythrin was diluted in DMSO to a concentration of 1 µg/ml concentration. The solution was studied spectrofluorometrically using a Perkin-Elmer LS-50B luminescence spectrometer with a red-sensitive photomultiplier installed (Perkin-Elmer, Norwalk, USA). Fluorescence was measured by exciting samples at 418 nm (Soret band) and detecting emission at 500–800 nm. A cut-off filter (545 nm) was used on the emission side. Subsequently, absorption was measured in a Perkin-Elmer Lambda-15 spectrophotometer equipped with an integrating sphere.

Fluorescence and absorption of cell-bound phylloerythrin

Cells were seeded out in 20 cm^2 dishes (Falcon NY, USA) and treated with 1 µg/ml phylloerythrin for 18 h. After incubation the cells were washed 3 times with ice cold Dulbecco's phosphate-buffered saline (PBS) and scraped off the dishes in 2 ml PBS. The cell suspension was measured spectrofluorometrically using a Perkin-Elmer LS-50B. The excitation wavelength was set to 422 nm, the maximum of the Soret band of phylloerythrin, and the emission was scanned from 500–800 nm. A cut-off filter (545 nm) was used on the emission side. Subsequently, absorption was measured in a Perkin-Elmer Lambda-15 spectrophotometer equipped with an integrating sphere, suited for measurements of scattering samples.

Uptake of phylloerythrin by V79 cells

V79 cells ($5x10^5$) were seeded out in 9.5 cm^2 dishes and labelled with 0.25 µg/ml phylloerythrin for up to 24 h. After incubation the cells were washed three times with ice-cold 0.9% NaCl and scraped off the substratum in 1 ml 0.9% NaCl. Part of the sample was collected for protein assay and the rest was centrifuged at 1,100 rpm for 10 min in a Beckman microlitre centrifuge (Beckman GS-15R, Beckman Instruments, Palo Alto CA, USA) and resuspended in 1 ml of a solution containing 50% methanol and 1 M $HClO_4$. Phylloerythrin was measured spectrofluorometrically using a Perkin-Elmer LS-50B and a 1 ml quartz cuvette (Hellma, Baden, Germany). Fluorescence was measured using an excitation wavelength of 422 nm and an emission wavelength of 650 nm. A cut-off filter (545 nm) was used on the emission side. For quantitative measurements, a standard of known concentration of phylloerythrin in DMSO was added to the samples to increase the fluorescence by 50–100%. The background, measured in dishes without cells but otherwise treated the same way, was subtracted. Protein was measured using the Bio-Rad assay as described by Bradford (1976).

Labelling with photosensitiser and irradiation

V79 cells were seeded out in 9.5 cm^2 dishes (Falcon NY, USA) and kept in the incubator for about 5 h to allow attachment to the substratum. The cells were exposed to 0.25, 0.5 and 1 µg/ml phylloerythrin in MEM medium. If not otherwise described, $50x10^4$ cells were incubated at 37°C with photosensitiser for 18 h, washed three times in MEM and incubated in the same medium for 1 h before exposure to light. Cells were subsequently exposed to blue light (400–500 nm) at a fluence rate of 9.0 mW/cm^2 from a bench of 4 fluorescent tubes (Osram 18W/67). After irradiation, the cells were washed with medium and kept in the incubator until fixation.

Cell survival assay

The cytotoxic effect of phylloerythrin, with or without light, was measured by incubating 400 cells with the photosensitiser for 18 h in 9.5 cm^2 dishes and treated as described above. Cytotoxicity was determined by measuring the colony-forming ability of the cells. After light exposure, cells were incubated for 5–6 days at 37°C. Cells treated with phylloerythrin, but without light exposure, were otherwise treated the same way. The colonies were then fixed with 96% ethanol, stained with methylene blue, and counted manually. Scored cell survival was calculated relative to parallel samples of V79 cells that were neither incubated with phylloerythrin nor irradiated. Cell death was defined as the fraction of cells not surviving in the cell-survival assay.

Fluorescence microscopy

The intracellular distribution of phylloerythrin was studied after incubation of $5x10^4$ V79 cells with 0.5 µg/ml phylloerythrin. Cells were washed three times in PBS at 37°C and a cover glass was placed gently on the top of the PBS layer. Surplus PBS was aspirated off. The cells were subsequently studied using a Zeiss Axioplan fluorescence microscope equipped with a cooled charge-coupled device (CCD) camera (TE 3/W, Astromed, Cambridge, UK), operated by an image processing program. The microscope was equipped with a 395 nm bandpass excitation filter, a 440 nm beam splitter and a 610 nm cut-off emission filter. In some

experiments, cells were incubated with phylloerythrin at 0°C and dishes subsequently put on ice in a room at 4°C.

Enzyme analysis

β-*N-acetyl-D-glucosaminidase (β-AGA)*

Cells were isolated by scraping and pelleted immediately after irradiation. They were then incubated for 3 h in a solution containing 0.1% Brij 35, 100 mM acetate buffer (pH 4.6), 0.1% Triton X-100 and 2 mM *p*-nitrophenyl-*N*-acetyl-β-glucosamine according to the method of Beaufay et al (1974). The reaction was stopped by adding ice-cold 0.5 M Na_2CO_3. The β-AGA-activity from the lysosomes was then measured by recording the formation of *p*-nitrophenol (from the substrate *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide), which can be registered spectrophotometrically at 410 nm. The presence of phylloerythrin in the cells did not influence the measurements.

NADPH cytochrome-c reductase

V79 cells were seeded out in 9.5 cm^2 dishes and treated with sensitiser as described above. The NADPH cytochrome-*c* reductase activity, a marker enzyme for the endoplasmic reticulum, was measured as described by Beaufay et al (1974). The method is based on the formation of cytochrome c_{red} from cytochrome *c*, induced by NADPH, which can be recorded spectrophotometrically at 550 nm.

UDP galactosyl transferase

V79 cells were seeded out in 9.5 cm^2 dishes and treated with sensitiser as described above. The UDP galactosyl transferase activity, a marker enzyme for the Golgi apparatus, was measured as described by Brandli et al (1988). The method is based on binding of radioactively-labelled uridine diphospho-D-[6-^{3}H] galactose to ovalbumin, which can be detected using an MR 300 automatic liquid scintillation system.

Cytochrome-c oxidase

V79 cells ($1x10^6$) were seeded out in 25 cm^2 flasks and treated with sensitiser as described above. Immediately after irradiation, the cells were trypsinated and mitochondria were isolated using electropermeabilisation. Repeated aspiration through a needle followed by differential centrifugation ruptured the permeabilised cells. The probes were first centrifuged at 2,500 rpm for 7 min, then the supernatant was centrifuged at 10,000 rpm for 20 min using a Beckman microlitre centrifuge. The cytochrome-*c* oxidase activity in the mitochondria was then measured as described by Gibson and Hilf (1983). The method is based on spectrophotometric recording of the disappearance of cytochrome $c_{red,}$ a reduced form of the substrate cytochrome *c* (Sigma) at 550 nm.

Statistical analysis

Results are presented as means and standard deviations (SD) or standard errors (SE). Linear regression calculations were conducted using the method of least squares in SimaPlot (SPSS Inc, Chicago IL, USA).

Results

Fluorescence properties of phylloerythrin

The fluorescence excitation spectrum of phylloerythrin in DMSO (Figure 2a) was characterised by a Soret band with a maximum at 418 nm, and a Q-band with vibrational structure (500–650 nm). The fluorescence emission spectrum had peaks at 643 and 706 nm. The absorption spectrum of phylloerythrin in DMSO was similar to the excitation spectrum (Figure 2a), although the Q-band (500–650 nm) was relatively weaker. The concentration curve vs absorbance at the Soret band of phylloerythrin in DMSO was linear for concentrations up to about 10 µg/ml. From this curve an extinction coefficient (ε) of 4.5 $x10^5$ was calculated (Figure 3).

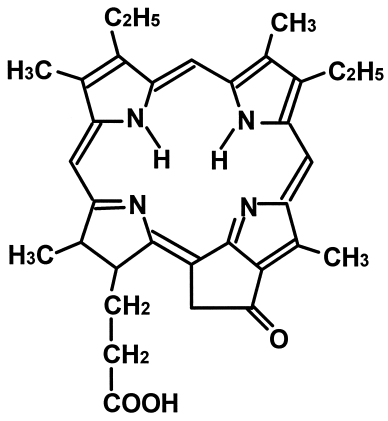

Figure 1. Chemical structure of phylloerythrin.

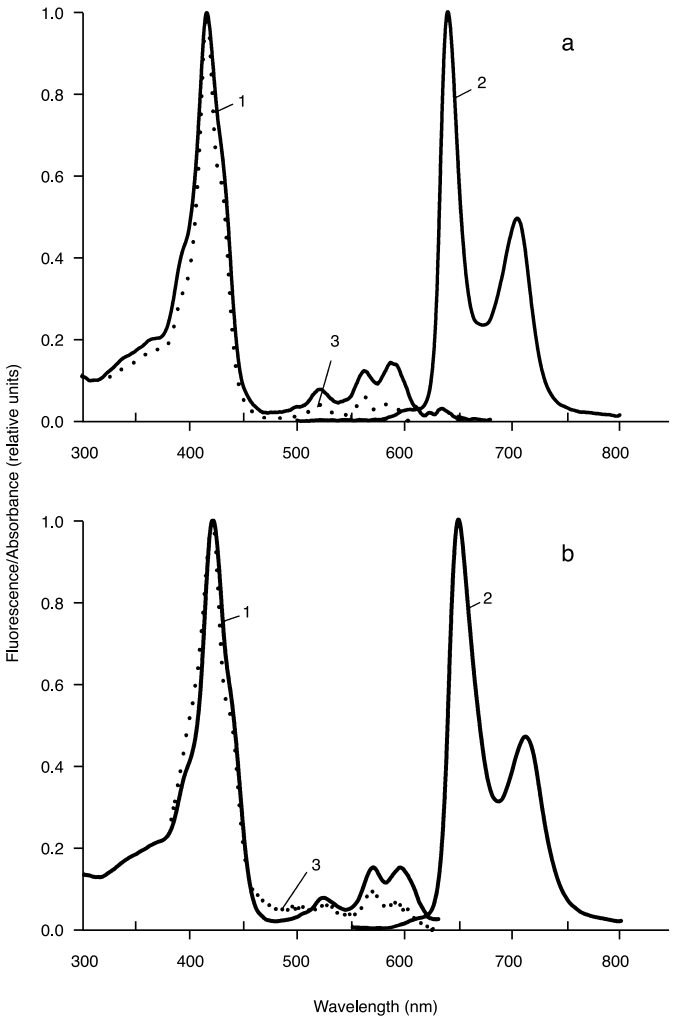

Figure 2. Fluorescence excitation (1), emission (2), and absorption (3) spectra of phylloerythrin (a) diluted in DMSO, and (b) in V79 fibroblasts. The fluorescence excitation- and absorption spectra of phylloerythrin in DMSO were normalised at 418 nm and the fluorescence-excitation and absorption spectra in cells were normalised at 422 nm. The cells were incubated for 18 h with 1 µg/ml phylloerythrin.

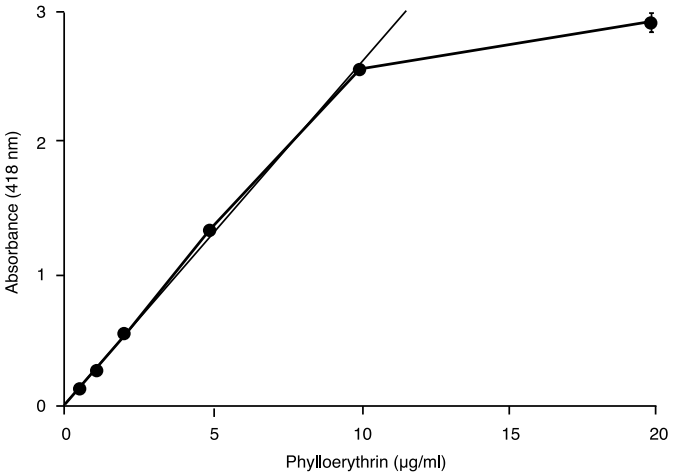

Figure 3. Absorbance-vs-concentration curve for phylloerythrin diluted in DMSO (●). The absorbance of phylloerythrin is a measurement of the peak intensity at 418 nm. The straight line indicates linear regression analysis based on results from the first five concentrations.

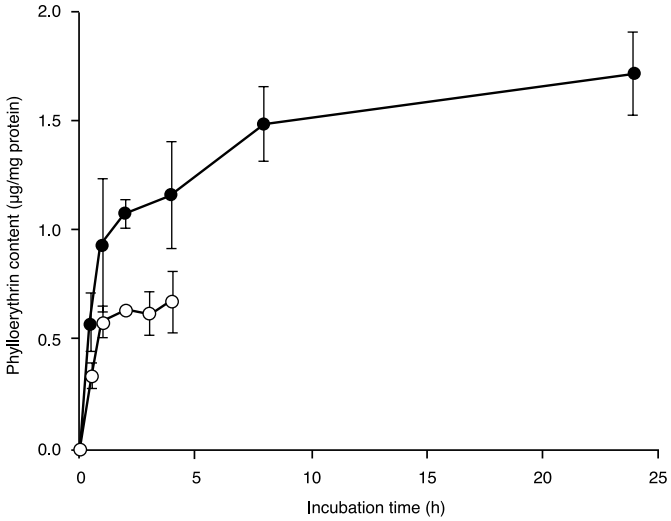

Figure 4. Time course and temperature dependency of cellular uptake of phylloerythrin in V79 fibroblasts. The cells were incubated with 0.25 µg/ml phylloerythrin in MEM at 0°C (○) and 37°C (●) and cell-bound phylloerythrin analysed. Bars=SD from three independent experiments.

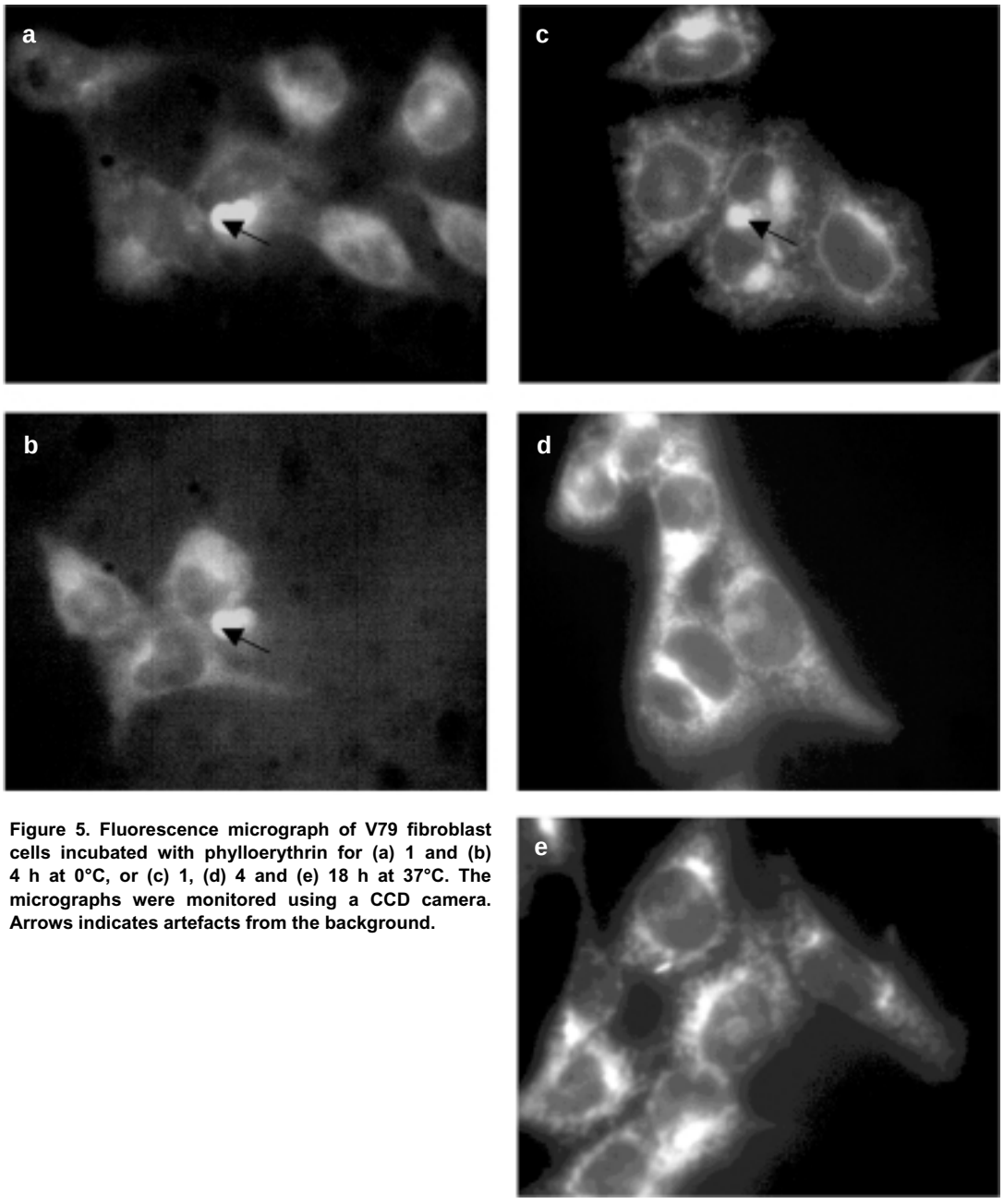

Figure 5. Fluorescence micrograph of V79 fibroblast cells incubated with phylloerythrin for (a) 1 and (b) 4 h at 0°C, or (c) 1, (d) 4 and (e) 18 h at 37°C. The micrographs were monitored using a CCD camera. Arrows indicates artefacts from the background.

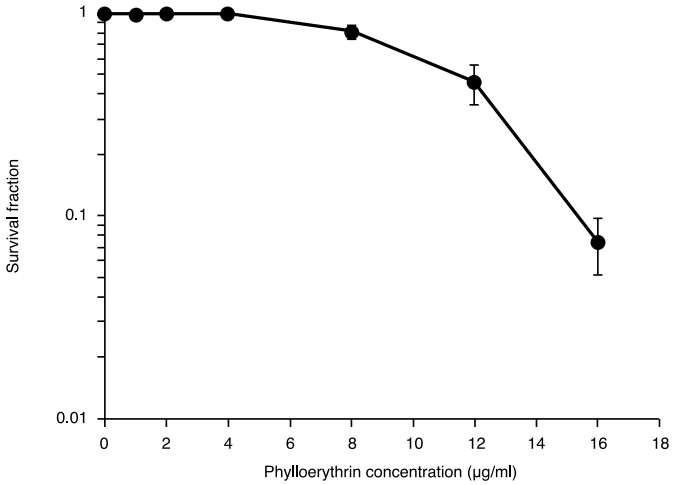

Figure 6. Concentration dependency of cellular survival after incubation for 18 h with phylloerythrin. V79 fibroblasts were incubated with different concentrations of phylloerythrin in the dark. Bars=SD from three independent experiments.

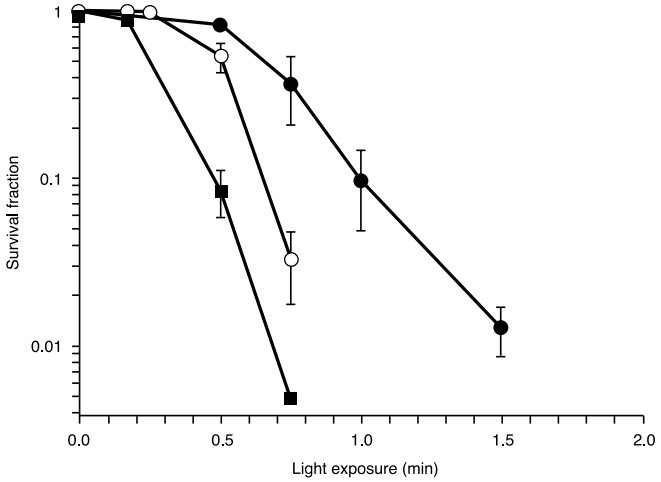

Figure 7. Survival curves for V79 fibroblast cells incubated for 18 h with 0.25 µg/ml (●) and 0.5 µg/ml (○) phylloerythrin and exposed to blue light. The cells were washed three times and incubated in sensitiser free medium for 1 h before light exposure. Survival curve of V79 cells incubated with 0.5 µg/ml phylloerythrin, but incubated directly in sensitiser free medium 1 h before light exposure (■). Bars=SD from three independent experiments.

The Soret band of phylloerythrin had its maximum at 422 nm when bound to cells, i.e. slightly red-shifted compared with the spectrum in DMSO. When the dye in cells was excited by 422 nm light, two fluorescence emission peaks were evident, at 650 and 712 nm (Figure 2b). The absorption and the corrected fluorescence excitation spectra for phylloerythrin bound to V79 cells were almost similar, and showed a main peak at about 422 nm (Figure 2b).

Cellular uptake of phylloerythrin

Cellular uptake of phylloerythrin was measured up to 24 h, and for the first hour of incubation uptake increased almost linearly with time (Figure 4). Uptake occurred quickly during the first few hours after addition of phylloerythrin, then reached a plateau within about 10 h; 55% of uptake over 24 h occurred within the first hour of incubation.

Temperature dependency of cellular uptake

The cellular uptake of phylloerythrin was almost linear over time

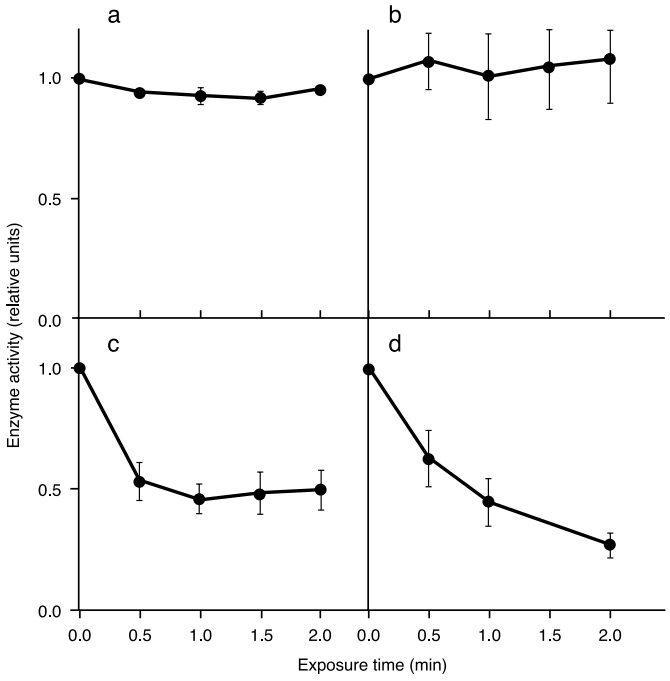

Figure 8. Relative activities of different intracellular enzymes in V79 fibroblast cells immediately after treatment with phylloerythrin and light. Inhibition of the activity of (a) β-N-acetyl-D-glucosaminidase located mainly in lysosomes, (b) NADPH cytochrome-*c* reductase in the endoplasmic reticulum, (c) UDP galactosyl transferase in the Golgi apparatus, and (d) cytochrome-*c* oxidase in the mitochondria. The cells were incubated with 0.25 µg/ml phylloerythrin for 18 h and incubated in sensitiser-free medium 1 h before exposed to various light doses. Bars=SE from three independent experiments.

for the first hour of incubation at 0°C. The uptake after 1 h at 0°C was about 60% of the uptake at 37°C and about 90% of the total uptake at this temperature (Figure 4). Linear regression analysis indicated an initial rate of phylloerythrin uptake of 0.60 µg/mg protein during the first hour of incubation at 0°C (R^2=0.94) compared with 0.96 µg/mg protein during the first hour of incubation at 37°C (R^2=0.80). The activation energy was estimated from these results to be about 10 kJ/mol, assuming a linear Arrhenius plot within this temperature range.

Fluorescence microscopy of cells exposed to phylloerythrin for 1 h at 0°C clearly showed the fluorescence originated from intracellular regions (Figure 5). When the treatment was extended to 4 h, the pattern of fluorescence was similar. A similar pattern was observed in cells treated with phylloerythrin at 37°C, although the fluorescence intensity was higher. After 1 h of incubation, the fluorescence pattern from phylloerythrin seemed to be almost independent of time of incubation at both 0°C and 37°C. The intracellular localisation of phylloerythrin was mainly extranuclear, was not homogeneous, and covered most of the cytoplasm. However, the fluorescence pattern was more granular after incubation at 37°C than after 0°C.

Cytotoxic effects in the absence and presence of light

Cytotoxic effects of phylloerythrin, measured both in the absence of light and after light exposure, are presented in Figures 6 and 7, respectively. Samples incubated with different concentrations of phylloerythrin in the dark for 18 h showed that the clonogenicity of the V79 cells was not influenced by treatment with up to 8 µg/ml phylloerythrin (Figure 6). However, cells exposed to 0.25 µg/ml phylloerythrin for 18 h followed by 1 h in sensitiser-free medium were highly sensitive to light exposure (Figure 7). About

90% of the cells were inactivated after 60 sec of light exposure, corresponding to 0.54 J/cm^2. After incubation with 0.5 µg/ml phylloerythrin only 40 secs of light exposure was required to inactivate 90% of cells. Incubation in sensitiser-free medium for 1 h before exposure to light reduced the sensitivity of cells to photoinactivation by only 15% (Figure 7).

Inactivation of intracellular enzymes

The enzymatic activity of UDP galactosyl transferase associated with the Golgi apparatus was reduced by approximately 55% after treatment with phylloerythrin and 1 min exposure to light (Figure 8c), while the activity of the mitochondrial cytochrome-*c* oxidase was reduced by approximately 70% after 2 min exposure to light (Figure 8d). Lysosomal β-AGA and NADPH cytochrome-*c* reductase located in endoplasmic reticulum showed no significant loss in enzymatic activities after treatment with phylloerythrin and 2 min light (Figure 8ab).

Discussion

The spectral characteristics of phylloerythrin were similar to those found for other porphyrins, which have their main absorption at about 400 nm, with a distinct Soret band near this wavelength (Blum 1941; Rimington 1960). Perrin (1958) reported that phylloerythrin in ether had a Soret band at 414 nm, whereas Rimington (1960) reported the spectral properties of phylloerythrin in acid solution with a Soret band at 421 nm and an extinction coefficient (ε) to 2.78x10^5. He also found that porphyrins obey Beer's law up to an absorbance (A) of about 1.0. The present work with phylloerythrin dissolved in DMSO showed linearity of absorption vs concentration up to about A=2.5. The extinction coefficient in this solution was found to be 4.5x10^5, i.e. slightly larger than that found by Rimington in 1960. The spectra in cells were slightly red-shifted compared with the spectra in DMSO, which may indicate membrane binding of the dye in cells.

In the case of porphyrins, it is well known that aggregates have a low fluorescence quantum yield compared to monomers (Moan and Sommer 1983) and absorption spectra that are different from those of monomeric dyes (Moan and Christensen 1981; Berg et al 1989). In the present study the absorption and the fluorescence excitation spectra of phylloerythrin in V79 cells were found to be similar in shape. This indicates little or no aggregation of phylloerythrin in V79 cells. This is to be expected for an efficient photosensitiser, since monomeric species are much more photoactive than aggregates (Moan 1984; Berg et al 1989). In DMSO, however, the Q-bands of the absorption and excitation spectra were different, indicating that the dye was not only in a monomeric form.

Photosensitisers may enter cells either by penetrating the plasma membrane by passive diffusion (Bohmer and Morstyn 1985; Dellinger et al 1986) or by endocytosis (Berg and Moan 1994). Plasma membrane transporters have not been reported to be involved in the cellular uptake of porphyrin-based photosensitisers. The present results indicate that the cellular uptake of phylloerythrin is due to a diffusion-controlled process. Although the uptake of phylloerythrin was slightly temperature dependent, the activation energy for the process was only about 10 kJ/mol, which is within the range of diffusion-controlled uptake mechanisms (Le Cam and Freychet 1977). In contrast, active transport mechanisms are more temperature dependent and have activation energies of more than 50 kJ/mol (Macy 1979). Active transport is normally completely inhibited at 0°C. These results demonstrate that phylloerythrin clearly penetrated the plasma membrane at 0°C. It is also evident from the fluorescence micrographs that phylloerythrin penetrated the plasma membrane and localised in intracellular compartments, even when the cells were incubated at 0°C. This would not be expected for compounds taken up by an active transport mechanism.

Photoinactivation of cells and tissues containing photosensitisers is strongly dependent on the localisation of the dye (Moan and Christensen 1981; Benstead and Moore 1988; Peng et al 1991). In the present study, localisation of phylloerythrin was investigated using fluorescence microscopy as well as measurements of photochemically induced damage to enzymes known to be markers for subcellular organelles. The activities of UDP galactosyl transferase, which localises in the Golgi apparatus, and cytochrome-*c* oxidase, a marker enzyme for mitochondria, were found to decrease in cells treated with phylloerythrin and light. This is in accordance with the fluorescence microscopic analysis, which showed a high concentration of fluorescence in the perinuclear area, i.e. where the Golgi apparatus is located, and a widespread extranuclear granular fluorescence that might resemble the localisation of mitochondria. Thus, our results are consistent with localisation of phylloerythrin in the Golgi apparatus and mitochondria in V79 cells and that these organelles are targets in phylloerythrin-sensitised photodynamic action. The lack of inactivation of the lysosomal marker enzyme, β-AGA, by treatment with phylloerythrin in combination with light indicates low or no lysosomal localisation of phylloerythrin in V79 cells. This is in agreement with the uptake studies, which indicated that endocytosis was not involved in the uptake of phylloerythrin in this cell line, as well as the intracellular localisation pattern evident using fluorescence microscopy (Figure 7). The marker enzyme for endoplasmic reticulum, NADPH cytochrome-*c* reductase, was not inactivated by phylloerythrin and light, indicating minor localisation of phylloerythrin in the endoplasmic reticulum.

Phylloerythrin is a lipophilic compound (Figure 1). Our results demonstrating that phylloerythrin located in both the Golgi apparatus and mitochondria support previous findings that lipophilic dyes in cells in vitro generally localise in membrane structures (including plasma, mitochondrial, endoplasmic reticulum and nuclear membranes), while hydrophilic dyes appear to accumulate in lysosomes (Moan et al 1989; Boyle and Dolphin 1996). Our results are not in accordance with results from a previous study that reported that phylloerythrin and light caused inactivation of the lysosomal enzyme, acid phosphatase, in frozen sections of rat tail skin (Slater and Riley 1966). However, in that study, phylloerythrin and light had only minor effects on release of acid phosphatase from isolated lysosomes from liver homogenate, and no comparisons with other enzymes or organelles were reported to indicate that lysosomes were a preferred target. Results from studies using frozen sections are difficult to equate with those such as ours that used live cells in culture.

The localisation of other porphyrins in mitochondria and photochemical damage to these organelles is well documented, and it has been suggested by several authors that the most efficient sensitisers of cells to photoinactivation localise in the mitochondria (Morgan and Oseroff 2001). This may explain the severe lesions to light seen in the skin of ruminants suffering from phylloerythrin-induced photosensitisation.

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