Violacein – part I: Theoretical foundations and foundations on pigment

https://doi.org/10.56238/sevened2024.003-036

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ABSTRACT

Purple pigments are very rare in nature and for this reason, since antiquity, the symbology and pricing of these pigments have been linked to nobility, which has also given the color purple the same meaning. Different microorganisms produce violacein, a compound derived from tryptophan that exhibits different properties and is applied from fabric dyeing to as a drug candidate. This chapter is the first of two texts dealing with violacein. This chapter deals with historical aspects and the physicochemical characteristics of the molecule, that is, from its biosynthesis to the properties of violacein, which make it an important natural bioactive with infinite applications. The text was prepared by students of the Graduate Program in Natural, Synthetic and Bioactive Products at UFPB, as a final project of the course.

Keywords: Microbial pigments, Natural bioactive compounds, *Chromobacterium violaceum*.

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INTRODUCTION

HISTORY OF VIOLACEIN

The first record of classification of a purple pigment-producing bacterium occurred in 1880 by Bergonzini, who studied and named the coccobacillus Chromobacterium *violaceum*, in detail in his study entitled "*Sopra un nuovo bacterio colorato*". In the text, Bergonzini used the term "exuberant" for the violet color produced, describing violacein, observed diffused in a rice-based preparation (DeMoss, 1967).

Nearly five decades later, Tobie developed the first method of producing, extracting, and purifying violacein. In the 1930s, *C. violaceum* was identified by pigmentation in skin lesions, but the pigmented areas had an interesting characteristic, that is, they were free of infections. This, at a time of historical expansion that antibiotic therapy was experiencing, suggested that that pigment produced an inhibitory effect caused by some metabolic by-product diffused in the region and that it was against some bacteria (Lichstein, 1945).

In 1942, Singh observed that the crude extract of *C. violaceum,* when mixed with a bacterial suspension, produced a "protective" action for them, in which it prevented phagocytosis by amoebas present in the soil. Only three years later, Herman and Virginia finally described the antimicrobial activity of violacein against Gram-positive bacteria, with the exception of *Clostridium welchii* (now *C. perfringens*). The authors also observed a small negative effect on the growth of all Gram-negative bacteria tested and only found no activity against *Neisseria meningitidis* (Lichstein, 1945).

Years of ostracism were experienced in terms of studies with the pigment until it was only after the 1970s that other goals in the field of understanding violacein were researched, such as photodynamic antimicrobial therapy. Over the next decade, new extraction protocols were tested. The 1990s were marked by the understanding of the synthesis of violacein, but new applications for the compound were proposed and achieved with the beginning of the twenty-first century, such as: antiviral activity, tuberculosis treatment, in which the pigment showed pyrazinamide-like activity, silk dyeing, treatment of leukemia and lymphoma, antileishimania activity, and production of the pigment from engineered bacteria (Durán et al., 2001).

More recently, violacein continues to be strongly studied as an antitumor compound, as well as in antiviral therapy, having also evolved in terms of new methodologies, for example, cell culture, obtaining the pigment and producing nanosystems that optimize and enhance the treatment of diseases (Durán, et. al. 2021; Doganci, et al., 2022; Lee, et al., 2022).

VIOLACEIN-PRODUCING MICROORGANISMS

Violacein is a pigment produced by phylogenetically distinct microbial species that have sequences in their genomes that are closely related to the molecule's biosynthesis genes (Hakvåg et

al., 2009). Despite this, violacein differs from species-specific microbial pigments, such as some found in nature, such as pyocyanin (Gonçalves and Vasconcelos, 2021) and staphyloxanthin (Clauditz et al., 2006).

Violacein has been described in edaphic, freshwater and marine species of betaproteobacteria, such as *Collimonas* (Hakvåg *et al.,* 2009), *Duganella* (Aranda; Montes-Borrego; Landa, 2011*), Microbulbifer* (Won et al., 2017) and Pseudoalteromonas (Thøgersen et al., 2016), in addition to the species *Janthinobacterium lividum and Chromobacterium violaceum*, *used as reference microorganisms for violacein production (Choi et al., 2015).*

Chromobacterium violaceum (Bergonzini, 1880) is a Gram-negative, facultative coccobacillus, a member of the saprophytic microbiota, inhabits soil and water in tropical and subtropical regions in different areas of the globe and is not normally considered pathogenic (Durán and Menk, 2001). The first studies of violacein metabolism were carried out with the species, and it was noticed that in non-oxygenated cultures, the production of the pigment was significantly reduced, indicating the importance of oxygen for its synthesis (Durán and Menk, 2001). In addition, *C. violaceum* also produces a purple minority pigment called deoxyviolacein. The compound differs from violacein in the 5-hydroxyndol region due to the absence of the hydroxyl group, making it less polar (Hoshino and Momem, 2000; Hoshino et al., 1995).

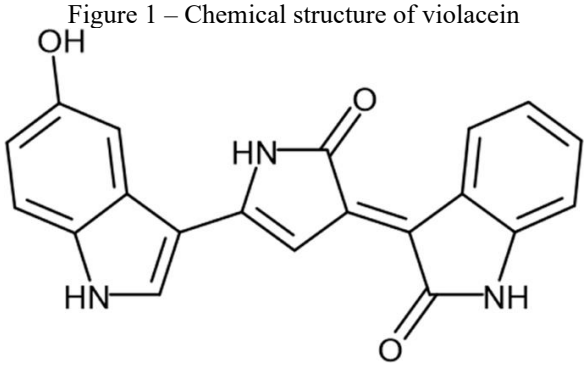
The second most studied violacein-producing species, *J. lividum* (Eisenberg, 1891) is an aerobic Gram-negative bacillus, commonly isolated from soils and fresh waters, using glycerol for synthesis of the compound. The extraction of violacein by *J. lividum* has been used in the dyeing of natural or synthetic fibers (Pantanella et al., 2006). The production of the pigment is encoded by the vioD, vioC, vioB *and vioA* genes *, whose expression is regulated according to the carbon source.*

CHEMISTRY OF VIOLACEIN

Violacein (3-[2-hydroxy-5-(5-hydroxy-1H-indole-3-yl)-1H-pyrrole-3-yl]indole-2-one) is a purple-colored pigment (Figure 1) produced by bacteria from the oxidative dimerization of tryptophan. Much of the knowledge about violacein has been obtained from studies with *C. violaceum.* As previously stated, violacein is the main pigment, however it is not specific to the bacterium. The molecule belongs to the class of hydroxyindoles, whose chemical formula is $C_{20}H_{13}N_3O_3$ and molecular weight of 343.3 g/mol (August et al., 2000; Hoshino et al., 1987).

The violacein molecule is composed of three distinct structural subunits, namely: 5-hydroxy-indole; 2-oxoindole and 2-pyrrolidone. All oxygen atoms present in the molecule originate from molecular oxygen (August et al., 2000; Hoshino et al., 1987).

Figure 1 – Chemical structure of violacein



Source: authors (2024)

Violacein synthesis is regulated by a *quorum sensing system* as a function of nutritional scarcity (Blosser and Grey, 2000). It is well known and comprises four phases. Briefly, the initial step occurs with the oxidative conversion of l-tryptophan into the corresponding alphaimin reactant, catalyzed by a flavonenzyme, VioA (l-tryptophan oxidase). The 1-tryptophan is converted to indole-3-pyruvic acid imine and then the imine is dimerized into a short-lived compound by iron-dependent VioB. It is spontaneously converted to chromopyrrolic acid or undergoes a displacement of the indole ring via VioE, resulting in protodeoxyviolacein acid. The final step, an autocatalytic oxidation, is regulated by means of two flavin-dependent oxidases, VioD and VioC, which convert protodeoxyviolacein acid to violacein. VioD hydroxyl an indole ring at position 5 to produce protoviolacein acid and then VioC hydroxyl the second indole ring at position 2, leading to a spontaneous oxidative decarboxylation that leads to the formation of violacein. In addition, VioC independently participates in the conversion of protodeoxyviolacein acid into deoxyviolacein, a minority pigment (Füller et al., 2016).

Violacein crystals are purplish-black in color, insoluble in water, and slightly soluble in ethanol. In addition, the molecule is moderately soluble in dioxane and acetone and very soluble in DMSO, methanol and ethyl acetate. Its melting point is greater than 290 ºC. The maximum absorption wavelengths in ultraviolet and visible light are at 258, 372 and 575 nm (Rettori and Durán, 1998).

Violacein exhibits a hydrophobic character and because of this low solubility in water, some experimental tests with violacein are unfeasible. However, ways to modify this characteristic are possible, for example when the molecule is added to glycosidic groups or subjected to the action of oxidative enzymes (Bromberg and Durán, 2001).

MECHANISM OF ACTION OF VIOLACEIN

Microbial pigments play a crucial role in the pathogenesis and progression of diseases by interfering with host immune mechanisms and/or exhibiting cytotoxic or pro-inflammatory characteristics (Liu and Nizet, 2009). In addition, pigments are also associated with the virulence of

the producing microorganism (Carević; Stojković; Ivanov, 2023) and with the understanding of the mechanisms of action, the biological and pharmacological functionalities of these compounds can assist in different therapies (Choi et al., 2021; Kothari; Sharma; Padia, 2017).

In this context, violacein shows commercial and therapeutic potential attributed to the biological activities it exhibits, such as antibacterial (Aruldass et al., 2018), antifungal (Choi et al., 2021), anticancer (Masuelli et al., 2016; Alshatwi; Subash-Babu; Antonisamy, 2015) and antiviral (Durán et al., 2016). With the advancement of studies with violacein, different mechanisms of action have been revealed, and the most recent literature reports four in particular.

The first mechanism of action of violacein concerns the property of causing damage to cell membranes, a response that is intrinsically associated with the antimicrobial activity of the molecule (Ahmed et al., 2021). It has been observed that violacein acts by directly interfering with the integrity of the membrane by leading to the loss of proton-ionic driving force, which results in efflux of cellular components and structural impairment of the cell. This interference leads to an osmotic imbalance that culminate in cell death (Aruldass et al., 2018).

A second mechanism relates violacein to the induction of oxidative stress, based on the formation of Reactive Oxygen Species (ROS) and consequent loss of membrane integrity. By generating peroxide, for example, violacein induces lipid peroxidation and increases cell membrane permeability, leading to forced loss of driving force and dissolution of K^+, soluble proteins, and sugars (Jiang et al., 2015; Durán et al., 2022).

Violacein has other targets of oxidative stress, such as pyridine nucleotides, oxygen uptake and alteration in the respiratory chain, and cytochrome C oxidation. Once cytochrome C is released into the cytosol, the apoptosome is formed, that is, a protein complex with caspase 9 and apaf-1, which cleaves and activates different proteins that together degrade the entire cell content. Different molecular targets that activate apoptosis can be affected by violacein, namely: tumor necrosis factor-α (TNF-α), mitogen-activated protein p38 kinase (p38 MAPK) and nuclear factor-κB (NF-κB) (Durán et al., 2007).

It is also noteworthy that by inducing the oxidation of pyridine nucleotides, violacein can directly interfere with the progression of the cell cycle. The way this occurs can be explained by two main mechanisms: the induction of cell cycle arrest in the G1 phase, as well as the increase of p53, p21 and p27 proteins. These events result in biochemical changes, directing the cell to apoptosis (Kodach et al., 2006; Obeng, 2021).

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