Chapter XXXVI
Modeling of Porphyrin Metabolism with PyBioS

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ABSTRACT

Photodynamic Therapy (PDT) involves administration of a photosensitizer (PS) either systemically or locally, followed by illumination of the lesion with visible light. PDT of cancer is now evolving from experimental treatment to a therapeutic alternative. Clinical results have shown that PDT is at least as efficacious as standard treatments of malignancies of the skin and Barrett's esophagus. Hemes and heme proteins are vital components of essentially every cell in virtually all eukaryote organisms. Protoporphyrin IX (PpIX) is produced in cells via the heme synthesis pathway from the substrate aminolevulinic acid (ALA). Exogenous administration of ALA induces accumulation of (PpIX), which can be used as a photosensitiser for tumor detection or photodynamic therapy. Although the basis of the selectivity of ALA-based PDT or photodiagnosis is not fully understood, it has sometimes been correlated with the metabolic rate of the cells, or with the differential enzyme expressions along the heme biosynthetic pathway in cancer cells. An in silico analysis by modeling may be performed in order to determine the functional roles of genes coding enzymes of the heme biosynthetic pathway like ferrochelatase. Modeling and simulation systems are a valuable tool for the understanding of complex biological systems. With PyBioS, an object-oriented modelling software for biological processes, we can analyse porphyrin metabolism pathways.

INTRODUCTION

The use of PDT for curative treatments of superficial tumors of the skin and for palliative treatments of disseminated tumors of skin and oral mucosa is well known (Daskalaki 2002). PDT also is efficacious as treatment of malignancies of Barrett's oesophagus **(**Foroulis and Thorpe 2006). PDT is based on a photochemical process, where photosensitizers (PS) act cytotoxic by generation of $^1O_2$ after laser irradiation.

The use of fluorescence measurements as quantitative indicators for PpIX accumulation after exogenous ALA administration is suitable for differentiating neoplastic, necrotic and inflammatory tissues from normal tissues. The modulation of ALA-induced PpIX accumulation and expression will provide more diagnostic information and more accuracy for the diagnosis of unhealthy tissue, especially in border-line cases. The modulation of fluorescence characteristics of ALA-induced PpIX with NAD has been used for differentiation between fibroblast and fibrosarcoma (Ismail et al. 1997).

The flow of substrates into the porphyrin pathway is controlled by tsynthesis of d–aminolevulinic acid (ALA), the first committed precursor in the porphyrin pathway. Although light is required to trigger ALA synthesis and differentiation of chloroplasts (Reinbothe and Reinbothe, 1996), a feedback inhibition of ALA synthesis by an end product of the porphyrin pathway is thought to be involved in the regulation of influx into the pathway (Wettstein et al., 1995; Reinbothe and Reinbothe, 1996). Both the nature of the product and the mechanism involved in effecting feedback inhibition remain unknown, probably because there have been no porphyrin pathway mutants identified so far that affect both chlorophyll and heme biosyntheses. Thus, modelling of porphyrin pathway may fill this gap and allow researchers to address these questions.

Downey (2002) tried to show how the porphyrin pathway may be an integral part of all disease processes through a model. Analytical techniques capable of measuring porphyrins in all cells are needed. Data gathered from plant and animal studies need to be adapted to humans where possible. An inexpensive, accurate and rapid analysis needs to be developed so porphyrins can be measured more routinely.

The committed step for porphyrin synthesis is the formation of 5-aminolevulinate (ALA) by condensation of glycine (from the general amino acid pool) and succinyl-CoA (from the TCA cycle), in the mitochondrial matrix. This reaction is catalyzed by two different ALA synthases, one expressed ubiquitously (ALAS1) and the other one only expressed in erythroid precursors (ALAS2) (Ajioka, 2006).

Heme inhibits the activity of ALA synthetase, the first and rate-limiting enzyme of the biosynthetic pathway, thereby preventing normal cells from drowning in excess production of its own porphyrins. This negative feedback control can be bypassed in certain malignant cells exposed to an excess amount of ALA, which is metabolised leading to overproduction of PpIX.

Excess accumulation of PpIX occurs because of the enzyme configuration in malignant cells (Kirby 2001). The enzyme ferrochelatase (FECH) catalyzes insertion of an iron atom into PpIX forming heme which is not photoreactive. However, cancer cells have a relatively low activity of ferrochelatase which leads to an excess accumulation of PpIX (Schoenfeld 1988). Another factor leading to augmented PpIX synthesis is an increased activity of the rate-limiting enzyme porphobilinogen deaminase in various malignant tissues (Wilson 1991). Kemmner W et al. (2008) recently showed that in malignant tissue a transcriptional down-regulation of FECH occurs causeing endogenous PpIX accumulation. Furthermore, accumulation of intracellular PpIX because of FECH small interfering RNA (siRNA) silencing provides a small-molecule-based approach to molecular imaging and molecular therapy.