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Review

# Serendipitous discovery of Chl *d* formation from Chl *a* with papain

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**Abstract**

Cancer photodynamic therapy (PDT) requires the availability of photosensitizers which have a high efficiency and selectivity for the destruction of tumor cells. Chlorophyll (Chl) *a* is one of the favorable photosensitizers, because it has a high extinction coefficient in the red light region, where light transmission through the human tissues is very high. However, Chl *a* had a serious problem that it cannot be dissolved in water, so we tried to prepare water-soluble chlorophyllide *a* from Chl *a* by several enzymes, and serendipitously came across a unique formation of Chl *d* from Chl *a* when papain was used in aqueous acetone. Similar oxidation was observed in Chl *b* and pheophytin *a*, although the reactions were very slow. Our finding will provide insight into the unsolved key question as to the biosynthetic pathway of Chl *d* *via* Chl *a* in a recently found novel cyanobacterium *Acaryochloris marina*.


*Keywords: Acaryochloris marina*; Chlorophyllide; Chlorophyll; Chlorophyll *a*; Chlorophyll *d*; Pheophorbide; Photodynamic therapy (PDT); Photosensitizer; Photosynthesis

**Contents**



## 1. Introduction

Cancer photodynamic therapy (PDT) requires the availability of photosensitizers, which have a high efficiency and selectivity for the destruction of tumor cells. Porphyrin derivatives, e.g. Photofrin® (Fig. 1), have been clinically used [1–3]. Photofrin® is most frequently used now, but is a complex mixture with variable composition, and thus interpretation of the localization and functional mechanism are not clear. Photofrin® has a serious problem that it has very low ability to absorb red light (see a broken line in Fig. 2), whereas the transmission of light through human tissues increases with increasing wavelength. Therefore,

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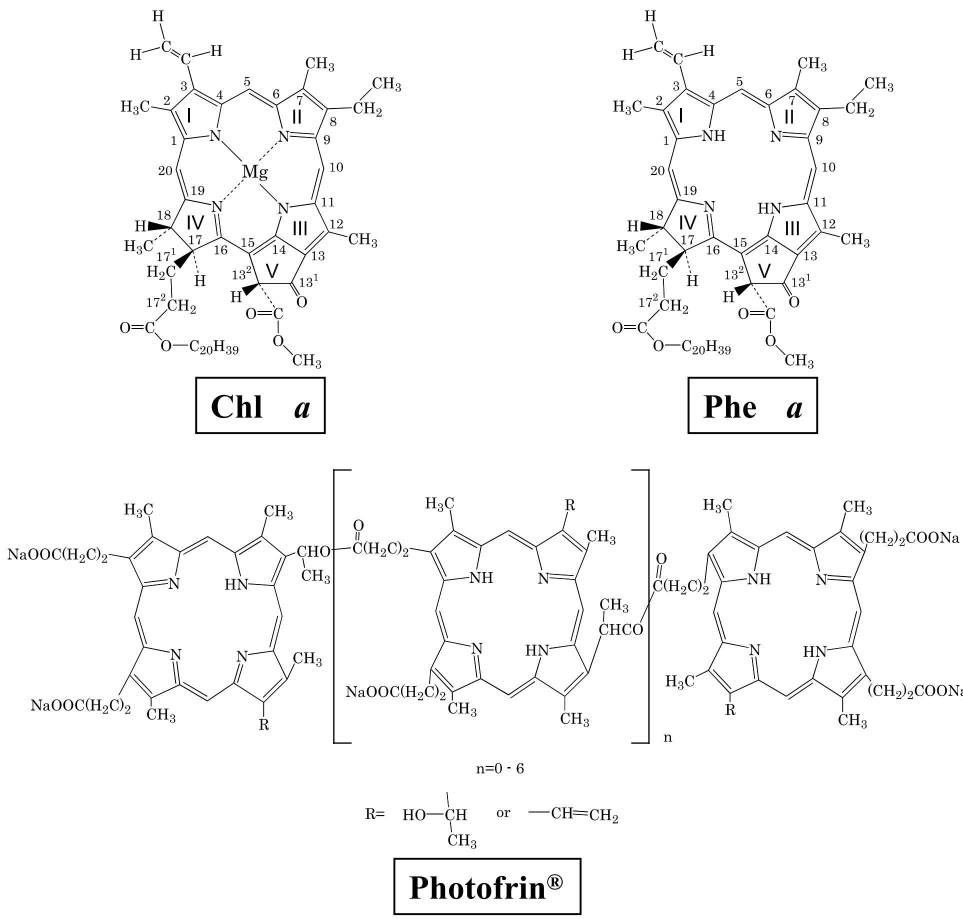


Fig. 1. Molecular structures of Chl *a*, Phe *a* and Photofrin®.

new photosensitizers, which are pure and have strong absorption in the red spectral region, are strongly expected.

Chlorophyll (Chl) *a* (Fig. 1) is one of the favorable candidates, since it can be easily prepared from green plants and has a high extinction coefficient in the red region (see a solid line in Fig. 2). However, Chl *a* has a fundamental problem: Chl *a* cannot be dissolved in water, because of the presence of a hydrophobic long alkyl chain ($-C_{20}H_{39}$). Release of the long chain by hydrolysis is effective to give the pigment solubility in water.

Chlorophyllase in green leaves is known to function as an esterase for Chl *a* (Fig. 3), but it is too difficult to extract it from plants and purify it. Therefore, we previously used acid for the hydrolysis of Chl *a* [4–7]. In this method, however, the central metal, Mg, was also released from Chl *a*, producing pheophorbide (Phde) *a* instead of Chlide *a*, as illustrated in Fig. 3. Phe *a* (Fig. 1) has almost the half absorption ability of Chl *a* for red light (Fig. 2), even though much higher than Photofrin® (please note that Chl *a* and Chlide *a* has the same absorption property, and Phe *a* and Phde *a* pair also).

Recently, we tried to hydrolyze Chl *a* by several esterases to yield Chlide *a*, but not succeeded [8]. So we next used some proteases, because a protease is known to function as an esterase in aqueous organic solvents [9–11]. During these studies, we serendipitously came across the formation of Chl *d* (Fig. 4) from Chl *a* when papain was used [8].

Until 1996, Chl *a* had been believed to be a major and essential pigment in oxygenic photosynthetic organisms without exception. However, Chl *d* is found to be dominant in a unique cyanobacterium *Acaryochloris marina* (*A. marina*) [12]. The biosynthetic pathway of Chl *d* in *A. marina* has not yet been clarified. From the molecular

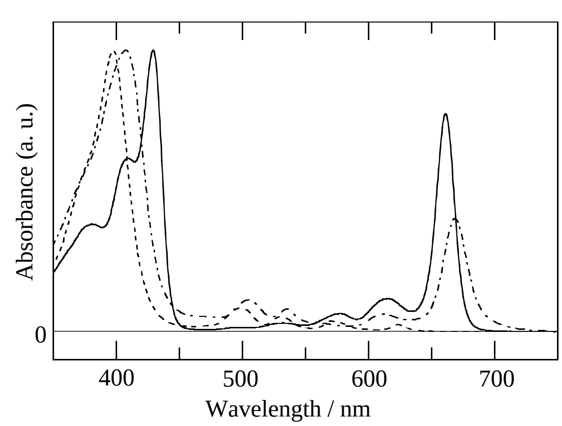


Fig. 2. Absorption spectra of Chl *a* (—), Phe *a* (-· -· -) and Photofrin® (- - -) in acetone. Soret-band maxima are arbitrarily scaled to a common height.