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5-Aminolaevulinic acid (ALA) induced formation of different fluorescent porphyrins: A study of the biosynthesis of porphyrins by bacteria of the human digestive tract

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Abstract

Aminolaevulinic acid (ALA) induces porphyrin formation in almost all living cells. The fluorescence spectra of porphyrins produced from a variety of 31 bacterial strains from the human oral cavity and other parts of digestive tract have been examined. Many of the bacteria exposed to ALA were able to induce protoporphyrin IX (PpIX) fluorescence, but under aerobic condition some bacteria can also produced different fluorescent porphyrins, in particular water-soluble porphyrins that can arise from an oxidation of the corresponding porphyrinogen precursors. The formation of fluorescent porphyrins can be different from one bacterial strain to another, but also one specific bacterium can form different fluorescent porphyrins. Irradiation of the ALA incubated cultures led to a rapid formation of water-soluble porphyrins exhibiting fluorescence maxima at wavelengths of 618–620 nm. This light induced formation of water-soluble porphyrins could be attributed to a photooxidation of the non-fluorescent (Uro/Copro)-porphyrinogen precursors. Addition of detergents to some of the bacterial cultures led to a strong PpIX fluorescence increase, indicating that some of the PpIX originally produced can be present in a non-fluorescent, probably aggregated, form. The large abundance of bacteria in the oral cavity and other parts of digestive tract, with their capacity to easily produce fluorescent porphyrins, indicates that such bacterial fluorescence should be suppressed during the ALA-based diagnosis of tumours in order to eliminate false positive results.

Keywords: 5-Aminolaevulinic acid; Bacteria; Porphyrin biosynthesis; Porphyrinogen photooxidation; Water-soluble porphyrins; Tumour fluorescence diagnosis

1. Introduction

The 5-aminolaevulinic acid (ALA) induced formation of the photosensitizing and fluorescent protoporphyrin IX (PpIX) is gaining in popularity as a method for treatment and diagnosis of superficial tumours. This is achieved by simply providing the body with its own porphyrin precursor ALA [1]. The facts that ALA can be administered topically, and that PpIX has a natural clearance mechanism, has led to wide usage of this approach, especially in dermatology [2], and is being actively studied for use in other areas such as urology, gastroentology and neurology [3].

Since PpIX has an appreciable quantum yields of fluorescence it can actually be seen by the naked eye, thus providing a promising monitoring means for early detection and demarcation of malignant neoplasms. However, the specificity of tumour detection can be greatly reduced and false positive findings can result when not taking into account the potential of the native bacterial flora to produce fluorescent porphyrins which can interfere with the

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tumour fluorescence [4]. If one introduces ALA into the oral cavity or other parts of the gastrointestinal tract in order to induce PpIX fluorescence from a tumour, one can also observe similar PpIX fluorescence from the bacteria of the local microflora. The ability of fluorescence emission after ALA incubation of numerous bacteria strains specific for the human oral cavity and other parts of the gastrointestinal tract has been examined in this study.

Fluorescence spectroscopy is a direct approach and the most sensitive method to follow the course of ALA induced porphyrin formation in vivo and in vitro. However, besides the normally observed PpIX fluorescence spectrum the appearance of additional emission bands were reported peaking in the region of 617–620 nm [5–10]. In [5], it was stated that these emission bands result from the formation of water-soluble porphyrins, mainly uroporphyrin and coproporphyrin, which were produced in the studied of Ehrlich ascite carcinoma (EAC) cells after ALA incubation. Up to now, however, the assignment of these fluorescence spectra at 617–620 nm has not been without controversy. While the fluorescence peak at 618 nm appearing in rat carcinoma cells has been assigned to an additional photoproduct [10], the authors [7] assumed that the presence of the observed hydrophilic porphyrins results by transportation from other parts of the organism in the area of investigation.

In order to understand the conditions under which different fluorescent porphyrins were produced, we have investigated the emission properties of ALA induced porphyrins upon the presence of light and oxygen. These studies were also performed on bacteria of the native microflora.

All nucleated cells have the capacity to convert ALA into PpIX, and it is well known [11] that bacteria also have this capacity. Examining the porphyrin synthesis in bacteria may give useful insights into the biosynthesis of nucleated cells. Pp IX is the last porphyrin intermediate in the haeme (and chlorophyll) biosynthesis. Due to its highly photodynamic susceptibility, all living organism must avoid its toxic action. Normally, PpIX does not accumulate in significant amounts in any cells [12], the whole process of PpIX formation being enzymatically regulated by the body's need for haeme. However, the question arise then whether PpIX can accumulate in a photodynamically inactive form in cells. We examined this possibility on ALA incubated bacteria.

2. Materials and methods

2.1. Bacteria

All strains of bacteria investigated in this study originated from the collection of the Institute of Medical Microbiology of the University Hospital of Jena, Germany. They were isolated from clinical specimens of patients of the hospital and identified by means of standard routine techniques of clinical microbiology.

The bacterial strains included in this study are given in Fig. 1.

2.2. Culture conditions of bacterial lawns and ALA incubation

All bacterial strains were subcultured on appropriate culture media. Columbia agar (Oxoid, Basingstoke, UK), as well as Columbia agar supplemented with 8% defibrinated sheep blood, was used for the cultivation of aerobic bacterial strains (24–48 h, 36 °C). Schaedler agar (Oxoid, Basingstoke, UK), supplemented with 8% defibrinated sheep blood, served as the medium for anaerobic cultivation in an anaerobic jar (48–72 h, 36 °C). *Haemophilus parainfluenzae* was cultivated on Columbia agar supplemented with 8% cooked sheep blood (24 h, 36 °C, 5% CO_2).

In order to obtain bacterial suspensions, 2 mL sterile 0.9% saline solutions were transferred into a tube and the bacteria were inoculated from agar plates to Mc Farland standard 4 by means of a sterile cotton swab. After vortexing, 0.1 mL aliquots of the suspension were transferred onto fresh agar culture media, plated regularly by a cotton swab, and incubated under the appropriate conditions (pressure, temperature) as described above for 24 h. After this incubation period a regular colony lawn was grown.

In order to facilitate the attainment of fluorescence spectra directly from the bacterial culture, and to avoid the strong light scattering occurring in suspensions, the bacteria were allowed to settle as homogeneous colony lawn on a membrane. Pieces (2 cm × 2 cm) of the membranes were transferred on the sides of the agar plates containing the colony lawns. Colonies were thus allowed to grow aerobically at 36 °C with aeration into the membranes. After period of 2 h the membranes were transferred to new fresh agar plates in such a way that the side with the colonies were in contact with the agar surfaces.

A freshly prepared 0.4% amino laevulinic acid (ALA) solution containing 0.9% NaCl and adjusted to pH 6.5 was added covering the membranes as a thin film. Incubation occurred at 36 °C for 24 h in the dark (standard conditions).

After the incubation period, the membranes with the colony lawn were lifted from the agar medium and the sides covered with the bacteria lawn were exposed to the excitation light.

The membranes with the anaerobically cultivated and incubated bacteria were removed from the anaerobic jar and exposed to the excitation light under air contact as well.

2.3. Chemicals

Aminolaevulinic acid (ALA), supplied by Medac, Hamburg, Germany, was freshly prepared in a concentration of 0.4% in sterile saline and the pH was adjusted to 6.5. Iodine was applied by addition of Lugol solution (5% Iodine, 10% Kaliumiodide, 85% water).