# INTRACELLULAR LOCALIZATION OF A PHOTOSENSITIZER AND CHLORIDE CHANNEL ACTIVATION IN ELECTROCYTES

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## ABSTRACT

The intracellular localization of a lipophilic alkyl long-chain tetraphenylporphyrin in electrocytes, *Psammobatis extenta* (Rajidae) is described. In contrast to the usual case, this porphyrin derivative was localized in electromotor nerves and in nuclei chromatin of electrocytes. Both structures exhibited intense fluorescence. However, the mitochondria were slightly fluorescent. These data are discussed in relation to electrocyte death in a weak electric fish. Additionally, the electron probe X-ray microanalysis suggest the activation of the chloride and cationic channels.

## RESUMEN

La localización intracelular de un derivado lipofílico de tetrafenilporfirina, unido a cuatro grupos alquilo de cadena larga, en electrocitos de la especie *Psammobatis extenta* (rayas) es descrita. En contraste con el comportamiento normal, este derivado de porfirina se localiza en los nervios electromotores y en los núcleos (cromatina) de los electrocitos. Ambas organelas presentaron una fluorescencia intensa. Sin embargo, la fluorescencia presentada por las mitocondrias fue débil. Estos datos son discutidos en relación con la muerte de los electrocitos de peces eléctricos de descarga débil. Adicionalmente, los datos obtenidos en el microanálisis por rayos-X (EDAX) sugieren la activación de los canales de cloro y catiónicos.

## 1. INTRODUCTION

The development of new generation photosensitizers to improve the efficiency of photodynamic therapy (PDT) is an area of intensive research. In PDT, the activation of a photosensitizer by light generates singlet molecular oxygen, a highly reactive form of oxygen that reacts with many biomolecules, including lipids, proteins, and nucleic acids.[1] These biomolecules are chemical modified and therefore can not accomplish their function, with the subsequent cell death. This therapy is applied for treatment of cancers, as well as for bacterial and viral eradication.[2-6] The fluorescence exhibited by photosensitizers allows also tumor detection.[7] Nevertheless, the application of PDT remains restricted due to the limited penetration of light in tissues, the photosensitization of normal tissues and the residual skin photosensitivity that is observed for several weeks. The advantages of this method, as compared to other conventional cancer treatment modalities, are its low systemic toxicity and its ability to destroy tumors selectively. The lipophilic degree of photosensitizers determines localization, and therefore site and type of damage of the cells.[8,9] In general, lipophilic photosensitizers accumulate in the membrane of the cell and its organelles.[5] In the other hand, hydrophilic, as well as aggregated states of photosensitizers, enter into the cell by pinocitosis and are localized mainly in lysosomes and endosomes.[10] There are many photosensitizers, usually porphyrin or phtalocyanine derivatives, under clinical study and can be classified as lipophilic or hydrophilic.[11] However, there are few examples of such macrocycles with long alkyl-chain studied for PDT. It was found that the efficiency and selectivity of tumors targeting slightly increased upon increasing the length of the alkyl groups connected to the Zn(II)-phthalocyanine complex.[12,13]

In this work, we have investigated uptake, intracellular localization of 5,10,15,20-tetrakis(4-*n*-dodecylphenyl)-porphyrin (TPP), elemental composition and morphological changes in electrocytes of *Psammobatis extenta*. The *P. extenta* belongs to Rajidae, one of three groups of weakly electric fish. We choose electrocytes for this study because they are big cells with very few organelles, this facilitate the study of the intracellular localization of the photosensitizer. In addition, electric ray electrocytes have myoproteins.[14,15] The electrocytes are highly polarized and multinuclear cells. They are semicircular in shape and have their concave face receiving innervations (IF) from electromotor neurons of the spinal cord. The other face, convex, is

non-innervated (NIF) and shows a system of caveolae.[14] The nuclei are localized at the posterior region of the cytoplasm.[15]

## 2. MATERIALS AND METHODS

**Chemicals**

The TPP derivative was synthesized following the litterature procedure.[16,17] The purity was controled by $^1$H-NMR and elemental analysis. max ($CHCl_3$) = 421 nm. This compound was already studied as for its thermal properties. It was found to exhibit two discotic lamellar phases. The phases change and the transition temperatures are: C - 31°C – $D_L$ – 52°C – $D_{L'}$ – 155°C – Isotrópico[16,17]

**Fluorescence microscopy**

Cryostat sections about 10 m were incubated for 3 min. with 7,8 x $10^{-5}$ M solution of the TPP derivative in chloroform. Sections were fixed for 5 min at 4ºC in a mixture of 3.7% formaldehyde and 0.5% glutaraldehyde in 0.05 M phosphate buffer, pH 7.4, and maintained for 30 min. at 4°C. After fixation, sections were washed in PBS for 20 min., mounted with Citifluor and observed with an epifluorescence Nikon Optiphot microscope equipped with filter G and 580W supplementary filter. Photomicrographs were taken using a Nikon camera and ILFORD HP 35 400 ASA film. As negative control the sections were treated as described above but with omission of the TPP derivative and did not produce any fluorescence.

**EDAX-SEM analysis**

For EDAX-SEM analysis, after fixation, samples, sections of 20 m, were washed in the same buffer and in ice-cold distilled water for 2 hours, and then dehydrated by washing many times with acetone. After dehydration, samples were routinely critical-point dried and metalized with gold (200A) in a sputter coater model 3 (Pelco, Ted Pella inc.) and oriented for observation by a JEOL 35 Scanning Electron Microscope equipped with EDAX Si(Li) energy dispersive X-ray detector. Microanalysis was carried out at the non-innervated face of the electrocytes.

## 3. RESULTS AND DISCUSIONS

The intracellular distribution of this photosensitizer in electrocytes is shown in figure 1 and 2.

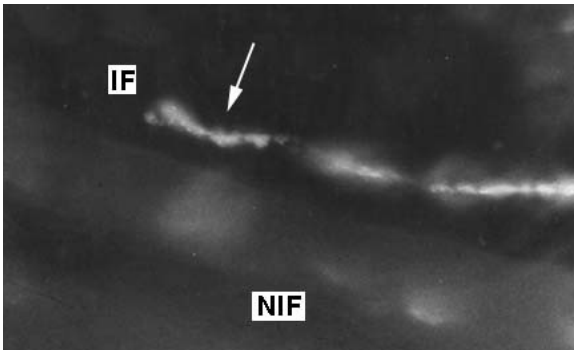


**Figure 1.** Localization of the TPP derivative on the terminal nerves (arrow) of an electrocyte (x600).

The red fluorescence pattern of the TPP derivative was observed as a thick band that corresponds to the nerves and nerve terminals (Fig.1). The affinity of this lipophilic photosensitizer for the nerves is explained because this tissue is rich in fatty acids. In addition, the nuclei show an intense red fluorescence and its localization were similar to the semicircular distribution of the chromatin of these cells (Fig. 2), suggesting that the TPP derivative interacts with DNA. In contrast, the mitochondria show slight fluorescence. This localization is unusual for a photosensitizer of similar polarity. For example, a hydrophobic phthalocyanine localize on the lysosomes.[18]

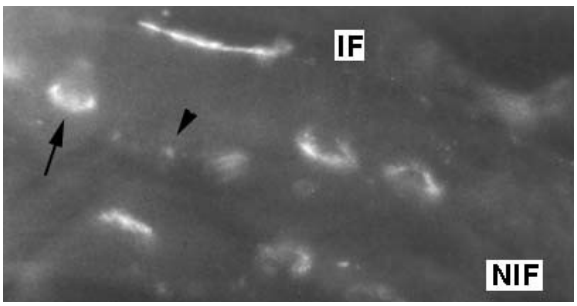


**Figure 2.** Localization of the TPP derivative on the nuclei (arrow) and in mitochondria (arrow head) of an electrocyte (x600).

Immediately after the penetration of the TPP derivative in the electrocytes, they start to swell and the concave face loses all their invaginations (Fig. 3). In order to understand the reason of the swelling of cells, microanalysis by energy-dispersive X-ray spectra (EDAX) of the same region was carried out (Fig. 4). After treatment of the electrocyte with the TPP derivative, the relative semi-quantitative weight % (*K* ) for oxygen, sodium and chloride ions were: 39, 17 and 15, respectively. Compared to negative controls, the peak for $Na^+$ is 5-fold bigger and for oxygen the variation is not significant. Also, the peak of $Ca^{2+}$ is 2-fold bigger but for $K^+$ is 6-fold minor. The simultaneous increasing of $Na^+$ and $Ca^{2+}$ concentration and decreasing of $K^+$ concentration is a good evidence of the cationic channel activation with the porphyrin derivative. However, the most important change is the new big peak observed for $Cl^-$ anion. This constitutes an evidence for chloride channel activation. The massive intracellular accumulation of $Cl^-$ and specially the influx of $Na^+$ lead to cell swelling, and the subsequently necrotic response of cells.[19,20] It should be pointed out that chloride channel activation is not usual for weak electric fish. However, voltage-gated chloride channel was demonstrated in non-innervated plasma membrane of *Torpedo* electrocyte, a strong electric fish.[21]

The activation of chloride channel by this TPP derivative may constitute an alternative in the study and treatment of the cystic fibrosis, which is related to a dysfunction of the chloride ion transport.[22] In contrast, the increase of $Ca^{2+}$ concentration in electrocytes suggest a participation of an apoptotic mechanism.[1] Moreover, the interaction of the TPP derivative with DNA, and even the low amount of mitochondrial-bound porphyrin, may contribute to cell death by apoptotic mechanism after excitation with light.

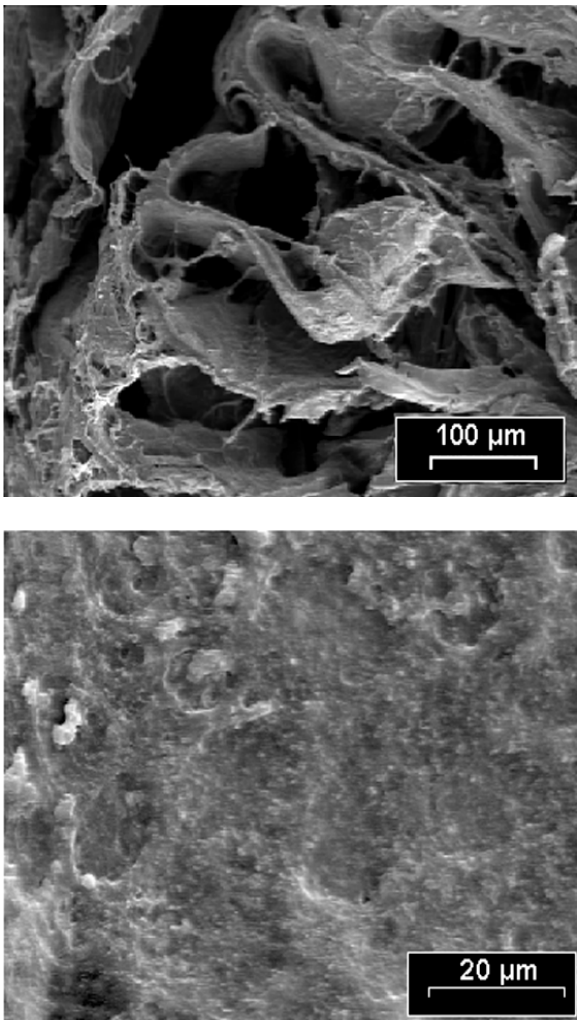


**Figure 3.** Scanning electron micrograph of non-innervated face of an electrocyte before (up) and after treatment with the TPP derivative (bottom).

The photosensitizer used in this work has liquid crystal properties.[16,17] It will be very interesting to compare these results with another photosensitizer with similar chemical structure with no mesomorphic properties.

## 4. CONCLUSIONS

The lipophilic TPP derivative used in this work has high afinity for terminal nerves and nuclei cromatine but little one for mitochondria. The presence of this compound on these organelles provokes morphological and physiological change in electrocytes leading to cationic and chloride channel activation.

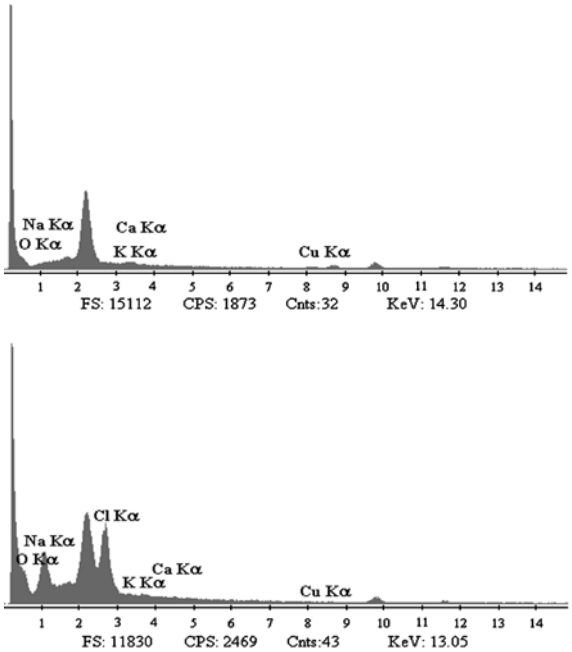


**Figure 4.** Microanalysis by EDAX of the same zone observed in figure 3, non-innervated face, before (up) and after treatment (bottom) of an electrocyte with the TPP derivative.

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