

New Perspectives for the Control of Parasitic Diseases Through the Use of Photodynamic Products

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Abstract

Parasitic diseases such as malaria, dengue or Schistosomiasis are a plague for millions of people mainly in underdeveloped and developing countries and often prohibit the economic development of the affected areas [1-9]. Great efforts have been made in the past to extinct or at least control these diseases by means of medical treatment, pesticides, converting wetland etc. - but with limited success. An example of this fact is the epidemic of dengue in Brazil, more specifically in the state of Rio de Janeiro and yellow fever in the center west region of Brazil. An important problem of underdeveloped areas suffering from parasitic diseases is the poverty, which cuts off the supply with material necessary for countermeasures. In the frame of this project we intend to develop methods for pest control, which are very inexpensive and non-toxic and by this are also applicable for poor regions in the world which are infested by severe tropical diseases. The method of pest control is based on the action of photodynamic substances. In contrast to previous attempts we will use inexpensive natural substances which can easily be extracted from plants, such as chlorophylls and their derivatives. Chlorophyll does not show any toxicity in humans: chlorophyll is a colorant in the food industry (E-140/E-141).

Keywords: Chlorophyll; Parasitic diseases; Photodynamic substances; Malaria; Dengue; Mosquitos

Research in pest control by means of photodynamic substances

Experiments conducted with photodynamic substances yielded very promising results. In a recent work performed by Abdel-Kader et al. [10] it was demonstrated that larvae of *Culex* are sensitive against hematoporphyrine (0.07 $\mu\text{mol/ml}$). Also larvae of *Musa domestica* could be killed with this substance (10 $\mu\text{mol/ml}$). In the course of his PhD thesis Allah el Tayeb, [11] could demonstrate the effectiveness of hematoporphyrine against *Culex* and eggs of the snail *Lymnaea natalensis* (vector of the trematode *Fasciola hepatica*). In this work the penetration of the photosensitizer inside the organism was shown by means of fluorescence microscopy as well as the pronounced effects on the ultrastructure of inner organs and muscles [10,11].

Wohllebe et al. [12], under laboratory conditions, the use of chlorophyllin/pheophorbid as photodynamic substances for pest control in water bodies promises to be not only effective and ecologically beneficial but also cheap. The LD_{50} (50% of mortality in the tested organisms) value in *Culex sp.* larvae was about 6.88 mg/l, in *Chaoborus sp.* larvae about 24.18 mg/l, and in *Daphnia* 0.55 mg/l. The LD_{50} values determined for pheophorbid were 8.44 mg/l in *Culex*, 1.05 mg/l in *Chaoborus*, and 0.45 mg/l in *Daphnia*, respectively. In some cases, chlorophyllin and pheophorbid were also found to be (less) active in darkness. The results presented in this paper show that chlorophyllin is about a factor of 100 more effective than methylene blue or hematoporphyrine, which were tested earlier for the same purpose. It is also much cheaper and, as a substance found in every green plant, it is 100% biodegradable.

Erzinger et al. [13] was demonstrated that mosquito larvae can be killed by means of photodynamic processes after the larvae have incorporated the photosensitizer chlorophyllin or pheophorbid, and were treated with light. The water-soluble substances were applied to and incorporated by the larvae in darkness. With *Chaoborus sp.* a

dark incubation of about 3 h is sufficient to yield mortality of about 90% and ≥ 6 h resulted in almost 100% mortality during subsequent illumination. Temperature did not influence mortality of the larvae significantly in a treatment of 6 h dark incubation and subsequent 3 h illumination. At 10°C, 20°C, or 30°C, between 80% and 100% of the treated larvae died when the light intensity from a solar simulator was above 30 W/m^2 . Lower irradiances were less effective.

Wohllebe et al. [14] have demonstrated for the first time that the photodynamic treatment of *C. crystallinus* larvae with chlorophyllin induces necrosis and apoptosis in these organisms. The intrinsic pathway of apoptosis plays a key role in this model.

Preservation of photodynamic activity of chlorophyllin

Erzinger et al. [13] described a major problem for the use of chlorophyll and its derivatives are related allomerization reactions. Allomerized chlorophyll a differs from the original material in that the product of hot alkaline hydrolysis is a mixture of unstable chlorins instead of the initial product chlorin. The same unstable chlorins are obtained from unallomerized chlorophyll or the magnesium-free compounds (pheophytin a or methyl pheophorbide a by saponification with alcoholic alkali at or below room temperature). Allomerization of chlorins is a dehydrogenation caused by oxygen of the air. It is

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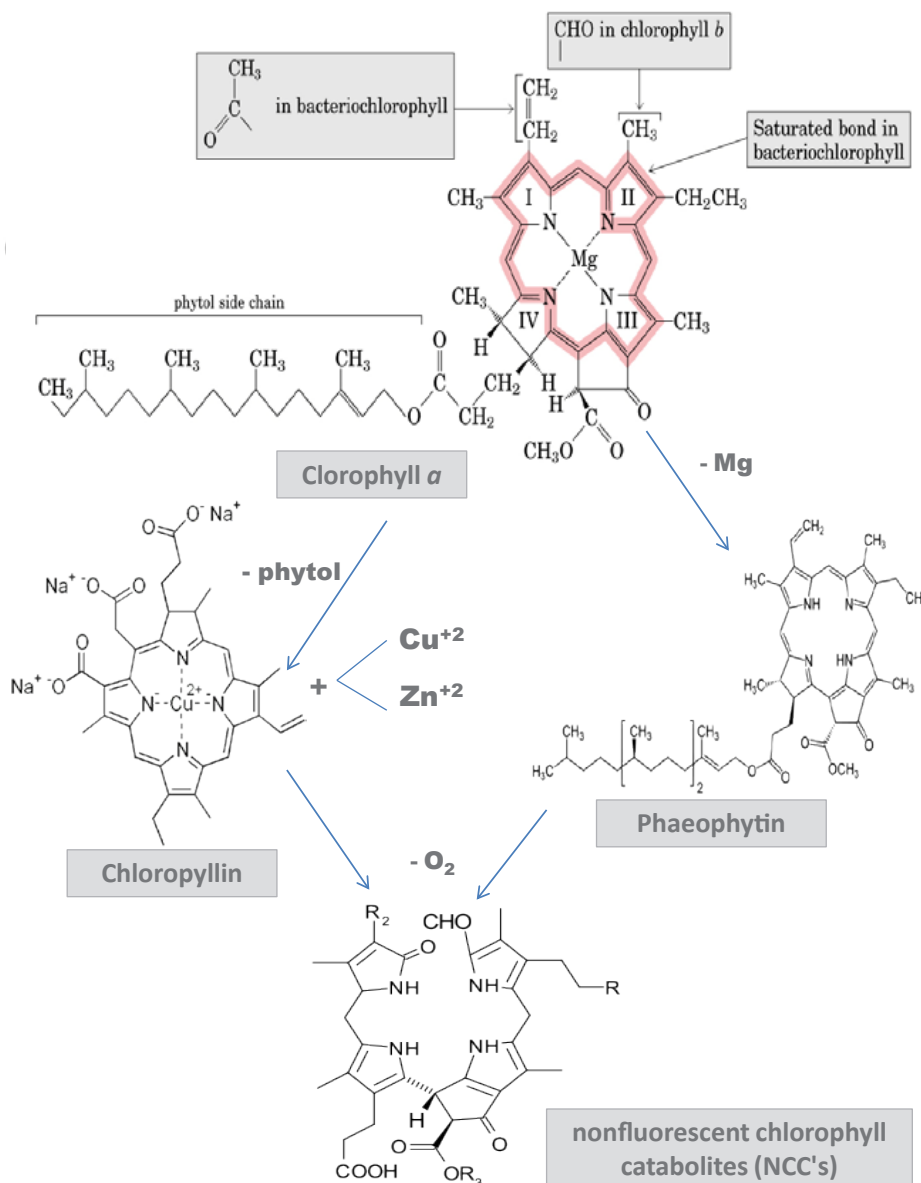


Figure 1: Chlorophyll and metabolic derivatives.

interesting that the dehydrogenation of a very easily oxidizable group in chlorophyll is without pronounced effect on its color, although marked color changes, as a rule, accompany the oxidation of colored substances. The unusual behavior of chlorophyll in this respect is due to the fact that the chromophoric group is not the most easily oxidized group [15-17] (Figure 1).

One of the alternatives considered were to make structural changes in chlorophyll derivatives by substitution of magnesium or in the process of conservation. The LD_{50} value of magnesium chlorophyllin was about 22.25 mg/l and for Zn chlorophyll 17.53 mg/l, while Cu chlorophyll (LD_{50} 0.1 mg/l) was shown to be toxic also without light. Chlorophyllin, which was lyophilized immediately after extraction,

was far more lethal to the larvae (LD_{50} 14.88 mg/l) than air-dried Mg chlorophyllin. Lyophilization immediately after isolation increases photodynamic activity of chlorophyllin and ensures its long-term stability [13].

Development of a new product

In 2010 Erzinger and Häder [18] developed and patented at INPI (National Institute of Intellectual Property) a new Bioinsecticide Nontoxic Biodegradable from a new semi-synthetic derivative of chlorophyll and in conjunction with a formulation system was able to get a product with high stability front light and maintained the same lethal power of chlorophyll and chlorophyllin for mosquito

larvae described in previous work. Currently is being done to study the possible environmental impacts of the continued use of this new product.

Conclusions and Perspectives

The derivatives of chlorophyll is a very interesting substance for photodynamic pest control. For this reason we conducted some primarily experiments in which we tested the effects on larvae of *Chaoborus* and *Culex* flies. We extracted chlorophyll from different plant sources (spinach, grass, dandelion etc.). Some experiments we conducted with chlorophyll converted into pheophytin or into a water-soluble chlorophyllide. We have developed a cheap new strategy for reducing the number of mosquito larvae, which may be very important for reducing the amount of mosquito bites and thereby the incidence of malaria and other parasitic diseases in the future.

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