

DERIVATION AND VERIFICATION OF KINETICS EQUATION FOR DEGRADATION OF CHLOROPHYLL IN EXTRACT OBTAINED FROM LEAVES OF SOME WEEDS

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ABSTRACT

Degradation of chlorophyll occurs in light as well as darkness and is also influenced by various factors. Isolated chlorophyll is particularly not stable. In the present study, weeds have been used for the analysis which otherwise do not have any utility. The species used for the purpose include- *Euphorbia hirta*, *Chenopodium album*, *Amaranthus hybridus*, *Lantana camara*, *Parthenium hysterophorus*, *Tridax procumbens*, *Xanthium strumarium*, *Cannabis sativa* and *Croton spp.* An expression for the rate of degradation of chlorophyll a has been derived using the various steps involved in the mechanism of degradation of chlorophyll a and chlorophyll b. The rate has been found to vary inversely with Chl b concentration. This was verified using the following plants- *Cannabis sativa*, *Amaranthus hybridus*, *Xanthium strumarium*, *Tridax procumbens*, *Parthenium hysterophorus* and *Euphorbia hirta*. The rate of degradation of Chl a in terms of concentration of Chl b has further been calculated for all the plant species mentioned earlier.

KEY WORDS: Degradation kinetics, chlorophyll absorption, first order kinetics, steady state approximation

The chlorophyll molecule is a central player in harvesting light energy channeled for photosynthesis. Chlorophyll degrading processes have been observed in diverse species across the entire taxonomic range: red algae (Marquardt, 1998), diatoms (Spooner et al., 1994), green algae (Doi et al., 1997) and prokaryotes and the leaves of many bryophytes, pteridophytes and gymnosperms, which lose chlorophyll during a process that closely resembles foliar senescence in angiosperms (Behera and Biswal, 1990; Nebel and Matile, 1992; Ong et al., 1995). In the last two decades the breakdown of chlorophyll in plants has begun to yield some of its mysteries (Krautler and Matile, 1999; Hortensteiner and Krautler, 2000). Both Chl a and Chl b degradation appeared to follow First Order Kinetics. (Canjura et al., 1991; Weemaes et al., 1999).

$$\ln (C/C^0) = -kt$$

Natural environments presumably provide favourable conditions for the degradation of chlorophyll to pheophorbides a and/or pyropheophorbide a (Park et al., 2003). This affects the energy levels within the molecules, causing its absorbance spectrum to alter.

Chlorophyllase, which has been found in all green vegetables (Mayer, 1930) catalyses the hydrolysis of the phytol esters of chlorophylls and pyrochlorophylls to pheophytins and pyropheophytins respectively. The occurrence of another enzyme- Magnesium dechelatase has

been demonstrated, which catalyses the removal of magnesium from chloropigments (Owens and Falkowski, 1982).

The purpose of this study was to derive a relation between chlorophyll a degradation and the concentration of chlorophyll b and verify the expression using different weeds.

MATERIALS AND METHODS

For this purpose, 100 mg of fresh leaf tissue of each weed were cut into very small pieces, homogenized in 80% acetone and a pinch of sodium bicarbonate (NaHCO_3) was added. After centrifugation at 5000 rpm for 10 min the supernatant was collected and the final volume was made upto 10 ml with acetone. The absorbance was measured at 663 nm and 645 nm on a UV-visible spectrophotometer (Systronics 2201) with 80% acetone as blank (Arnon, 1949). Chl a, Chl b and total chlorophyll content were calculated using Arnon's formulae as mentioned below.

$$\text{Chl a (mg/ml)} = \frac{12.7 A_{663} - 2.69 A_{645}}{d \times 1000 \times w (\text{gm})} \times V (\text{ml})$$

$$\text{Chl b (mg/ml)} = \frac{22.9 A_{645} - 4.68 A_{663}}{d \times 1000 \times w (\text{gm})} \times V (\text{ml})$$

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where, A_{663} = Absorbance at 663 nm

A_{645} = Absorbance at 645 nm

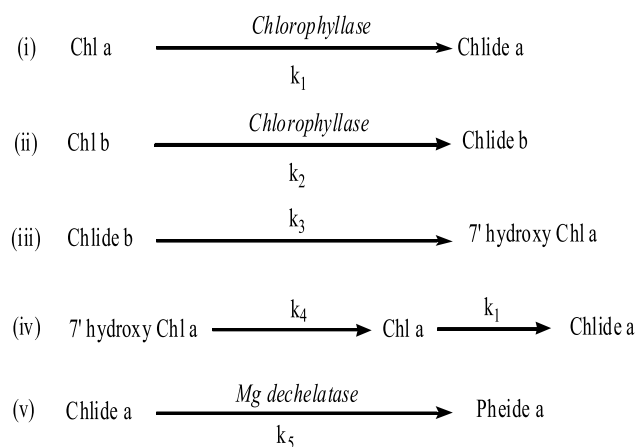
d = diameter of cuvette

w = wt. in grams of leaves taken

V = Final volume of the chlorophyll extract (in ml)

Mathematical Derivation of Kinetics of Degradation of Chl a

An expression for the rate of degradation of Chl a has been derived in terms of rate constants and the concentrations of Chl a and Chl b, using the earlier suggested mechanism for chlorophyll degradation, which involves the decomposition of Chl b via Chl a. (Folly and Engel, 1999). Thus the stepwise formation of Pheide a can be summarized as follows-



The above mechanism can be used to derive the relation for the rate of degradation of chlorophyll in all the plants considered for the study.

Equations were derived for the rate of Chl a degradation in different plants, using the stepwise degradation mechanism of chlorophyll involving different reaction intermediates. Steady State Approximation was applied to the intermediates formed during the degradation assuming the concentrations of enzymes chlorophyllase and Mg dechelataase to remain constant throughout the degradation.

The formula derived has then been used for the determination of the final expression for rate of degradation of Chl a in different plants in terms of Chl b concentration. For this purpose ratio of Chl a and Chl b was calculated in

different weeds and then the concentration of Chl a was put in the respective formulas for the final expression.

RESULTS AND DISCUSSION

The initial steps of degradation of chlorophyll involve the conversion of Chl b to Chl a which further gets converted to Pheide a. Thus, both Chl a and Chl b are degraded by formation of a common intermediate- Pheide a.

The breakdown of chlorophyll is a multi step enzymatic process

(i) Pheo a oxygenase appears to be highly specific, the enzyme uses Pheo a as substrate but refuses Pheo b (vide supra) (ii) Independent proofs have shown that the Chl a (b) converting enzymes are present in higher plants (vide supra) (iii) The proposed cation is stabilised by resonance through the extended electronic 18π system of the chlorophyll macrocycle, whereas the formation of a corresponding cation from 7'hydroxy Chl b catabolite would be less favoured by resonance.

The Rate of decomposition of Chl a can be written as-

$$-\frac{d \text{ Chl a}}{dT} = k_1 [\text{Chl a}] - k_4 [\text{7' hydroxy Chl a}] \quad \dots 1$$

The Rate of formation of Pheide a can be put as-

$$\frac{d [\text{Pheide a}]}{dT} = k_5 [\text{Chlide a}] \quad \dots 2$$

$$\frac{d [\text{Chlide a}]}{dT} = k_1 [\text{Chl a}] - k_5 [\text{Chlide a}] \quad \dots 3$$

Since Chlide a is formed as intermediate in the degradation of chlorophyll and is not isolated from the extract, it is assumed to be consumed during the degradation hence steady state approximation may be applied in case of Chlide a, i.e. rate of formation of Chlide a may be taken as equal to its rate of decomposition. Thus equation 3 may be put as-

$$k_1 [\text{Chl a}] = k_5 [\text{Chlide a}]$$

The concentration of Chlide a can be now written as-

$$[\text{Chlide a}] = \frac{k_1}{k_5} [\text{Chl a}] \quad \dots 4$$

Putting the value of [Chlide a] from eq (4) to eq (2), the rate of formation of pheophorbide a in terms of Chl a can be written as-

$$\frac{d [\text{Pheide a}]}{dT} = k_1 [\text{Chl a}] \quad \dots 5$$

Now, 7' hydroxy Chl a is also one of the intermediates formed during the degradation in Step (iii) and consumed in step (iv)

$$\frac{d [7' \text{ hydroxy Chl a}]}{dT} = k_3 [\text{Chlide b}] - k_4 [7' \text{ hydroxy Chl a}] \quad \dots 6$$

Applying Steady State approximation to 7' hydroxy Chl a we have-

$$k_4 [7' \text{ hydroxy Chl a}] = k_3 [\text{Chlide b}]$$

Thus the concentration of 7' hydroxy Chl a can be put as-

$$[7' \text{ hydroxy Chl a}] = \frac{k_3}{k_4} [\text{Chlide b}] \quad \dots 7$$

Similarly, the rate of formation of Chlide b can be given by-

$$\frac{d [\text{Chlide b}]}{dT} = k_2 [\text{Chl b}] - k_3 [\text{Chlide b}] \quad \dots 8$$

Applying steady state approximation to Chlide b also

$$[\text{Chlide b}] = \frac{k_2}{k_3} [\text{Chl b}] \quad \dots 9$$

Putting the value of Chlide b from eq (9) to eq (7) we have-

$$[7' \text{ hydroxy Chl a}] = \frac{k_2}{k_4} [\text{Chl b}] \quad \dots 10$$

Thus a final equation can be derived for the degradation rate of Chl a in different plants, in terms of [Chl b] by using the above equations. It can be shown that rate of degradation of Chl a is dependent on the concentration of Chl b and is inversely proportional to Chl b concentration.

$$-\frac{d \text{ Chl a}}{dT} = k_1 [\text{Chl a}] - k_2 [\text{Chl b}] \quad \dots 11$$

Now, the values of [Chl b] can be determined in terms of [Chl a] or vice versa, for different plants and can be used to derive a final expression for rate of degradation of Chl a in different plants (Table 1).

Similarly, the rate of degradation of Chl a in terms of concentration of Chl b can also be calculated.

Effect of Chlorophyll b Concentration on Rate of Degradation of Chlorophyll a

Chlorophylls b and c may significantly interfere with chlorophyll measurements depending on the amount present (Arar and Collins, 1997).

It has been observed in some weeds that rate of degradation of Chl a is dependent on the active concentration of Chl b. The weeds in which the observation has been made include- *Cannabis sativa*, *Amaranthus hybridus*, *Tridax procumbens*, *Euphorbia hirta*, *Parthenium hysterophorus* and *Xanthium strumarium*. (Table 2).

It was found that plants having lower concentration of chlorophyll b possess higher rate of degradation of chlorophyll a.

Thus, it was observed that rate of degradation of Chl a was highest in *Euphorbia hirta* and slowest in case of *Cannabis sativa*, amongst the plants mentioned above; whereas the Chl b content has been found to be highest in case of *Cannabis sativa* and lowest in case of *Euphorbia hirta*.

The rate of degradation of Chl a follows the order-

Euphorbia hirta > *Parthenium hysterophorus* > *Tridax procumbens* > *Xanthium strumarium* > *Amaranthus hybridus* > *Cannabis sativa*.

The order of Chl b content value is-

Euphorbia hirta < *Parthenium hysterophorus* < *Tridax procumbens* < *Xanthium strumarium* < *Amaranthus hybridus* < *Cannabis sativa*.

Thus the above results support our hypothesis that rate of degradation of Chl a is inversely related to Chl b concentration.

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Table 1: Ratio of Chl a and Chl b concentration

Plants	Chl a	Chl b	Final Expression
<i>Croton spp.</i>	1	0.49	$-\frac{d \text{ Chl a}}{dT} = [\text{Chl a}] (0.49k_2 - k_1)$
<i>Cannabis sativa</i>	1	0.43	$-\frac{d \text{ Chl a}}{dT} = [\text{Chl a}] (0.43k_2 - k_1)$
<i>Xanthiumstrumarium</i>	1	0.42	$-\frac{d \text{ Chl a}}{dT} = [\text{Chl a}] (0.42k_2 - k_1)$
<i>Tridax procumbens</i>	1	0.38	$-\frac{d \text{ Chl a}}{dT} = [\text{Chl a}] (0.38k_2 - k_1)$
<i>Parthenium hysterothorus</i>	1	0.36	$-\frac{d \text{ Chl a}}{dT} = [\text{Chl a}] (0.36k_2 - k_1)$
<i>Lantana camara</i>	1	0.33	$-\frac{d \text{ Chl a}}{dT} = [\text{Chl a}] (0.33k_2 - k_1)$
<i>Amaranthus hybridus</i>	1	0.31	$-\frac{d \text{ Chl a}}{dT} = [\text{Chl a}] (0.31k_2 - k_1)$
<i>Chenopodium album</i>	1	0.31	$-\frac{d \text{ Chl a}}{dT} = [\text{Chl a}] (0.31k_2 - k_1)$
<i>Euphorbia hirta</i>	1	0.27	$-\frac{d \text{ Chl a}}{dT} = [\text{Chl a}] (0.27k_2 - k_1)$

Table 2: Rate of Degradation of Chl a is inversely proportional to Chl b concentration

Plants	Rate of Chl a degradation (mol L ⁻¹ sec ⁻¹)	Chl b concentration (mol L ⁻¹)
<i>Cannabis sativa</i>	6.17 x 10 ⁻⁹	0.925 x 10 ⁻³
<i>Amaranthus hybridus</i>	14.88 x 10 ⁻⁹	0.665 x 10 ⁻³
<i>Xanthium strumarium</i>	17.50 x 10 ⁻⁹	0.480 x 10 ⁻³
<i>Tridax procumbens</i>	18.00 x 10 ⁻⁹	0.336 x 10 ⁻³
<i>P. hysterothorus</i>	18.08 x 10 ⁻⁹	0.315 x 10 ⁻³
<i>Euphorbia hirta</i>	19.06 x 10 ⁻⁹	0.310 x 10 ⁻³

REFERENCES

- Arar E.J. and Collins G.B., 1997. *In vitro* determination of Chlorophyll a and Phaeophytin a in marine and freshwater algae by fluorescence. National Exposure Research Laboratory US. Environmental Protection Agency.
- Arnon D.J., 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiology, **24**: 1-15.
- Behera Y.N. and Biswal B., 1990. Differential response of fern leaves to senescence modulating agents of angiospermic plants. J. Plant Physiol., **136**: 480-483.
- Canjura F.L., Schwartz S.J. and Nunes R.V., 1991. Degradation kinetics of chlorophylls and chlorophyllides. J. Food Sci., **56**(6): 1639-1643.
- Doi M., Shima S., Egashira T., Nakamura K. and Okayama S., 1997. New bile pigment excreted a *Chlamydomonas reinhardtii* mutant: a possible breakdown catabolite of chlorophyll a. J. Plant Physiol., **150**: 504-508.
- Folly P. and Engel N., 1999. Chlorophyll b to Chlorophyll a conversion precedes Chlorophyll degradation in *Hordeum vulgare* L. J. Biol.Chem., **274**:21811-21816.
- Hortensteiner S. and Krautler B., 2000. Chlorophyll breakdown in oilseed rape. Photosynth. Res., **64**: 137-146.
- Krautler B. and Matile P., 1999. Solving the riddle of chlorophyll breakdown. Acc. Chem. Res., **32**: 35-43.
- Marquardt J., 1998. Effects of carotenoid depletion on the photosynthetic apparatus of a *Galdieria sulphuraria* (Rhodophyta) strain that retains its photosynthetic apparatus in the dark. J. Plant Physiol., **152**: 372-380.
- Mayer H., 1930. Untersuchungen über die chlorophyllase. Planta, **11**: 294-306.
- Nebel B. and Matile P., 1992. Longevity and senescence in needles of *Pinus cembra*. Trees, **6**: 156-161.
- Ong B.L., Ng S.L. and Wee Y.C., 1995. Photosynthesis in *Platycerium coronarium* (Koenig ex Mueller) Desv. Photosynthetica, **31**: 59-69.
- Owens T.G. and Falkowski P.G., 1982. Enzymatic degradation of chlorophyll a by marine phytoplankton *in vitro* Phytochemistry, **21**: 979-984.
- Park Y-J., Kim W-S., Ko S-H., Lim D-S., Lee H-J., Lee W-Y. and Lee D-W., 2003 Separation and characterization of Chlorophyll Degradation Products in Silkworm Using HPLC-UV-APCI-MS. J. of Liquid Chromatography and related technologies, **26**: 3183-3197.
- Spooner N., Harvey H.R., Pearce G.E.S., Eckardt C.B. and Maxwell J.R., 1994. Biological defunctionalisation of chlorophyll in the aquatic environment. 2. Action of endogenous algal enzymes and aerobic bacteria. Org. Geochem., **22**: 773-80.
- Weemaes C.A., Ooms V., Van Loey A.M. and Hendricks M.E., 1999. Kinetics of Chlorophyll degradation and color loss in heated Broccoli Juice. J. Agric. Food Chem., **47**: 2404-2409.