



Synthesis and Oxidation of Cobalt(II) Pheophytin-*a* Complex

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Abstract: The mechanism of oxidation of natural pheophytin-*a* incorporated with cobalt as the central metal ion has been investigated. Natural pheophytin-*a* extracted from spinach was metallated with cobalt(II) to form the complex, cobalt(II) pheophytin-*a* [Cophe]. The complex was characterized by Ultraviolet and Visible, Fourier Transform Infrared and Electrospray ion Mass Spectroscopy. The synthesis of cobalt(II) pheophytin-*a* was carried out and the effect of the substitution on the chlorophyll macrocycle was studied by the reaction of hexaaquachromium(III) cation. The presence of cobalt as the central metal ion increases the energies of the chlorophyll main absorption transitions. The oxidation of the cobalt(II) pheophytin-*a*, [Cophe] by hexaaquachromium(III) cation in dilute hydrochloric acid has been studied and found to follow first-order kinetics. Rate constants for the oxidation reaction at 313, 322.8 and 332.9 K were found to be 5.4×10^{-5} , 1.8×10^{-4} and 5.9×10^{-4} /s respectively. An outer-sphere mechanism has been proposed for the oxidation of cobalt(II) pheophytin-*a*.

Keywords: Cobalt(II) pheophytin-*a*, Kinetic reaction, Extraction, Chlorophyll-*a*, Electron transfer

Introduction

Studies of metals coordinated to pheophytin in place of magnesium in chlorophylls have been done¹. Photosynthetic pigments such as chlorophyll-*a* with magnesium as the central metal ion, bind light and moderately heavy elements with a simple Frontier NS² electronic configuration. This permits the complex to retain both photochemical and redox activity. With such versatile design, a single type of pigment has the capacity to participate either in energy absorption and transfer or in electron transfer. The situation becomes less simple when tetrapyrroles chelate transition metal ions which contribute their *d*-electrons and

interfere with the ligand *p*-electron system. The nuclei of heavy central ions introduce another type of interaction, the so called heavy atom effect. Such interactions, via the enhancement of spin-orbit coupling cause a mixing of states of differing spin multiplicity and thus promotes otherwise formally spin forbidden transitions³.

Chlorophyll-*a*, plays a vital role in green plant photosynthesis, the process by which solar energy is converted into the chemical energy required for plant metabolism². Chlorophyll-*a* has therefore been studied thoroughly by a variety of techniques²⁻⁶.

A study of the effects of central metal ion (Mg, Zn) and solvent on singlet excited state energy flow in porphyrins, concluded that Zn-containing arrays are quite robust while Mg-containing arrays are slightly labile towards demetallation and photooxidation⁷.

It has been known for some time that the substitution of the central metal in various porphyrin systems and transmetallated chlorophylls alters their properties². Complexes of cyclic tetrapyrroles with metals other than magnesium have been studied to better understand their spectroscopic and redox properties⁴. The substitution of the central metal in chlorophyll from magnesium to zinc increases chemical (acidic) stability and other physicochemical properties⁹. Rare earth elements such as lanthanum⁸ have been coordinated to chlorophyll in place of magnesium. A suitable amount of rare earth element has been found to improve photosynthesis in plants⁸.

Electron transfer processes involving metallated chlorophyll-*a* complexes has not been studied to determine the rate of metal-metal electron exchange reactions. In this study, we have investigated the mechanism of electron transfer by the oxidation of cobalt(II) pheophytin-*a* with hexaaquachromium(III) ions in dilute hydrochloric acid by UV-Vis spectroscopy between 313 and 332.9 K.

Experimental

Spinach leaves used in all analysis were obtained locally in Mafikeng, South Africa. The leaves were frozen for 24 hours before analysis. All reagents (Petroleum ether (60-80 °C), diethyl ether, glacial acetic acid, acetone and methanol) were of analytical grade and used without further purification. Cobalt acetate and hydrochloric acid were obtained from Unilab, Saarchem Pty, Muldersdrift 1747, South Africa.

Characterization

A Pye unicam 8700 and a Cary 50 (Varian Mulgrave USA) spectrophotometers were used to determine the UV-Vis spectrum and for kinetic measurement of absorbance in quartz cuvettes in 1 cm path length.

A Finnigan LCQ-MS spectrometer with an ESI source was employed for the mass spectrum determination. The determination conditions were as follows: sheath gas flow rate 0.55 L min⁻¹; auxillary gas flow rate, 0.05 L mi; ion spray voltage, 3.5 kV; capillary temperature, 200 °C, capillary voltage 27V; tube lens offset, 55 V. The sample was injected by a Finnigan syringe pump connected to the ESI source by a 100 µm id fused-silica capillary, flow rate was 3.5 µL/min. IR spectra were recorded on a Nicolet impact 410 Fourier transform infrared spectrometer in the 400 – 4000 cm⁻¹ spectral region with potassium bromide cells.

Preparation of cobalt(II) pheophytin-a

Pheophytin-*a* was obtained from chlorophyll *a* extracted from fresh spinach leaves as described before⁹. Cobalt pheophytin-*a* was prepared as follows: cobalt(II) acetate (2.0 mg)

was dissolved in acetic acid (20 cm³). To this solution, pheophytin-*a* (3.0 mg) was added by stirring. The mixture was gently heated on a steam bath for 10 minutes and allowed to cool. The mixture was extracted with chloroform and evaporated to dryness to yield cobalt(II) pheophytin-*a*. The product was re-crystallized from diethyl ether. The purity of the product was checked by visible absorption, infrared and mass spectroscopy.

Pigment extraction

100 g of Fresh frozen spinach leaves were ground with pestle and mortar and exhaustively extracted with 80% acetone. The whole extract was washed with petroleum ether (60-80 °C) and diethyl ether. After solvent reduction in a rotavapour, the extract was chromatographed by column chromatography. The extract was acidified with glacial acetic acid, (1 cm³ of glacial acetic acid: 5 cm³ extract) and then heated to boil. The extract was metallated with an excess of 0.1 mol/dm³ cobalt acetate in acetic acid solution. Each extraction product was analyzed by UV-Vis spectroscopy and by electrospray ion mass spectroscopy (EPI-MS). The extraction process is shown in the flow diagram in Figure 1

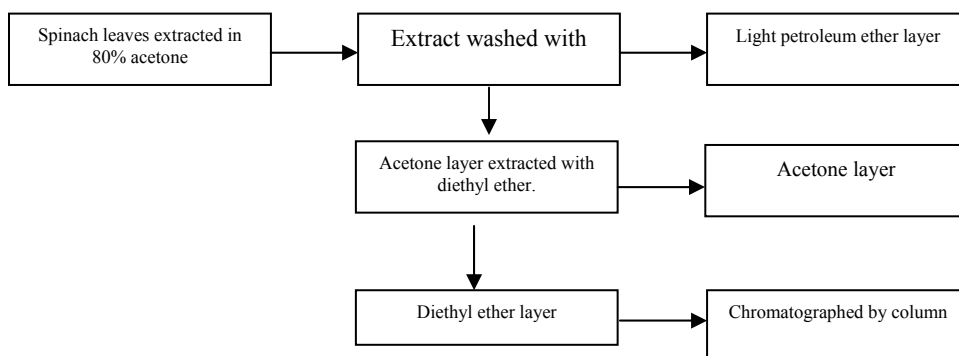


Figure 1. Extraction flow diagram

Rate measurement

The reaction of cobalt(II) pheophytin-*a* complex with hexaaquachromium(III) cation was followed spectroscopically with a Cary 50 spectrophotometer. Cobalt(II) pheophytin-*a* (6.45 × 10⁻⁵ mol/dm³) in 0.1 mol/dm³ hydrochloric acid solution and hexaaquachromium(III) cation ([Cr(H₂O)₆]³⁺) 0.01 mol/dm³, in 0.1 mol/dm³ hydrochloric acid solution were reacted at 313 K, 322.8 K and 332.9 K. The two solutions were mixed and quickly transferred to a 1 cm quartz cell in a thermostated compartment of the UV-Vis spectrometer. The UV-Vis absorption spectrum 300 - 800 nm was then scanned at specific time intervals. Maintenance of isosbestic point throughout the entire reaction was an indication of a simple reaction. Pseudo-first order kinetics were derived from the equation.

$$[A] = A_0 e \quad (1)$$

$$\ln \frac{[A]_t}{[A]_0} = - \ln \frac{(A_t - A_\infty)}{(A_0 - A_\infty)} = - \quad (2)$$

Where [A]₀ is the initial concentration of A (at t = 0). We have replaced the concentration [A]_t by (A_t - A) and [A]₀ is replaced by (A₀ - A). The equation of the first order reaction can therefore be presented as the concentration at infinity subtracted from the initial concentration and from the concentration at all time intervals.⁹

$$\text{Therefore} \quad \ln \frac{[Cr(H_2O)_6]^{3+} - [Cr(H_2O)_6]^{2+}}{[Cr(H_2O)_6]^{2+} - [Cr(H_2O)_6]^{3+}} = - \quad (3)$$

Results and Discussion

The yield from 100 g of freeze-dried spinach leaves was 23 - 24 mg of chlorophyll-*a* (obtained in triplicate). The identification and purity of the chlorophyll-*a* extract was determined by UV-Vis, IR and mass spectrometry. Spectrophotometric monitoring was carried out wherever possible at every stage.

The insertion of the metal cobalt in the centre of the tetrapyrrole ring of chlorophyll-*a* in place of magnesium, was successful. This is evident in the electrospray ion mass spectra ESI-MS obtained. The most important signal of the ESI-MS is the signal at m/z 648.66 that is due to cobalt(II) pheophytin-*a* complex without the phytol chain. The observed m/z of this signal at 648.66 and the calculated m/z of this signal at 648.53 is also an indication that cobalt was inserted successfully. The signal of cobalt(II) pheophytin-*a* complex at m/z 648.66_(obs), 648.53_(cal) is found in the ESI-MS of chlorophyll-*a* at m/z 613.2_(obs), 613.973_(cal) and in pheophytin-*a*, at m/z 593.2_(obs), 592.0_(cal) respectively. Whilst the relative abundance of this signal is about 100% in the ESI-MS of chlorophyll-*a* and pheophytin-*a*, in cobalt(II) pheophytin-*a*, the signal has an abundance of about 85%.

The other signals of interest in the cobalt(II) pheophytin-*a* ESI-MS are those at m/z 825.49_(obs) and 1002.32_(obs) these signals were also observed in the ESI-MS spectra of chlorophyll-*a* and pheophytin-*a*. These signals may have resulted from chlorophyll-*a* and pheophytin aggregation or dimerization in the solvent^{8,10-14}.

The UV-Vis spectra of pheophytin-*a*, show several absorption bands, which are characteristic of pheophytin-*a* confirming that the chlorophyll-*a* obtained is chlorophyll-*a* and the pheophytin obtained is also pheophytin-*a*¹⁴. The absorption spectra of chlorophyll-*a*, and pheophytin-*a*, show strong absorption bands in the red region, (called the Q_x band) and in the blue region (called the soret band, Q_y) of the spectrum. The band in the blue region, near ultraviolet is characteristic of porphyrins¹⁵. These absorption bands arise from the promotion of electrons from the porphyrin π HOMO to the π^* LUMO^{16,17}. The soret band at 430 nm (Q_y) in chlorophyll-*a* is shifted to 410 nm in pheophytin-*a*, while the band in the red region at 663 nm (Q_x) in chlorophyll-*a*, is shifted to 671 nm in pheophytin-*a*. The spectra of pheophytin-*a* show absorption bands notably at 505 nm and at 671 nm¹⁴.

Pheophytin-*a* metallated by the acetate method with cobalt acetate forms cobalt(II) pheophytin-*a* complex. The absorption spectra of the complex, showed two absorption bands at 415 and 654 nm. Metallation of pheophytin-*a* with a metal such as cobalt to form cobalt(II) pheophytin-*a* complex causes further changes in the absorption spectrum¹⁶.

The Q_x band in the red region is shifted from 671 nm to 654 nm while the soret band Q_y, is shifted from 410 nm to 415 nm in the cobalt(II) pheophytin-*a* complex. The relationship between the central metal and the band energies in chlorophyll-*a* is confirmed by the soret band energy versus the Q_y energy in several metal substituted complexes¹⁸. The change in this absorption spectra is a confirmation of the successful metallation of the pheophytin-*a* with the metal cobalt¹⁵.

The reaction of cobalt(II) pheophytin with hexaaquachromium(III) cation is a complementary reaction in which the cobalt(II) is oxidized to cobalt(III) and the chromium(III) is reduced to chromium(II), as shown in equation (4).



The oxidation reaction of cobalt(II) pheophytin-*a* by hexaaquachromium(III) cation followed a pseudo first order kinetics, Figure 2. The oxidation reaction was slow, an indication

that the cobalt ion is not easily oxidized, due largely to the fact that the chlorophyll macrocycle is a large ligand⁶. Rate constants for the reactions were determined from the slope of the plot of equation (3) against time over fixed temperatures of 313, 322.8 and 332.9 K. Rate constants at these temperatures are presented in Table 1.

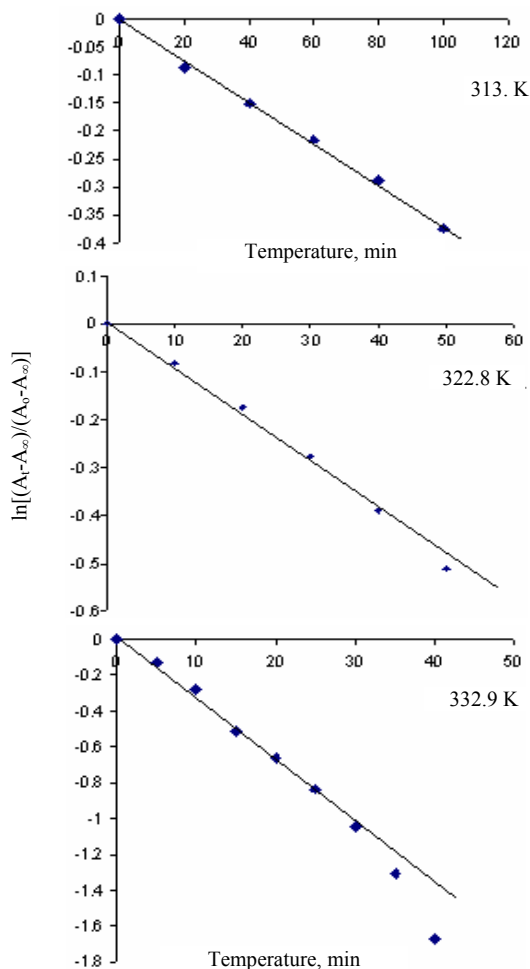


Figure 2. Kinetic plots for the oxidation of $[\text{Cophe}]^{2+}$ with $[\text{Cr}(\text{H}_2\text{O})_6]^{3+}$ in dilute hydrochloric acid solution at different temperatures. Absorbance change was monitored at 629 nm. A_0 , A_t and A_∞ are absorbance values at time = 0, t and ∞ respectively.

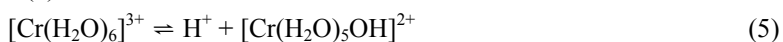
Table 1. Rate constants for the oxidation of $[\text{Cophe}]^{2+}$ with $[\text{Cr}(\text{H}_2\text{O})_6]^{3+}$.

Temperature (K)	313	322.8	332.9
Rate constants (/s)	5.4×10^{-5}	1.8×10^{-4}	5.9×10^{-4}

The kinetic stability of the cobalt ion in the centre of the chlorophyll macrocycle is comparable to results obtained by other workers¹⁹⁻²¹ in this field, which show that transition metals coordinated to the chlorophyll macrocycle provide an increase in the stability²² of the chlorophyll macrocycle. This stability is reflected in the activation energy of the electron transfer reaction obtained.

The cobalt(II) pheophytin-*a* complex in this study is stable and less prone to degradation. The pseudo first order reaction observed could also be explained by invoking the inductive effect of the central metal by comparing the Mg—N bond and the Co—N bond. Higher ionicity is also expected for the Mg—N bond because the electronegativity (E_N) of magnesium ($E_N = 1.2$) is also lower than that of cobalt ($E_N = 1.7$)⁶.

The higher electron density in the chlorophyll-*a* macrocycle and on the nitrogen atoms is expected to assist in electrophilic attack of proton on nitrogen resulting in lability of the magnesium when acidified. As a result, magnesium is easily exchanged by acidification to form the derivative or degradation product pheophytin in solution. This is also followed by a colour change from green to olive green. The hexaaquachromium(III) cation²³⁻²⁶ is acidic according to equation (5).



Since this could occur by the loss of a proton from coordinated water, the complementary oxidation reaction between cobalt and chromium may take place by electron transfer in this manner¹⁷. The mechanism of the oxidation of cobalt(II) pheophytin-*a* by hexaaquachromium(III) cation in solution is an outer-sphere reaction mechanism. This is because the pheophytin-*a* ligand to which the cobalt is coordinated to does not have a bridging group which is common to the coordination sphere of both cobalt(II) pheophytin-*a* and the hexaaquachromium(III) cation. Both reactants also have to rearrange before electron transfer could take place. The chlorophyll macrocycle being relatively large takes time for this rearrangement to take place. This explains the relatively slower rate of this reaction compared to the normal rates experienced in outer sphere mechanisms²⁷. As a result, the oxidation of cobalt(II) pheophytin-*a* by hexaaquachromium(III) cation in hydrochloric acid medium is a complementary outer-sphere electron transfer reaction in which, the cobalt is oxidized ($\text{Co}^{2+} \rightarrow \text{Co}^{3+}$) and the chromium is reduced ($\text{Cr}^{3+} \rightarrow \text{Cr}^{2+}$). This reaction could therefore be represented as shown in equation (4),

Conclusion

This paper presents the results of the electron transfer reaction between cobalt(II) pheophytin-*a* and hexaaquachromium(III) cation. The rate of reaction obtained presents evidence of the difficulty that would be associated with natural photosynthetic electron transfer process if the central metal ion in the chlorophyll macrocycle were a transition metal atom such as cobalt.

It is interesting to note that with cobalt as the central metal ion, the ligand ground state properties in chlorophyll shifts in their absorption maxima. The substitution of cobalt in the centre of the chlorophyll macrocycle, is discernible in the spectral properties of the Cophe complex. The high electronegativity of the cobalt as the central metal in the chlorophyll macrocycle results in the electronic transition of the complex to shift to higher energies.

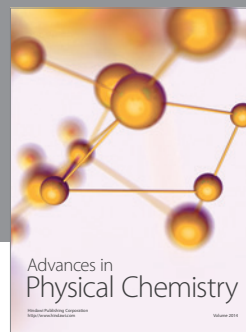
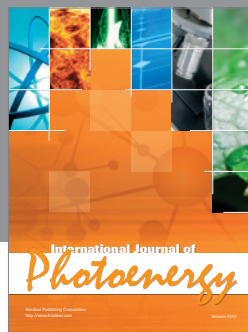
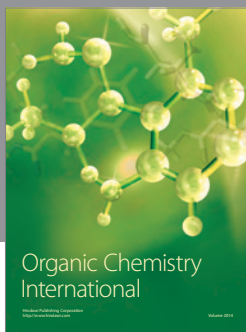
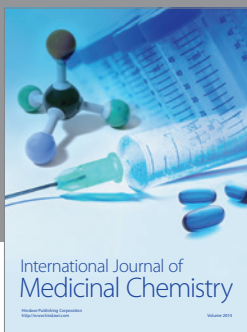
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