The substitution of Mg$^{2+}$ with Mn$^{2+/4+}$ metal ions in chlorophyll structure isolated from *Gliricidia sepium* leaves

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Abstract. One of the critical issues of using chlorophyll (Mg-Chl) as a dye photosensitizer in DSSC is related to a rapid decay of this material. Changing the alkali metal using transition metal ions could theoretically increase the stability of chlorophyll. In this research, the substitution of Mg$^{2+}$ with Mn$^{n+}$ ions ($n = 2$ and $4$) in chlorophyll structure isolated from *Gliricidia sepium* leaves had been carried out. The column chromatography technique was chosen to separate the chlorophyll followed by modifying the metal centre using two different compounds containing manganese, namely MnCl$_2$ and MnO$_2$. The results show that there are differences in Rf values after substitutions, [0.90 (Mg-Chl), 0.81 (Mn$^{2+}$-Chl), and 0.96 (Mn$^{4+}$-Chl)]. The Soret bands experience a redshift from 330 to 338 nm for Mn$^{2+}$-Chl and a blue shift from 411 to 401 nm for Mn$^{4+}$-Chl while the Q band has a redshift from 665 to 668 nm for Mn$^{2+}$-Chl but a blue shift from 665 to 526 nm for Mn$^{4+}$-Chl. The obtained products have similar IR absorption patterns to Mg-Chl but differ only in its transmittance with slightly higher wavenumbers shifts. Shifting of UV-Visible and Infra-red characteristics indicate different molecular electronic nature of the obtained compounds. This implies successful substitution for the metal centre without damaging the structure of the chlorophyll.

1. Introduction

Global consumption of fossil fuels has been increasing at an alarming rate since we are almost running out of this carbon based type of fuel as our main source of energy. The search for new and sustainable energy platforms is essentially needed to cope with this challenge. At the same time, providing solutions for reducing the global warming effect derived from our non-renewable energy consumption is critically needed as well. Solar light harvesting research offers a promising future for green energy consumption especially in regions situated in the equator including Indonesia with the abundance of sunlight [1,2]. The third generation of solar cell known as dye-sensitized solar cell (DSSC) proposed by Michael Gratzel [3] has been extensively studied. Attention has been focused on increasing the efficiency of the cell using a wide range of approaches such as modification of solid-based semiconducting materials [5], development of liquid-based DSSC [6,7], and improvement of dye molecule design to enhance performance in harvesting lights [8]. Natural dyes from plant leaves such as chlorophyll seem to have more advantages compared to other dyes because it is environmentally friendly and naturally abundant. However, the efficiency of cells using this type of dye is considerably low owing to the rapid decay of this photosensitizer. Several studies have been developed by changing the metal centre of the
chlorophyll using transition metals such as Fe$^{3+}$ [9], Fe$^{2+}$ [10,11], and Zn$^{2+}$ [12] to maintain the stability of the structures used as photosensitizers. Manganese, a transition metal atom abundantly found especially in NTT province has not been used for this purpose. *Gliricidia Sepium* is also a typical plant grown without difficulty in the dry land of NTT potential as a chlorophyll source. With the abundance of manganese minerals and chlorophyll-rich plants, we aim to modify the structure of chlorophyll using Mn metal atom by substituting Mg$^{2+}$ and studying the impact of oxidation states of the manganese metal on the substitution.

2. Methods
The experiments were conducted using standard procedures [11-13].

2.1. Chlorophyll isolation
200 g of *Gliricidia sepium* leaves were destructed using blender followed by maceration for 5 days using 500 ml of cold acetone. The mixture was then filtered using Whatman no.1 filter paper. The green extract obtained was evaporated to separate the solvent. This green mixture containing chlorophyll was subsequently subjected to column chromatography and eluted using petroleum ether:acetone (3:1) until all yellow colours were completely separated. Further elution was performed to obtain the next green colour band identified as chlorophyll after solvents were evaporated.

2.2. Metal substitution
150 ml chlorophyll A ($2.2 \times 10^{-4}$ M) was added with 10 ml HCl 0.1M at 40°C for 30 minutes. After that, n-hexane was added to the acetone-water mixture of the sample to extract pheophytin. The extracted pheophytin was further dissolved using 20 mL acetone together with 4 ml buffer acetate (pH = 5) and reacted with MnO$_2$ or MnCl$_2$ in 40 ml methanol. The mixture was continuously stirred at the same level of temperature under a nitrogen atmosphere for 1 hour. After the reaction was completed indicated by a colour change from green to deep brown, the solution was evaporated to obtain the desired product.

3. Results and discussion
The maceration of crude chlorophyll of *Gliricidia sepium* sample was carried out in a dark container since chlorophyll is sensitive to light exposure. The separation of chlorophyll from other compounds in the extract was done through column chromatography with the eluent of petroleum ether:acetone (3:1) because these solvents are proven to effectively separate minor pigments such as chlorophyll a and b as reported by Diaz and Ferreira [13].

The processes of substitution of the alkali metal with transition metals in the chlorophyll structure are highly dependent on its experimental condition. In this research, a set of glassware designed for this purpose was carefully assembled to maintain an oxygen-free environment by gently pumping nitrogen gas into the system and simultaneously monitoring the gas bubbling system to ensure a constant flow of nitrogen throughout the reaction. At the initial stage of the substitution reaction, hydrochloric acid was added to maintain a stable pH of the solution during the dechelatase process to pull the magnesium metal out from the chlorophyll structure. This will result in the formation of pheophytin and a side product of MgCl$_2$ in which this condition allows pheophytin to open for potential binding with outside metal added to the solution. The presence of Mn metal ions in the solution acting as the Lewis acid allows interaction with the pheophytin ring with four nitrogen atoms in the pheophytin structure donating lone pairs to form Mn-chlorophyll complexes. The overall chemical reaction occurred can be summarized in figure 1 as follows:
Figure 1. Substitution reaction of Mg using Mn (adapted from [11] with the modification of the metal center).

Thin Layer Chromatography (TLC) experiment (figure 2) shows a single spot for each product when using MnCl$_2$ or MnO$_2$ as the source of manganese metal. The substitution using a lower oxidation state of manganese metal from MnCl$_2$ gives an Rf value of 0.81 which differs when the manganese metal was changed to a higher oxidation state using Mn from MnO$_2$, 0.96. This two Rf values indicate different products compared to Mg(II)Chl as the starting material.

Figure 2. Thin-layer chromatography of Mg(II)-Chlorophyll and synthesized Mn-Chlorophylls.
Figure 3. UV-Vis spectrum of Mg-Chlorophyll and synthesized Mn-Chlorophylls.

The line graph (figure 3) provides information about UV-Vis characteristics of the synthesized compounds compared to Mg Chlorophyll (green line) and pheophytin (purple dots). When Mg metal ion was removed from the structure upon the addition of hydrochloric acid to form pheophytin, the shoulder of the Soret band experiences a slight shifting to higher wavelength (~420 to 425 nm). After the substitution, both products assigned as Mn(II) and Mn(IV) chlorophyll complexes (blue and brown lines, respectively) have significant shifts in the Soret bands to shorter wavelength (hypsochromic effect) or higher energy with less intense absorptions (hypochromic effect) compared to Mg-Chlorophyll and Pheophytin. The hypsochromic and hypochromic effects occurrence are due to less conjugation in the structure of Mn-Chlorophyll because of the presence of some empty d orbital of manganese metal attracting electron in the conjugated bonds leading to an increase of energy gap between HOMO and LUMO [14,15]. These changes of Soret bands are similar to the substitution of Mg$^{2+}$ with Fe$^{2+}$ using chlorophyll from papaya leaves as studied by Arifin and co-workers [11] in which the maximum wavelength of the Soret bands shifted from 412 nm (Mg-Chl) to 384 nm (Fe-Chl). Besides, Mg$^{2+}$ substitution using Fe$^{2+}$ with a different source of chlorophyll from spinach leaves conducted by Sandinintyas and Suendo [10] had also confirmed comparable UV-Vis shifting characteristics, from 413 nm (Mg-Chl) to 393 nm (Mn-Chl).

For the Q bands, the substitution using Mn$^{2+}$ causes a small change to longer wavelength or lower energy. However, the Q bands for modification with Mn$^{4+}$ metal ion is not clearly identified as for Mn$^{2+}$. This might be due to the fact that the ionic radius of Mn(II) is slightly closer to Mg$^{2+}$ which results in similar stability of the Mn(II)-chlorophyll. On the other hand, the ionic radius of Mn(IV) is theoretically smaller which forms longer metal-ligand bonding leading to possibly different electronic structures of the compounds to absorb light for the whole range of the visible spectrum, therefore, causing an unnoticeable Q band. Changes of the metal centre affect the molecular electronic of the compound giving rise to the shifting of the UV-Vis absorption bands.
Figure 4. FTIR spectra of Mg-Chlorophyll and synthesized Mn-Chlorophylls.

Since the UV-Vis spectrum of the modified structure indicates noticeable changes, it is important to confirm whether the Mn metal ions were successfully introduced into the pheophytin ring. IR spectrum of the modified structure of Mn(II) and Mn(IV) complexes (blue and red colours), display a similar pattern compared to Mg-Chlorophyll. However, the substitution of Mg$^{2+}$ with Mn$^{2+}$ and Mn$^{4+}$ gives a broader IR absorption in 3000-3600 cm$^{-1}$ with slight shifting to higher wavenumber (Mn$^{2+}$) and lower wavenumber (Mn$^{4+}$) in this region. The same absorption behaviors are also displayed in 1500-1700 cm$^{-1}$. This typical shifting is associated to atomic radii of the metal centres (Mg$^{2+}$= 72, Mn$^{2+}$= 83, Mn$^{4+}$= 53) [16]. The successful introduction of a higher atomic radius of metal causes a shift to higher wavenumber while a lower atomic radius shifts the IR absorption into lower wavenumber as proposed by Ziminov and team [17]. In addition, the transmittance percentages of modified structures are not similar to Mg-Chlorophyll indicating different absorption natures of the obtained products. For example, the changes of metal ions using manganese decreases % transmittance around 2800 – 3000 cm$^{-1}$ and 1000 – 1500 cm$^{-1}$. On the other hand, the presence of transition metal ions increases the transmittance in the far-infrared region (400 – 700 cm$^{-1}$) associated to the formation of Mn-N bonds. The transmittance characteristic data aligns with IR spectra of studied Fe(III)-Chlorophyll reported by Darmakoesomo and co-workers [9]. The alignment between UV-Vis and IR data confirms that the electronic structure of the synthesized compounds is different but their general characteristics such as the pheopthyn ring together with the attached functional groups remain the same. This means that only the magnesium metal ion was successfully substituted with manganese metal ions without damaging the structure.

4. Conclusion
Magnesium metal ion in chlorophyll isolated from *Gliricidia sepium* was successfully modified using Mn metal ions. The change of the oxidation states of the metal centre (Mn$^{2+}$ and Mn$^{4+}$) influence the electronic structure of the compound leading to different UV-Vis characteristic. The IR slight shifting and different transmittance with consistent characteristic patterns of the absorptions indicates a successful substitution of the metal centre without damaging the structure of chlorophyll.

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