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# INFLUENCE OF LECITHIN LIPOSOMES ON CHLOROPHYLLASE-CATALYZED CHLOROPHYLL HYDROLYSIS

## COMPARISON OF INTRAMEMBRANEOUS AND SOLUBILIZED *PHAEODACTYLUM* CHLOROPHYLLASE

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## Summary

1. Chlorophyllase-catalyzed chlorophyll hydrolysis is greatly enhanced by the addition of divalent cations ($Mg^{2+}$) combined with a reducing agent (dithiothreitol, ascorbate). A similar effect is obtained by the addition of lecithin. In the presence of lecithin, dithiothreitol has only slight or no influence on chlorophyll hydrolysis. $Mg^{2+}$ eliminates the activating effect of lecithin.

2. In the absence of $Mg^{2+}$ + dithiothreitol or of lecithin, Triton X-100 has a slight activating effect on chlorophyllase-catalyzed chlorophyll hydrolysis, but only at low concentrations (0.01—0.02%). In the presence of $Mg^{2+}$ and dithiothreitol or of lecithin, Triton X-100 (greater than or equal to 0.02%) inhibits this reaction.

3. Whereas chlorophyllase combines with chlorophyll, no combination of chlorophyllase and lecithin could be detected.

4. Solubilized chlorophyllase is stabilized by its substrate, chlorophyll. Enzyme stabilization is eliminated by lecithin, whereas in the absence of chlorophyll, denaturation is somewhat increased by dithiothreitol.

5. No clear difference was found between the actions of intramembraneous and solubilized chlorophyllase. The results suggest that chlorophyllase is situated within membranes in such a way that the active group protrudes into the aqueous medium surrounding the membrane.

6. A hypothesis explaining the activating effects which $Mg^{2+}$ combined with

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Abbreviation: Tricine, *N*-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine.

a reducing agent and lecithin have upon chlorophyllase-catalyzed chlorophyll hydrolysis is presented.

## Introduction

Chlorophyllase (chlorophyll chlorophyllidohydrolase, EC 3.1.1.14) catalyzes the hydrolysis of chlorophyll into chlorophyllide and phytol. The enzyme is found in homogenates of several higher plants and algae; it is particularly abundant in preparations of the marine diatom *Phaeodactylum tricornutum* [1]. As chlorophyll hydrolysis is observed only after cell disintegration, the in vivo enzyme, presumably inactive, was called pro-chlorophyllase [2,3]. The elucidation of the function and action mechanism of the (pro)-enzyme within the cell is the ultimate goal of our investigations.

In previous experiments, it was shown that the hydrolysis of chlorophyll into chlorophyllide and phytol can be catalyzed by intramembraneous as well as by isolated, solubilized *Phaeodactylum* chlorophyllase [4,5]. The aim of the investigation described below was to check whether the activity of the enzyme depends on if it is situated inside or outside chloroplast membranes, or, in other words, whether membrane components influence chlorophyllase activity.

Some experiments to determine the influence of phospholipid and membrane proteins on chlorophyllase from *Phaseolus vulgaris* L. were performed by Moll and Stegwee [6,7]. Moll and Stegwee [6] showed that chlorophyll embedded in lecithin-cholate liposomes can be used as a substrate for chlorophyllase. Preliminary experiments by Moll [7] were interpreted to indicate that addition of pigment-free lecithin-cholate liposomes to chlorophyllase, or incorporation of chlorophyllase into chlorophyll-containing liposomes, suppresses enzymatic chlorophyll hydrolysis.

The experiments described in this paper were performed with *Phaeodactylum* chlorophyllase, either situated in natural membranes or in a solubilized form. In both cases it was found that hydrolysis of added chlorophyll was greatly enhanced by lecithin.

## Materials and Methods

*Cultivation. P. tricornutum* was cultivated as described in Ref. 8.

*Membrane fragments.* Photosynthetic membrane fragments ('heavy membrane fragments') were obtained by treating the cells in a French press, followed by differential centrifugation and washing [3].

*Solubilized chlorophyllase.* 'Acetone precipitate extract', was prepared according to the method of Terpstra [5], treatment 8. The preparation consists of the water-soluble fraction of an 80% acetone precipitate of $Na_4P_2O_7$-washed *Phaeodactylum* membrane fragments.

*Chlorophyllase activity.* See Ref. 4. Reaction mixtures are given with the tables and figures. Unless stated otherwise, the mixtures were sonicated in a Bransonic bath for 5 min and subsequently incubated for 17 h at 23°C; chlorophyll and chlorophyllide were separated by addition of acetone (final concentration 80%) and extraction with petroleum spirit, b.p. 40—60°C. Chlorophyll

was added as an acetone extract of spinach chloroplasts. In this way, possible complications due to methylchlorophyllide formation [6], could be avoided. Another advantage of using acetone was that chlorophyll was found to be more stable in this solvent than in methanol which we used in previous experiments. Acetone concentration in the reaction medium did not exceed 7%.

*Lecithin vesicles (liposomes).* These were prepared in two ways. (a) Sonication (cf. Ref. 9). An egg lecithin solution (Sigma) in chloroform/methanol or hexane was evaporated to dryness in an $N_2$ current. The residue was then shaken with 0.02 M Tris-HCl buffer, pH 8.0 (1 ml/10 mg lecithin); the suspension was sonicated in a Bransonic bath sonicator until a more or less opalescent preparation was obtained (usually 0.5—1 h). (b) French press passage [10]. The lecithin suspension, instead of being sonicated, was passed twice through a French press needle valve (930 kg/cm$^2$) and then centrifuged for 15 min at 43 000 $\times g$; the pellet was discarded. The results obtained with liposomes prepared in both ways were found to be similar.

## Results and Conclusions

*Chlorophyllase activation by $Mg^{2+}$ combined with a reducing agent*

Both *Phaeodactylum* membrane fragments and solubilized, purified, *Phaeodactylum* chlorophyllase show increased enzyme activity on the addition of a combination of $Mg^{2+}$ and dithiothreitol; monovalent cations have only a slight effect or no effect [4,5].

Although solubilized chlorophyllase in freshly prepared acetone precipitate extracts tends to show a higher sensitivity to $Mg^{2+}$ + dithiothreitol than the enzyme in membrane fragments, no essential difference in the reaction of intramembraneous and solubilized chlorophyllase was found. The membrane fragments as well as the solubilized enzyme exhibit a low chlorophyllase activity without added $Mg^{2+}$ + dithiothreitol.

In the presence of $Mg^{2+}$, dithiothreitol can be replaced by another reducing agent, sodium ascorbate, without much loss of chlorophyllase activation action [4,5], (Table I). Furthermore, *Phaeodactylum* chlorophyllase is not sensitive to thiol reagents (*p*-chloromercuribenzoate [4], *N*-ethylmaleimide, Table I).

TABLE I

INFLUENCE OF REDUCING AGENTS AND OF A THIOL REAGENT ON *PHAEODACTYLUM* CHLOROPHYLLASE ACTIVITY

Reaction mixtures: solubilized chlorophyllase (acetone precipitate extract) sufficient for a 10—60% chlorophyll hydrolysis, $MgCl_2$ (10 mM), chlorophyll (140 μg in about 0.1 ml acetone), Tris-HCl or Tricine-NaOH (20 mM, pH 8.0); added reagents as indicated. Final aqueous reaction volume 1.5 ml.

| Reagent added (mM) | Relative chlorophyllase activity |
|---|---|
| None | 8 |
| *N*-Ethylmaleimide (1) | 8 |
| Dithiothreitol (6.7) | 100 |
| Sodium ascorbate (6.7) | 90—100 |
| Sodium ascorbate (6.7) + *N*-ethylmaleimide (1) | 90—100 |

This suggests that the chlorophyllase-activating action of dithiothreitol, in the presence of $Mg^{2+}$, is not due to reduction of essential disulfide groups.

*Effect of lecithin on chlorophyllase-catalyzed chlorophyll hydrolysis*

Egg lecithin liposomes affect chlorophyll hydrolysis catalyzed both by intramembraneous chlorophyllase and by solubilized chlorophyllase:

(a) Hydrolysis of added chlorophyll increases upon addition of lecithin; chlorophyll conversion is roughly similar to that observed when $Mg^{2+}$ + dithiothreitol is added instead of lecithin (Table II).

(b) The activating effect of lecithin is not significantly influenced by dithiothreitol. $Mg^{2+}$ inhibits chlorophyll hydrolysis in the presence of lecithin (Table II). The latter effect may be due to an association of lecithin with $Mg^{2+}$ [11—17].

(c) The activating action of lecithin depends on its concentration. Under the experimental conditions used, maximum effect was found with about 0.1% lecithin (Fig. 1).

(d) No significant difference was observed between the lecithin effects with intramembraneous and with solubilized chlorophyllase.

It is concluded that chlorophyllase-catalyzed chlorophyll hydrolysis can be activated to approximately the same extent by $Mg^{2+}$ + dithiothreitol and by lecithin with or without dithiothreitol.

*Effect of lecithin on chlorophyll; comparison with Triton X-100*

The possibility that the activating action of lecithin upon chlorophyllase-catalyzed chlorophyll hydrolysis may be due to its influence upon the reaction substrate, chlorophyll, was considered.

Lecithin associates with chlorophyll under our experimental conditions, as was deduced from a slight change in the absorption spectrum between 690 and

TABLE II

INFLUENCE OF $MgCl_2$, DITHIOTHREITOL AND LECITHIN LIPOSOMES ON CHLOROPHYLL HYDROLYSIS CATALYZED BY *PHAEODACTYLUM* PHOTOSYNTHETIC MEMBRANE FRAGMENTS OR SOLUBILIZED *PHAEODACTYLUM* CHLOROPHYLLASE

Reaction mixtures: membrane fragments containing 6—9 μg protein, solubilized chlorophyllase (acetone precipitate extract) containing 0.7—2 μg protein, Tris-HCl (20 mM, pH 8.0), chlorophyll (140 μg in about 0.1 ml acetone), dithiothreitol (6.7 mM), $MgCl_2$ (10 mM), lecithin (1.5—2 mg); total volume 1.5 ml. Data indicate variations found with three to six experiments.

| Chlorophyllase preparation | Reagents added | Relative chlorophyllase activity |
|---|---|---|
| Membrane fragments | dithiothreitol | 23— 31 |
| | dithiothreitol + $MgCl_2$ | 100 |
| | dithiothreitol + lecithin | 97—111 |
| Solubilized chlorophyllase | dithiothreitol | 17— 27 |
| | dithiothreitol + $MgCl_2$ | 100 |
| | dithiothreitol + lecithin | 71—122 |
| | lecithin | 89— 98 |
| | dithiothreitol + lecithin + $MgCl_2$ | 12— 25 |

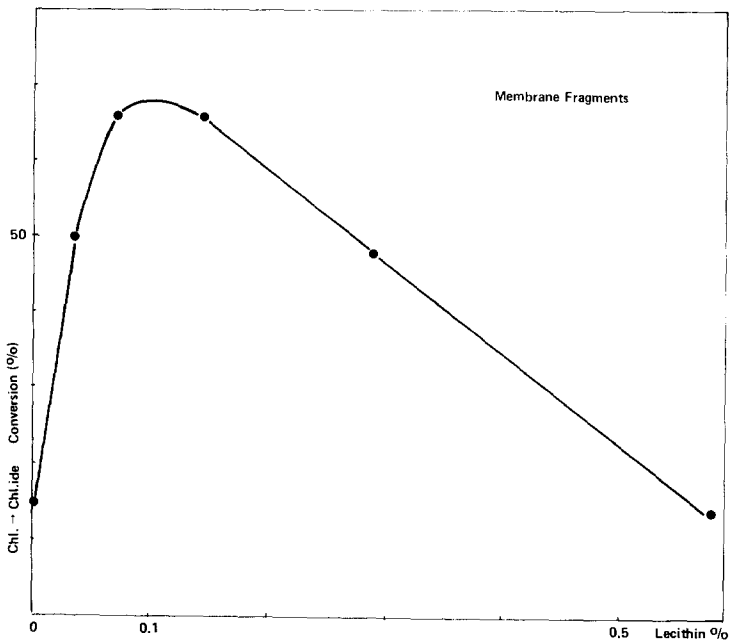


Fig. 1. Influence of lecithin concentration on chlorophyll hydrolysis catalyzed by *Phaeodactylum* photosynthetic membrane fragments. Reaction mixtures: membrane fragments containing 9 μg protein, Tris-HCl (20 mM, pH 8.0), lecithin as indicated, chlorophyll (140 μg in 0.1 ml acetone). Final aqueous volume 1.5 ml. Chl., chlorophyll; Chl.ide, chlorophyllide.

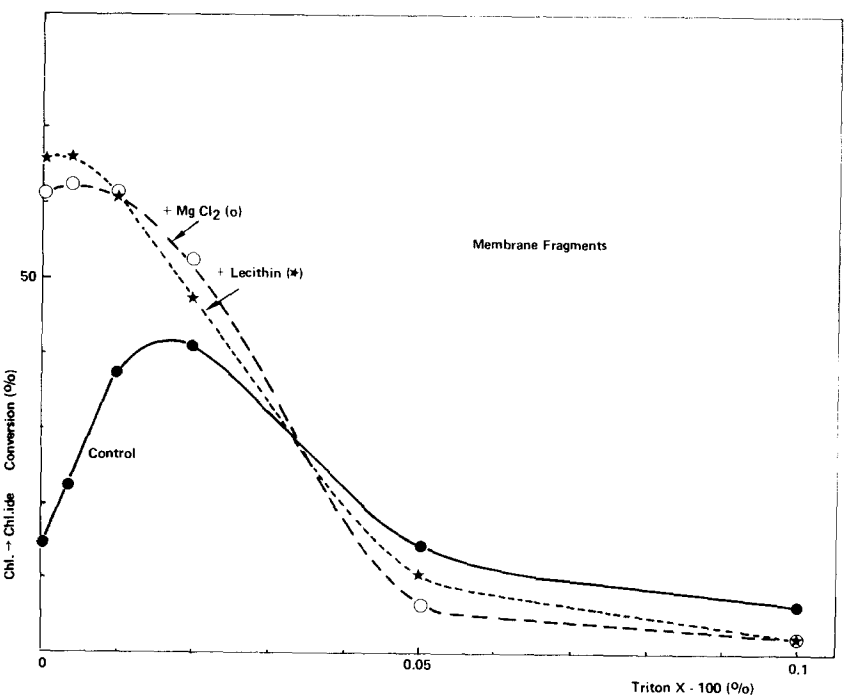


Fig. 2. Influence of Triton X-100 on chlorophyll hydrolysis catalyzed by *Phaeodactylum* photosynthetic membrane fragments in the absence and presence of $MgCl_2$ or lecithin. Reaction mixtures: see Fig. 1. Dithiothreitol, 6.7 mM; $MgCl_2$, 10 mM; lecithin, 2 mg. Chl., chlorophyll; Chl.ide, chlorophyllide.

700 nm, and an increase of 685 nm fluorescence upon addition of lecithin liposomes to aqueous colloidal chlorophyll.

If the effect of lecithin on chlorophyllase-catalyzed chlorophyll hydrolysis were due to its 'solubilizing' action upon colloidal chlorophyll, a similar effect would be expected to occur with, for instance, the detergent Triton X-100. In fact, at concentrations below 0.05% (about the critical micellar concentration, cf. Ref. 18), Triton does exert some activating action on chlorophyllase-catalyzed chlorophyll hydrolysis in the absence of $Mg^{2+}$ + dithiothreitol or lecithin; this action is at its maximum at 0.01—0.02% Triton X-100. At concentrations greater than 0.05% Triton has an inhibiting effect (Fig. 2). In the presence of $Mg^{2+}$ + dithiothreitol or lecithin no significant increase of chlorophyllide formation in the presence of Triton X-100 is measured and inhibition occurs already with Triton at concentrations greater than 0.01% (Fig. 2).

The results obtained with intramembraneous and solubilized chlorophyllase were essentially similar.

It is concluded that the results obtained with Triton X-100 do not contradict the hypothesis that the activating effects which both lecithin and Triton X-100 have upon chlorophyllase-catalyzed chlorophyll hydrolysis are due to their interaction with chlorophyll. The experiments also indicate that Triton concentrations exceeding 0.02% should be avoided in reaction mixtures used for estimating chlorophyllase [6,7].

*Does chlorophyllase associate with lecithin liposomes?*

Lecithin liposomes activate chlorophyll hydrolysis; in the experiments described the reaction mixture was sonicated for 5 min in a bath sonicator, but even without this treatment the activation was observed. One wonders whether, under these circumstances, chlorophyllase becomes incorporated into the lecithin vesicles. These vesicles can be precipitated by centrifugation at 266 000 × *g* (tube bottom) for 90 min, whereas solubilized chlorophyllase does

TABLE III

CENTRIFUGATION OF SOLUBILIZED CHLOROPHYLLASE WITH $Mg^{2+}$ OR LECITHIN IN THE PRESENCE AND ABSENCE OF CHLOROPHYLL

Reaction mixtures: solubilized chlorophyllase (acetone precipitate extract) containing 15 μg protein, dithiothreitol (6.7 mM), Tris-HCl (20 mM, pH 8.0). Added components: $MgCl_2$ (10 mM), lecithin (about 3 mg), chlorophyll (20 μg in 0.065 ml acetone). Final aqueous volume 3.0 ml. The mixture was sonicated for 5 min (Bransonic bath) and centrifuged for 1.5 h at 226 500 × *g* (tube bottom). Temperature 17°C. Controls were stored for the same time at 17°C. Centrifuged mixtures were divided into upper and lower halves; 1.3 ml were supplemented with 150 μg chlorophyll and tested for chlorophyllase activity (reaction volume 1.5 ml).

| Components added | Chlorophyllase activity after centrifugation (% of uncentrifuged control) | |
|---|---|---|
| | Upper half of tube | Lower half of tube |
| $Mg^{2+}$ | 70—100 | 70—100 |
| $Mg^{2+}$ + chlorophyll | 10 | 120 |
| Lecithin | 100 | 70—100 |
| Lecithin + chlorophyll | 45 | 130 |

not precipitate upon this treatment. Upon mixing chlorophyllase with lecithin liposomes, the enzyme is pelleted only if chlorophyll is also present in the reaction mixture.

Distribution of colloidal chlorophyll in Tris-HCl buffer is only slightly influenced by centrifugation, but in the presence of liposomes chlorophyll precipitates with the liposomes. This precipitation occurs both in the absence and in the presence of chlorophyllase. In buffer containing $Mg^{2+}$, colloidal chlorophyll precipitates upon centrifugation. Chlorophyllase which is added to a medium with $Mg^{2+}$ precipitates only if chlorophyll is present (Table III).

It is concluded that, under the conditions we used, chlorophyllase binds to chlorophyll (as was already indicated by former experiments [8]), but is not incorporated into lecithin liposomes. The experiments provide further evidence that chlorophyll combines with the lecithin liposomes.

*Denaturation and stabilization of chlorophyllase*

In the course of the experiments it was observed that chlorophyllase activity is influenced by pre-incubation in some reaction mixtures.

Table IV shows the influence of pre-incubation of the enzyme for 3 h at 23°C with dithiothreitol, $Mg^{2+}$, lecithin or chlorophyll, alone or in various combinations. From these results the following conclusions were drawn:

TABLE IV

EFFECT OF PREINCUBATION WITH CHLOROPHYLL ON CHLOROPHYLLASE ACTIVITY DETERMINED IN THE PRESENCE OF $Mg^{2+}$ WITH OR WITHOUT DITHIOTHREITOL (A) OR LECITHIN WITH OR WITHOUT DITHIOTHREITOL (B)

Reaction mixtures: solubilized chlorophyllase (acetone precipitate extract) containing 0.5 μg protein, Tris-HCl (20 mM, pH 8.0). Dithiothreitol (6.7 mM), $MgCl_2$ (10 mM), lecithin (about 1.5 mg), chlorophyll (10 μg in 0.04—0.045 ml acetone). Final aqueous volume 1.25 ml. Sonication 5 min (Bransonic Bath), then preincubation (3 h, 23°C). Thereafter, the mixtures (A) that lack dithiothreitol and/or $MgCl_2$ are supplemented with these reagents, and the mixtures (B) are supplemented in a similar way with lecithin. Then, after addition of 150 μg chlorophyll, chlorophyllase activity is determined. Data show the averages of threee experiments.

| Preincubation with (3 h, 23°C) | Chlorophyll chlorophyllide conversion (%) |
|---|---|
| (A) | |
| — | 39.4 |
| Dithiothreitol | 36.4 |
| $MgCl_2$ | 41.5 |
| $MgCl_2$ + dithiothreitol | 39.5 |
| Chlorophyll | 53.5 |
| Dithiothreitol + chlorophyll | 53.8 |
| $MgCl_2$ + chlorophyll | 51.1 |
| $MgCl_2$ + dithiothreitol + chlorophyll | 53.9 |
| (B) | |
| — | 46.9 |
| Dithiothreitol | 37.8 |
| Lecithin | 42.1 |
| Lecithin + dithiothreitol | 35.7 |
| Chlorophyll | 60.4 |
| Dithiothreitol + chlorophyll | 56.4 |
| Lecithin + chlorophyll | 41.7 |
| Lecithin + dithiothreitol + chlorophyll | 34.5 |

(a) Chlorophyllase preincubated with a small amount of chlorophyll shows higher activity than chlorophyllase preincubated without chlorophyll.

(b) Preincubation of chlorophyllase with dithiothreitol and/or $Mg^{2+}$ has no significant effect if the enzyme activity is determined with $Mg^{2+}$ + dithiothreitol.

(c) Preincubation with dithiothreitol, in the absence of chlorophyll, decreases activity if determination occurs with lecithin.

(d) Preincubation with lecithin in the presence of chlorophyll abolishes the favourable effect of chlorophyll.

The 'activation' of chlorophyllase by chlorophyll (cf. a) could be due to (1) allosteric enzyme activation or (2) enzyme stabilization.

(1) If allosteric enzyme activation occurs, an S-shaped curve of reaction velocity vs. substrate concentration would be expected. In order to avoid significant enzyme denaturation during the reaction, a relatively short reaction time (30 min) was chosen. Fig. 3 shows that no disproportionately slow reaction velocity at low substrate concentrations was found; this indicates that chlorophyll is not an allosteric chlorophyllase activator.

(2) The possibility that chlorophyll protects chlorophyllase from denaturation was checked by preincubation of the enzyme at 23° C for various lengths

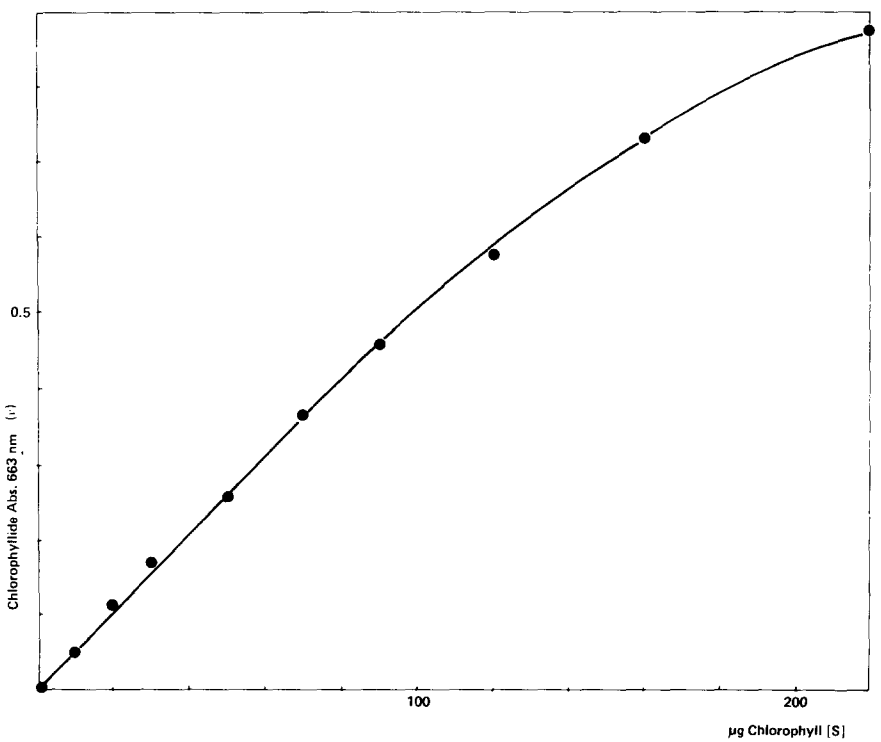


Fig. 3. Chlorophyll hydrolysis catalyzed by *Phaeodactylum* solubilized chlorophyllase; reaction velocity vs. substrate concentration. Reaction mixtures: solubilized chlorophyllase ('acetone precipitate extract'), 20 µg; dithiothreitol, 6.7 mM; $MgCl_2$, 10 mM; Tris-HCl (20 mM, pH 8.0); chlorophyll as indicated in 0.15 ml acetone; final aqueous volume 1.5 ml. Incubation 30 min at 25° C.

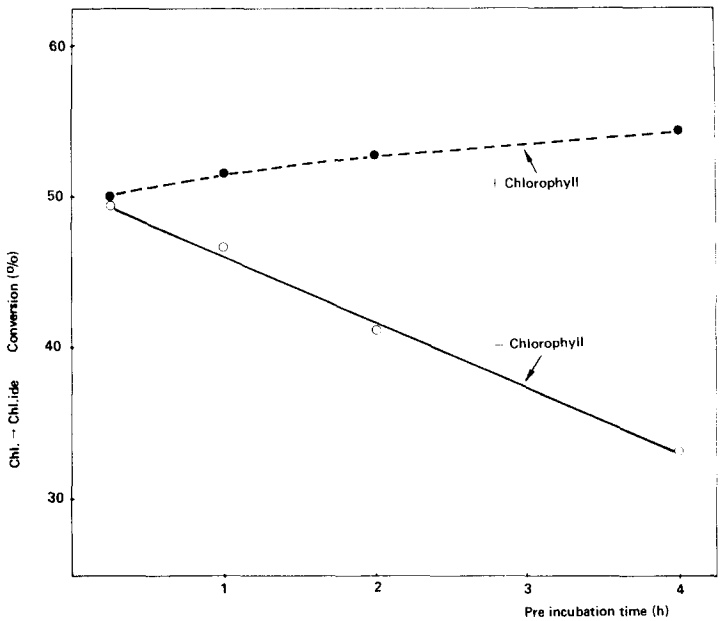


Fig. 4. Influence of preincubation of chlorophyllase upon enzyme activity. Reaction mixtures: *Phaeodactylum* solubilized chlorophyllase (acetone precipitate extract), 0.5 μg; dithiothreitol, 6.7 mM; $MgCl_2$, 10 mM; Tris-HCl (20 mM, pH 8.0); chlorophyll, 10 μg in 0.05 ml acetone (●), or 0.05 ml acetone (○); final aqueous volume 1.5 ml. After preincubation at 23°C, time as indicated, 140 (●) or 150 (○) μg chlorophyll in 0.1 ml acetone are added for determination of enzyme activity. Chl., chlorophyll, Chl.ide, chlorophyllide.

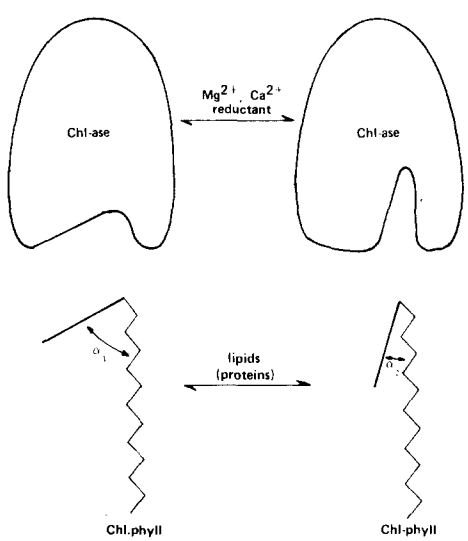


Fig. 5. Schematic presentation of hypothetical chlorophyllase (Chl-ase) and chlorophyll (Chl.phyll) conformations.

of time in the presence and absence of chlorophyll.

Fig. 4 shows that chlorophyllase activity gradually decreases during preincubation at 23°C, whereas this effect is eliminated in the presence of chlorophyll. It is concluded that chlorophyll protects chlorophyllase from denaturation.

Chlorophyllase activity of membrane fragments is fairly stable for several weeks, provided the fragments are stored in buffer, pH 8.0, in a refrigerator. The activity of the solubilized enzyme in acetone precipitate extracts, however, decreases under these circumstances (about 50—75% during 8 days). Activity also decreases at −20°C. Dithiothreitol does not counteract this inactivation (see also Table IV). In view of the experimental results described above we tried to store the enzyme in the presence of chlorophyll (4.5 μg/100 μg protein per ml). Although enzyme inactivation was slower under these circumstances, a 20—45% loss of activity still occurred during storage in the refrigerator for 8 days.

## Discussion

The activation of chlorophyllase-catalyzed chlorophyll hydrolysis by $Mg^{2+}$ combined with dithiothreitol has formerly been ascribed to a favourable action of $Mg^{2+}$ on chlorophyllase conformation. Moreover, if the enzyme is to be active, it must be in a reduced form [4,5].

It is now found that lecithin has an activating effect on chlorophyll hydrolysis similar to that of $Mg^{2+}$ and dithiothreitol. Lecithin may affect (1) chlorophyll or (2) chlorophyllase. Chlorophyll associates easily with lecithin vesicles [19—22]. This occurs also in the circumstances of our experiments (see Results and Conclusions). There is evidence that, within a lecithin bilayer, the chlorine ring system of chlorophyll is oriented toward the interior of the membrane and makes contact with the aqueous phase; the hydrophobic region of the ring system is in the vicinity of the phytol-ester link [23—28]. This position of chlorophyll in the membranes would expose the phytol-ester link to enzymes present in the aqueous phase.

The experimental results may be explained if it is assumed that, when associated with lecithin, chlorophyll has the right sterical structure for combination with chlorophyllase, whereas for association with free ('colloidal') chlorophyll the enzyme has to be activated by $Mg^{2+}$ + dithiothreitol.

Walz [29,30] suggests that the orientation of the chlorine ring may depend on the aggregational state of the lipid membrane (cf. also Ref. 31). In our case, it is suggested that the average angle, $\alpha$, between the chlorine ring system and phytol in chlorophyll could depend to some extent on adjacent lipids (and possibly also proteins), whereas the shape of the active site of the enzyme depends on surrounding ions and reductants. Both sites will have to fit into each other spatially for an enzyme reaction to be possible. A schematic representation of this hypothesis is given in Fig. 5.

Another possibility (2) that was considered was activation of chlorophyllase by lecithin. Similar enzyme activations have been described for protein kinase [32], ATPase [33,34] and D-β-hydroxybutyrate apodehydrogenase [35]. With chlorophyllase, this would mean that the enzyme can be activated in two ways, either with $Mg^{2+}$ + dithiothreitol or with lecithin. The latter activation would

in this case most probably depend on the presence of the tertiary ammonium group (cf. Ref. 35).

In membrane fragments, chlorophyllase is incorporated in a natural membrane consisting of lipids and proteins. Is seems reasonable to assume that if chlorophyllase could be activated by certain lipids the enzyme within the membrane would be present in its activated form. However, chlorophyll hydrolysis with intramembraneous chlorophyllase is appreciably stimulated by lecithin; although, generally, the stimulation was somewhat less than that with solubilized chlorophyllase, the difference does not warrant the conclusion that the conformation of intramembraneous chlorophyllase differs from that of the solubilized enzyme. These considerations, together with the experimental result that chlorophyllase does not perceptibly associate with pigment-free lecithin vesicles in the circumstances of our experiments, lead us to prefer the hypothesis that the activating effect of lecithin on chlorophyllase-catalyzed chlorophyll hydrolysis is due to its action on steric chlorophyll conformation.

Apparently, the activity of chlorophyllase does not depend on whether it is situated inside or outside the thylakoid membrane. As $Mg^{2+}$ + dithiothreitol activate the intramembraneous as well as the solubilized enzyme, the intramembraneous enzyme probably protrudes into the aqueous phase surrounding the lipid membrane. If the active site is situated in this hydrophilic part of the enzyme, the catalysis of the hydrolysis of added chlorophyll becomes understandable. The conversion of intramembraneous chlorophyll [2] could occur by chlorophyllase in adjacent membranes [36]. However, this subject needs to be further investigated.

The conclusions drawn above differ from those of Moll [7]. This author observed that pigment-free lecithin-cholate liposomes exerted an inhibiting effect on the hydrolysis of chlorophyll contained in similar liposomes in the presence of solubilized chlorophyllase. He concludes that phospholipids protect chlorophyll molecules from degradation. However, no control experiment without liposomes has been reported. Lecithin concentration may have been supraoptimal in these experiments (cf. Fig. 1). Therefore, Moll's experiments need not invalidate our conclusion that enhanced chlorophyllase-catalyzed chlorophyll hydrolysis in the presence of lecithin can be explained by the influence of lecithin on the substrate only. It is not necessary to assume any alteration of the chlorophyllase conformation.

Our results show that before one starts experiments with enzymes incorporated into artificial membranes, it is advisable to check what influence the compounds constituting the membrane have on the various components of the enzyme-catalyzed reaction.

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