Photoinactivation of *Propionibacterium acnes* by Near-Ultraviolet Light

B. Kjeldstad

Institute of Physics/NLHT, University of Trondheim, N-7055 Dragvoll, Norway

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Photodestruction of *Propionibacterium acnes* was investigated by broad-band near-ultraviolet light. The inactivation of the bacteria was found to be oxygen dependent, and without O_2 practically no photoinactivation occurred. D_2O caused an increased inactivation ($D_{10} = 5$ kJ/m^2 in D_2O as compared to $D_{10} = 11$ kJ/m^2 in normal water). Decreased temperature during illumination increased the ability to form colonies. The results are compared with corresponding results for other types of cells and the destruction mechanism is discussed.

Introduction

Propionibacterium acnes (*P. acnes*) belongs to the normal microbial flora of the human skin [1], and seems to play a role in the pathology of acne vulgaris [2]. Among therapy methods used against acne, UV-treatment is common [3]. The reactions of *P. acnes* to UV-light are therefore of practical interest; in addition they are of interest to compare with corresponding reactions in other cells, *e.g.* human cells, *E. coli* cells and yeast cells.

P. acnes produces porphyrins [4]. Porphyrins make yeast cells and human cells [5, 6] more sensitive to UV-irradiation, and the porphyrins in the *P. acnes* cells could therefore render the bacteria sensitive to UV. Singlet oxygen might be involved in the destruction mechanisms [7].

The aim of the present report is to investigate the photoinactivation of *P. acnes* to UV-light, the participation of oxygen in the destruction mechanisms and the temperature sensitivity of the UV-destruction.

Materials and Methods

Bacterial strain and growth conditions

P. acnes, serotype I (CN 6278), was kindly supplied from Dept. of microbiology, University Hospital, Trondheim, Norway. The laboratory cultures were grown on blood agar plates. The cultures used in experiments were incubated for 5 days on Eagles medium, modified (Flow laboratories, U.K.) with added agar noble (Difco laboratories, Detroit, Mich.). The medium was buffered with 60 mM phosphate buffer, KH_2PO_4 and $Na_2HPO_4 \cdot 2H_2O$ (Merck) at pH 6.7.

Cultures were incubated under dark conditions at 37 °C in anaerobic jars. The jars contained a mixture of H_2 and CO_2 (95:5, v/v) and the oxygen content was less than 5% (Biomérieux-Charbonnieres les Bains, France).

Irradiation and determination of survival fractions

At the age of 5 days the cells were suspended in PBS (phosphate buffered saline, pH 6.7) either with H_2O or D_2O (99.8%, Norsk Hydro, Norway). The cell concentration was around 2×10^4 cells/ml. 2 ml suspensions were illuminated with blacklight (Philips tubes TL20W/09 covered with a thick glass plate; spectral range of light 330–410 nm with maximum at 366 nm). The intensity of the light at the sample position was 9 Wm^{-2}, measured by a Kettering power radiant meter. During illumination, two samples were taken out in 5 min intervals, and spread out on Petri dishes with complex medium (Difco bactoagar 20 g/l, Difco bactotryptone 10 g/l, Difco yeast extract 5 g/l, NaCl 10 g/l) on which the bacteria were incubated for 4 days. The colonies on each dish were then counted and used to calculate the survival fractions. Two replicates were used in each experiment to calculate the survival fraction.

The illumination was carried out at 25 °C if otherwise not stated. In some experiments the illumination was carried out at other temperatures and the sample was then kept at the relevant tem-

Reprint requests to B. Kjeldstad.

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perature for 20 min before irradiation started and during irradiation.

To remove oxygen, the sample was flushed by nitrogen. Flushing started 20 min before the illumination and continued during the illumination.

Controls were kept in darkness in all experiments.

Results

Fig. 1 shows survival curves for *P. acnes* demonstrating that the bacteria are sensitive for broad-band UV radiation. About 25 min illumination at the irradiance level used gave a survival fraction of 10% (Fig. 1, curve H_2O).

For illuminations at the same irradiance level, nearly no inactivation occurred for the de-oxygenated solutions (Fig. 1, curve N_2).

On the other hand, a change from H_2O to D_2O in the PBS, increased the inactivation (Fig. 1, curve D_2O). The D_{10} values (*i.e.* the doses which give a survival fraction of 10%) are presented in the figure text.

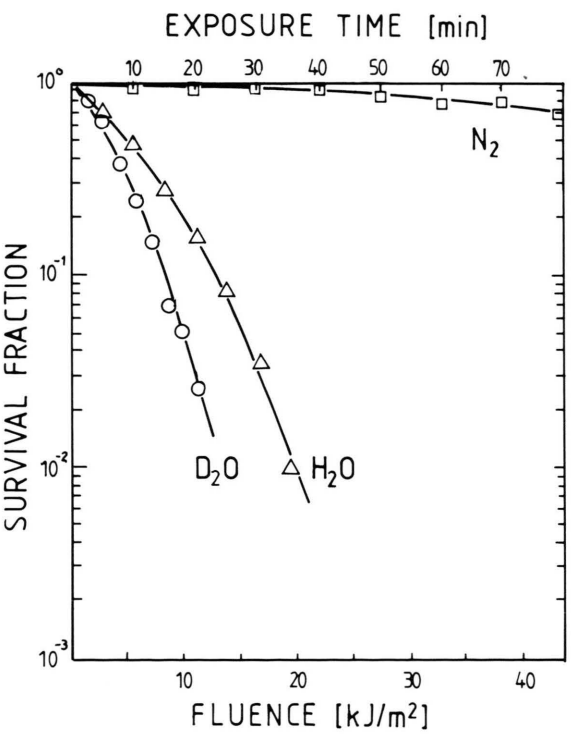

Fig. 1. Survival curves for *P. acnes* irradiated by broad-band near-UV-light, dissolved in PBS either with D_2O (○) or H_2O (△). D_{10}-values, based on 4 experiments, were D_2O: 5 ± 2 kJ/m², H_2O: 11 ± 1 kJ/m² (mean value ± S.E.). Sample flushed by nitrogen (3 l/h) gave the upper survival curve (□). Results from one out of 4 experiments are shown.

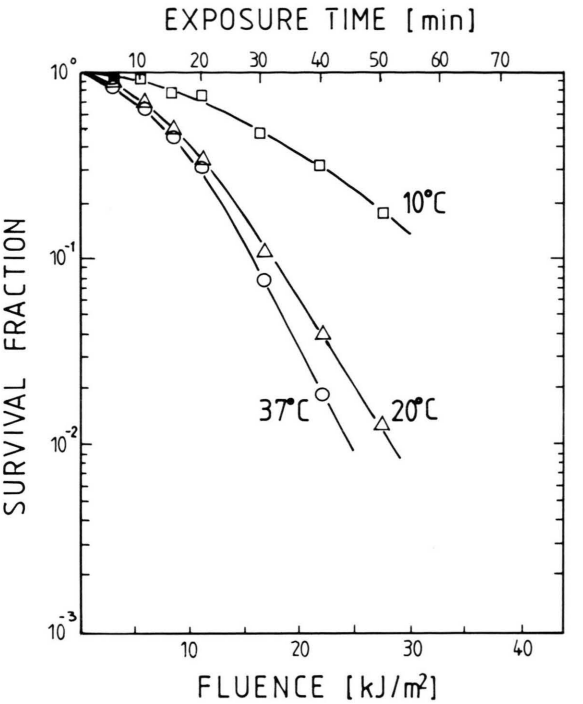

Fig. 2. Survival fractions of *P. acnes*, dissolved in PBS and irradiated with broad-band near-UV-light at different temperatures 10 °C (□), 20 °C (△), 37 °C (○). The experiment was repeated 4 times and D_{10}-values were calculated (mean value ± S.E.). 10 °C: 22 ± 8 kJ/m², 20 °C: 19 ± 5 kJ/m², 37 °C: 15 ± 2 kJ/m². Results from one out of 4 experiments are shown.

The temperature during the illumination was changed in a series of experiments. The photoinactivation increased as the temperature increased in the interval 10 °C to 37 °C (Fig. 2). D_{10} values are given in the figure text.

Discussion

The results in Fig. 1 showed that *P. acnes* was inactivated by blacklight as in the case for many microorganisms [8]. Near-UV-survival curves for microorganisms are shouldered, a break occurs typically for 10^6 J/m² at 366 nm [8]. The mean D_{10} value is 11 kJ/m² and therefore the *P. acnes* bacteria are comparatively sensitive to the UV-light.

Oxygen was required for inactivation (Fig. 1). The inactivation effect is therefore likely to be related to photosensitization, rather than to a direct

photo-chemical change [9]. D_2O gave an increased inactivation (Fig. 1). This might indicate that singlet oxygen is involved in the photosensitization process, since the lifetime of singlet oxygen is about 10X longer in D_2O than in H_2O [10]. A doubled inactivation due to D_2O is also found in yeast cells incubated with different sensitizers [7] and human cells incubated with hematoporphyrins [6]. The present D_2O results are therefore in quantitative agreement with corresponding results for yeast cells and human cells.

The temperature dependence of inactivation of *P. acnes* (Fig. 2) is consistent with findings in yeast cell experiments [9], also yeast cells sensitized by hematoporphyrin [5]. In contrast, increased temperature caused lower inactivation in human cells sensitized by hematoporphyrin [6]. The reason for this difference is unknown.

It is of interest to discuss the nature of the photoreceptor which makes *P. acnes* sensitive to near UV-irradiation. In *E. coli* one assumes that the absorbing chromophore 4-thiouride, a base in the 8'th position of t-RNA, is functioning as a target for near-UV light [8]. The nature of the chromophore in *P. acnes* has not been established but it is fairly natural to assume that one or several porphyrins function as photoreceptors. Further experiments to test this hypothesis are now in progress.

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