Chaos, Solitons and Fractals 77 (2015) 304–309

Contents lists available at ScienceDirect

Chaos, Solitons and Fractals

Nonlinear Science, and Nonequilibrium and Complex Phenomena

journal homepage: www.elsevier.com/locate/chaos

AFM imaging, fractal analysis and *in vitro* cytotoxicity evaluation of Zn(II) *vs.* Cu(II) porphyrins

Radu Socoteanu [a], Mihai Anastasescu [a,*], Gianina Dobrescu [a], Rica Boscencu [b], Georgiana Vasiliu [b], Carolina Constantin [c]

[a] *Romanian Academy, "Ilie Murgulescu" Institute of Physical Chemistry, 202 Splaiul Independentei, 77208 Bucharest, Romania*
[b] *"Carol Davila" University of Medicine and Pharmacy, Faculty of Pharmacy, 6 Traian Vuia St., 020956 Bucharest, Romania*
[c] *"Victor Babes" National Institute for Pathology and Biomedical Sciences, 99–101 Splaiul Independentei, 050096 Bucharest, Romania*

ARTICLE INFO

Article history:
Received 30 March 2015
Accepted 15 June 2015

Keywords:
Metalloporphyrins
AFM
Fractal analysis
U937 cell line
PDT

ABSTRACT

A pair of unsymmetrical porphyrinic complexes with Cu(II) and Zn(II) as metallic ion are studied first time by a combination of atomic force microscopy experiments with complex fractal analysis. In view of a possible use of these metalloporphyrins in diagnosis and photodynamic therapy of cancer, the morphological and structural investigations were coupled with cytotoxicity evaluation using U937 cell line. Atomic force microscopy coupled with fractal analysis was proved to be a useful tool to predict the self-similarity domains, in terms of morphology patterns with specific fractal dimensions which may be used to predict a better bio-activity in potential medial applications.

Our data point out that the investigated compounds did not alter membrane integrity, but acted as cytostatics at higher concentrations and prolonged incubation time. We highlighted that the investigated porphyrinic structures might interfere with cellular LDH, particularly after 24 h incubation.

1. Introduction

Copper and zinc divalent ions inserted in tetrapyrrolic core are extended subject in porphyrins related papers [1,2]. Meso-tetrasubstituted porphyrins were studied in relation with Zn(II) insertion in diagnosis and photodynamic therapy (PDT) efficacy, establishing relations involving membrane binding and cell uptake or even as doping agent in order to increase the efficiency as photosensitizer of certain nanofabrics [3].

Although copper porphyrins are not so often associated with PDT the involvement is in some particular cases only as precursor in routes to obtain compounds with potential photosensitizing behavior [4]. Copper porphyrinic complexes are not qualified for good PDT agents for many reasons, as in the case of the copper metallated octaethylbenzochlorin photosensitizer which has a too short triplet state lifetime [5]. In this particular case, the copper porphyrinic structure is opposed as reference for the zinc porphyrin due to the identity of the peripheral surroundings.

Atomic force microscopy (AFM) measurements of various type of porphyrinic compounds [6,7] and in particular on metalloporphyrins [8] proved to be an important tool for investigating their nanometric scale aggregates and self assembly. In order to quantitatively characterize the morphology of complex surfaces it can be used one of the modern concepts – scaling.

In this paper it is comparatively examined the morphology of the two type of M (II)-porphyrinic complexes, Zn(II)-5-(4-acetoxy-3-methoxyphenyl)-10,15,20–tris-(4-carboxymethylphenyl) porphyrin (Zn(II)TCMPOMo)

* Corresponding author. Tel.: +40 21 3167912; fax: +40 21 3121147.
E-mail addresses: manastasescu@icf.ro, manastasescu_ro@yahoo.com (M. Anastasescu), gianinadobrescu@yahoo.com (G. Dobrescu).

http://dx.doi.org/10.1016/j.chaos.2015.06.013
0960-0779/

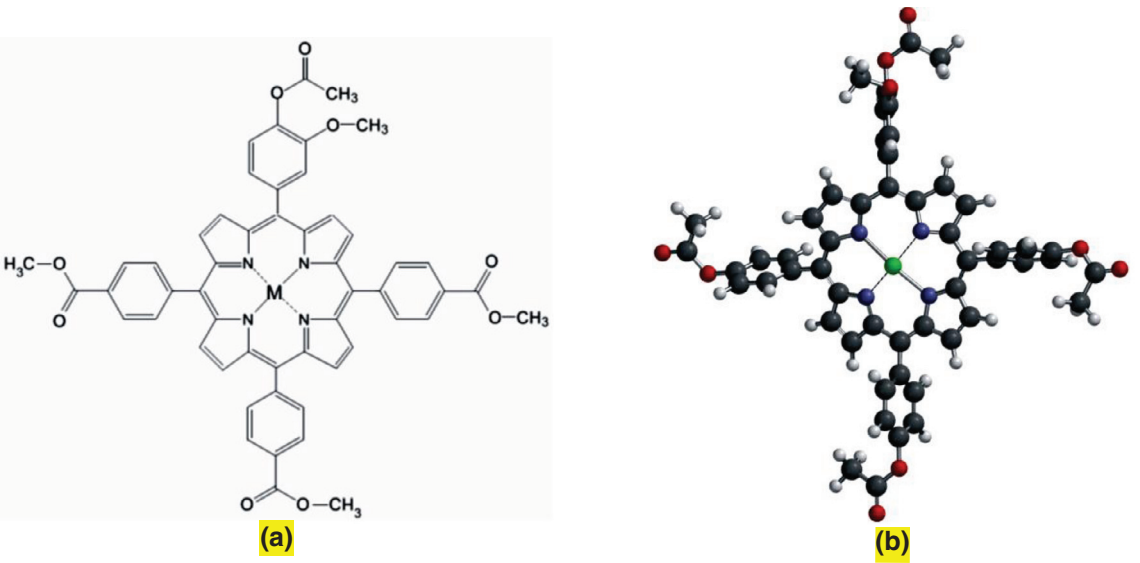

Fig. 1. Structure of M(II)-5-(4-acetoxy-3-methoxyphenyl)-10,15,20–tris-(4-carboxymethylphenyl) porphyrin (M = Cu, Zn) (a); Cu(II)TCMPOMo Spartan wavefunction™ optimized structure (b).

and Cu(II)-5-(4-acetoxy-3-methoxyphenyl)-10,15,20–tris-(4-carboxymethylphenyl) porphyrin (Cu(II)TCMPOMo) [9] (as presented in Fig. 1) and it has been studied the self-similarity of copper and zinc porphyrins using systematic fractal analysis performed on AFM micrographs.

The cytotoxicity evaluation of porphyrinic complexes is a prerequisite step in their development as potential drug. Moreover, prolonged incubation of cells with the same compounds might give an indication about their accumulation within cells and their possible delayed cytotoxic effects [10,11]. For this reason, the new complexes were *in vitro* evaluated using U937 cell line for different doses and incubation times.

The results obtained from AFM combined with fractal analysis of the investigated porphyrinic complexes are discussed and correlated with their *in vitro* cytotoxicity evaluation.

2. Methods and materials

2.1. Atomic force microscopy

Atomic force microscopy measurements at the scale of $8 \times 8\ \mu m^2$ were carried in true non-contact mode recommended for non-destructive sample scan with a XE-100 apparatus from Park systems equipped with flexure-guided, cross-talked eliminated scanners, using ultra-sharp tips (<8 nm tip radius; NCHR type from nanosensors™) of 125 μm length, 30 μm width and 42 N/m spring constant/~330 kHz resonance frequency. In order to prepare the specimens for AFM investigations, a quantity of powder was ultrasonically dispersed in ultra-pure water (Millie-Q system, >18 MΩ cm) as to obtain the dilution used in cytotoxic/cytostatic tests, and then a drop from this suspension was deposited on freshly cleaved highly oriented pyrolytic graphite (HOPG) and dried at room temperature. HOPG was used as to avoid any influence of the substrate on roughness and texture of the investigated samples. The AFM images were processed with XEI (v 1.8.0) program from Park systems.

2.2. Fractal analysis

A fractal is an object with an observed volume which depends on the resolution (length scale) and following power law behavior with a nontrivial exponent over several orders of magnitude. The most important property of fractals is self-similarity, which is the property of a part to look like the whole. Isotropic fractals are self-similar: they are invariant under isotropic scale transformation. When the object scales differently on different space directions, we call it a self-affine fractal. From this point of view, rough surfaces are usually self-affine structures [12]. Self-similarity has a mathematical description [12]:

$$N\left(\frac{r}{R}\right) \sim \left(\frac{r}{R}\right)^{-D} \tag{1}$$

where D is the fractal dimension and $N(r, R)$ is the number of boxes of size r which cover the object of linear size R; in other words, self-similarity is the property of an object to look the same when zooming in.

In our work we shall compute fractal dimensions by two methods: the height correlation function method (C) [13–15] and the variable length scale method (L) [16].

2.2.1. Correlation function method

Different parameters can be used to characterize the surface roughness. One of these parameters that describe self-affine surfaces is the roughness exponent ε. In addition to the roughness exponent ε, it is possible to associate a fractal dimension D with a self-affine function. The fractal dimension of a self-affine surface can be computed from the height correlation function [13–15]:

$$G(r) \equiv \langle C(\vec{x}, r)\rangle_x, \tag{2}$$