# Photodestruction of *Propionibacterium acnes* Porphyrins

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The fluorescence spectra of colonies of *Propionibacterium acnes* were studied under various experimental conditions.

The spectra contained peaks at 580 nm and 620 nm. These bands were due to two different components; the 580 nm component was likely to be a metalloporphyrin, and there are indications that the 620 nm component could be a coproporphyrin.

The 580 nm fluorescence was destroyed by the combined action of light and oxygen (no destruction under strict anaerobic conditions). A dark period interrupting the bleaching light stopped the destruction of this component for the time of the dark period.

The initial production of the 620 nm component was due to the oxygen exposure. Upon light irradiation this component was later destroyed by the combined action of oxygen and light.

## Introduction

It is well known that *Propionibacterium acnes (P. acnes)* produces fluorescent porphyrins [1, 2]. A cell-destructive spectro-fluorimetric assay for porphyrin determination has been developed by Granik *et al.* [3]. However, fluorescence measurements on *P. acnes* cultures provide an alternative method to study the bacterial porphyrins with respect to their amounts, production and photodestruction [2, 4].

Many porphyrins are photodynamic sensitizers [5]. In the present report fluorescence measurements will be used to investigate the photodestruction of porphyrins present in *P. acnes* cells. Such studies of *P. acnes* might elucidate molecular reaction mechanisms which play a role in the acne pathogenesis.

## Materials and Methods

*P. acnes* serotype I (CN 6278) was used in the present experiments. Cultivation of the bacteria was done as reported earlier [2]. Eagle's medium without buffer was used and the bacteria were grown semi-anaerobically (about 2% oxygen) at 37 °C in darkness.

The fluorescence instrumentation has also been described previously [2]. Pieces of agar, containing bacterial colonies, were placed in the sample house of the fluorimeter. The intensity of the exciting light at 415 nm was 6 $W \cdot m^{-2}$.

All the fluorescence spectra recorded in this way contain, besides the porphyrin fluorescence, an agar background signal. This signal decreases slowly with increasing wavelength. It is subtracted from all recorded spectra in the present paper. In the first experiments reported below, the cultures were adapted to oxygen in darkness for about one hour before the fluorescence spectra were measured. To obtain oxygen free conditions in the later experiments, the inoculated agar pieces were placed in special cuvettes which could be flushed with argon and sealed.

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## Results

### *Fluorescence emission spectra*

The fluorescence spectrum from *P. acnes* cultures fixed to glass plates is shown in Fig. 1. The spectrum had two major emission bands, one at 620 nm and another at 580 nm. This is due to two different emission components since the spectrum was changed when the excitation wavelength was changed from 415 to 385 nm (full *vs.* dotted line in Fig. 1). These two fluorescent components will be denoted the 580 and 620 nm component, respectively.

As will be mentioned in the discussion, the 580 nm component is likely to be a metalloporphyrin. The spectral shape of this component can be

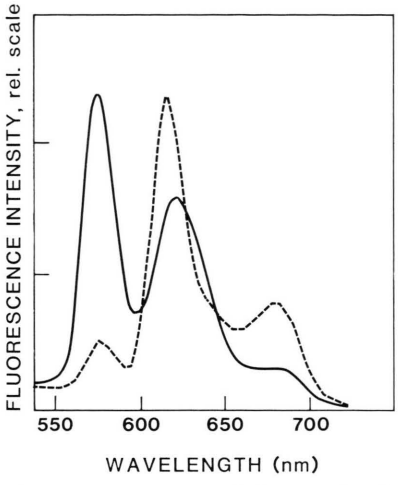


Fig. 1. Fluorescence emission spectra from *P. acnes* serotype I cells. Exciting light 415 nm (full line) or 385 nm (dotted line).

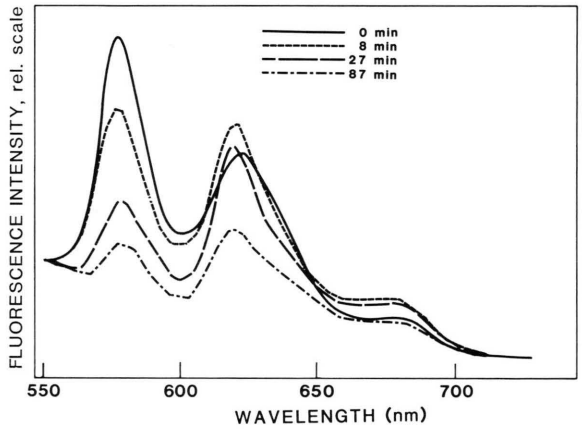


Fig. 2. Fluorescence spectra during bleaching. The culture was grown semi-anaerobically for 3 days, but was then exposed to oxygen and continuous light (415 nm) at time zero.

estimated under the assumption that only the 620 nm component is emitting in the 680–710 nm region. Fitting the two spectra of Fig. 1 together in this wavelength region and subsequently subtracting these two curves will as a result show the fluorescence emission spectrum of the 580 nm component. The procedure will thus allow a splitting up of the compound spectra of Fig. 1 in its 580 and 620 nm components, a procedure which will be used below.

*Fluorescence of semi-anaerobically grown cultures exposed to oxygen and light*

When a culture, grown under semi-anaerobic conditions but kept under strict anaerobic conditions during the measurements, was exposed to light, the fluorescence spectrum remained stable. This indicates that no photochemical reactions occurred (data not shown).

However, when such cultures were exposed to both oxygen (air) and light at 415 nm, different changes in the fluorescence emission were observed, as shown in Fig. 2:

1. The height of the 580 nm fluorescence component decayed when the culture was continuously illuminated.
2. There is an initial increase in the amount of the 620 nm component, monitored by fluorescence.
3. The action of light caused a subsequent reduction in the height of the 620 nm peak.

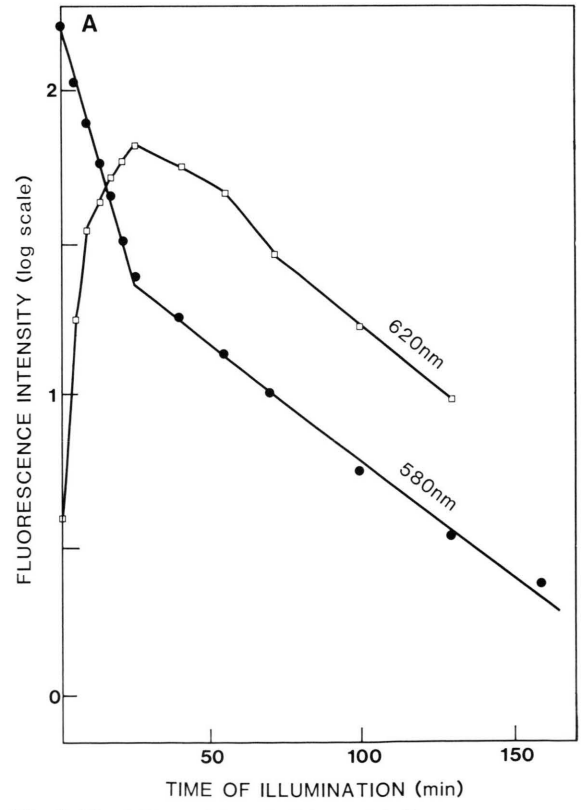


Fig. 3. The 580 nm (●) and 620 nm (□) fluorescence components of a culture exposed to oxygen and light. Calculated from data in Fig. 3.

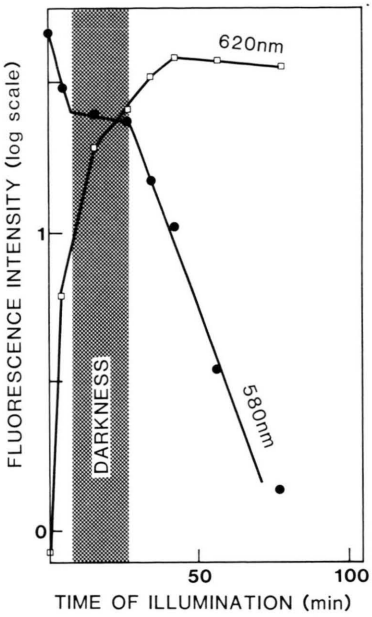


Fig. 4. Bleaching process interrupted by dark period. The 580 nm (●) and 620 nm (□) fluorescence components were calculated from data achieved as in Figs. 2 and 3. The bleaching was interrupted by a 20 min dark period.

In Fig. 3 the heights of the fluorescence peaks at 580 and 620 nm are plotted against time of illumination. Since the shape of the spectrum due to the 580 nm component was known, the intensity changes of the second component at the 620 nm wavelength were easily found.

In some experiments the cultures were exposed to oxygen and light but the illumination period was interrupted by a dark period, as exemplified in Fig. 4. The increase in the 620 nm peak proceeded as in the case of constant illumination whereas the 580 nm peak remained constant during the dark period. Thus, the processes leading to the intensity changes in the fluorescence spectra of the 580 and 620 nm components were not directly related.

The initial production of the 620 nm component required only the presence of oxygen whereas the decomposition of the 580 nm component required both oxygen and light.

## Discussion

The identity of the 620 nm component is not uniquely determined by fluorescence, since the fluorescence properties of, *e.g.*, uroporphyrin and coproporphyrin can be very similar. Recently it has, however, been shown that *P. acnes* cells contain large amounts of coproporphyrins [6] which have an emission band in this wavelength region.

A general comment on the existence of the 580 nm component can be given. It has been shown in this laboratory that the fluorescence spectra of *P. acnes* cells vary with age and external growth conditions [6]. The 580 nm peak as discussed in the present report has in some of our replicate experiments been very small, in others the height has been bigger than shown in Figs. 1 to 3. More work needs to be done in order to identify and control satisfactorily the growth parameters producing a constant and reliable 580 nm peak in the *P. acnes* spectra.

It is of interest to note that the 580 nm porphyrin fluorescence peak has attracted considerable attention in other contexts. Fluorescence emission spectra of hematoporphyrin and related compounds, especially hematoporphyrin derivative, have recently been reported to contain an emission band at 580 nm [7].

The results indicate that the formation of the 580 nm band is an interesting feature in the uptake mechanisms of the hematoporphyrin derivative and its component into cells. Sommer *et al.* (1984) provided evidence supporting that the 580 nm component is a Zn-porphyrin. It is known that metalloporphyrins are emitting in the 580 nm band [12].

The finding of the 580 nm peak in *P. acnes*, being a living non-tumorous system with a high endogenous concentration of porphyrins, could therefore be of a more general interest.

The bleaching of the 580 nm component is obviously governed by an oxygen requiring process. In so-called Type II reactions [13] singlet oxygen is formed by porphyrin photoreactions. The excited oxygen molecule produced can then attack unsaturated groups in the cells (lipid components of membranes, porphyrin molecules etc.) [14]. Aerobic conditions combined with light can therefore reduce the viability of the *P. acnes* cells. Indeed, it has been shown in this laboratory, that the viability of *P. acnes* is dramatically reduced after near UV-irradiation under aerobic conditions [4].

The initial increase in the 620 nm fluorescence as a result of exposure to air may be interpreted as an oxygenation of a porphyrinogen into a corresponding porphyrin. It can therefore be expected that the

pools of porphyrinogens present in the cells are readily oxidized on oxygen exposure.

The illumination time necessary to cause a 50% decrease in the 580 nm fluorescence is about 50 min (Fig. 3) at 415 nm and 6 $W \cdot m^{-2}$. This light dose is about one tenth of the dose which gives 37% survival of the cells at the same wavelength (to be published from this laboratory). This indicates that survival is not, at least not directly, coupled to the presence of the 580 nm component in the cells.

Several interesting aspects on the bleaching effects remain to be clarified. Identification of fluorescing components, details of the bleaching processes, possible value of phototherapy in acne treatment etc. should therefore be studied in the future.

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