PHOTOINACTIVATION OF CELLS STUDIED BY $^{31}$P-NMR.

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1. Introduction.

$^{31}$P-NMR techniques allow the continuous, non-destructive measurements of phosphorous compounds in intact cells. Immediate effects of photodynamic treatment and photosensitization processes can be mapped [2,6]. $^{31}$P-NMR also gives information about changes in the pH gradient across membranes and indirectly about ion pumping mechanisms controlling proton fluxes [3]. We have studied the bacterium Propionibacterium acnes, (P. acnes) which produces large amounts of endogenous porphyrins taking part in photosensitization [5]. Externally applied HpD is effective in increasing the photosensitivity [4]. In the present short report we would like to present some new experimental results where $^{31}$P-NMR has been used to analyse photodynamic reactions of red light and HpD treatment.

2. Material and methods.

P. acnes was grown semianaerobically. Cells were harvested in late exponential phase, 1-2 h before the experiments were carried out. Immediately before an NMR experiment, the cells were suspended in a buffer, pH 6.5 and treated as in [6]. Minimum sample volume was 1.5 ml with 40% cell density. Irradiation of cells was performed in a 70 times diluted buffer [6]. Four red light tubes (Philips TL 32/20W) in a row were used, covered with a 5 mm glassplate and red perspex filter (cut wavelength at 610 nm). The survival fraction under identical conditions was above 37%. After irradiation, cells were centrifuged and resuspended to the original density and further NMR-spectra were collected. The $^{31}$P-NMR spectra were recorded on a Bruker WM-400

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spectrometer at 161.8 MHz and the identification of spectral shifts were performed as described in [6].

3. Results.

Fig. 1A demonstrate the pronounced increase in the polyphosphate, PP, pool of HpD treated cells after red light irradiation, as determined by NMR. This increase could also be found after near-UV irradiation [6]. The increase occurred preferentially in cells which showed a PP-peak also before the light treatment started, (e.g. cells grown on Eagle’s medium [6]). Furthermore, a change in the position of the inorganic phosphorous peaks, P_i, could be seen, figure 1B. This signifies a change in the proton gradient across the membrane. Experiments (not shown) demonstrated that the two peaks coincided when nigericin (a proton ionophore) was added to the buffer.

![Figure 1](image_url)

Fig. 1. *P. acnes* grown on Eagle’s (A) and bactoagar (B) medium before (upper spectra) and after (lower spectra) irradiation with red light, irradiance 5.5 Wm⁻². Scales are different in the two curves in A: The P_i peak in the two spectra have the same size. Peaks are identified as described in [6]. Chemical shifts are referred to 85% orthophosphoric acid.