The participation of chlorophyllase in chlorophyll metabolism

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Abstract

Although the breakdown of chlorophylls can easily be visually recognized, the fate of these photosynthetic pigments as well as their catabolism is still largely unknown. In the past, the enzyme called "chlorophyllase" (chlorophyll-chlorophyllido-hydrolase, EC 3.1.1.14) had been thought to participate both in the biosynthesis and in the degradation of chlorophylls. It is, however, clear at present that *in vivo* this enzyme is involved rather in the first steps of chlorophyll degradation.

Key words: chlorophyllase, chlorophyll metabolism

INTRODUCTION

It is estimated that on average a billion tonnes of chlorophyll are degraded each year on land and in the oceans. Although chlorophyll breakdown is the most visually obvious of all biochemical processes, the fate of this plant photosynthetic pigment is largely unknown. Its biosynthesis is recognized early as the characteristic "greening up" of plants, its degradation manifests itself as "yellowing" due to preponderance of carotenoids. In agriculture, disappearance of chlorophyll predicts ripening of crops and fruits. It may also have a role in the control of senescence and plant longevity. Autumnal disappearance of chlorophyll in leaves is a major attraction of this season of the year. It is, however, surprising how limited is our deeper knowledge of the catabolism of this class of plant pigments. Chlorophyll has been shown to be a substrate for a number of oxidative and hydrolytic activities occurring in green tissues, but

the sequence in which these enzymes attack chlorophyll remains to be established.

Although there is still no coordinated scheme on the pathway of chlorophyll breakdown, the significantly and rapidly growing literature has forced us to restrict our considerations to only one, but the most controversial enzyme taking part in the above-mentioned phenomenon. It is chlorophyllase which has been implicated many times both in the degradation as well as in the biosynthesis of chlorophyll.

CHLOROPHYLLS AND THEIR DERIVATIVES

Since this review is not primarily devoted to detailed considerations of the origin and the chemical structure of chlorophylls, only some fundamental features and chemical properties helping understand the action of chlorophyllase on these pigments will be presented here. Chlorophylls are a family of compounds that are magnesium complexes of tetrapyrrole derivatives. In general, they are dihydroporphyrins with one of the four pyrrole rings of the porphyrin reduced (Fig. 1). In contrast, bacteriochlorophylls are based on the tetrahydroporphyrin structure, in which two pyrrole rings have been reduced (Fig. 1). Unusual exceptions are the algal chlorophylls *c1* and *c2* based on unreduced porphyrin. All chlorophyll porphyrins and their derivatives are esterified with phytol or, less commonly, the related terpenoids such as farnesol or geranyl geraniol (Holden 1976, Jackson 1976, Jones 1979, Castelfranco and Beale 1983). Chlorophyll *a* is the most abundant chlorophyll found in all oxygen-evolving organisms. In higher plants as well as in some algae, this chlorophyll coexists with chlorophyll *b*. They differ only in the substituent at the C-7 carbon atom, chlorophyll *a* having a $-\text{CH}_3$ group and chlorophyll *b* $-\text{CHO}$ group (Seely 1966). The ratio between chlorophyll *a* and chlorophyll *b* varies from 2 to 4.5 depending upon the species and

Fig. 1. Structure of porphyrin precursors of major chlorophyll pigments

irradiance (Björkmann 1981). Chlorophylls *c* and *d* occur in diatoms, dinoflagellates and brown algae. Chlorophyll *c*, in contrast to chlorophyll *a*, lacks the phytol moiety and its pyrrole ring is not reduced. Chlorophyll *d* is 2-desvinyl, 2-formyl chlorophyll *a*. In cyanobacteria and red algae chlorophyll *a* is the only chlorophyll present. Bacteriochlorophyll *a* and *b* are present in almost all photosynthetic bacteria. In *Chlorobacteriaceae* it is the minor chlorophyll. The major pigments are bacteriochlorophylls *c* and *d* (Jackson 1976, Jones 1978).

Chlorophylls can be readily converted, both *in vivo* and *in vitro*, to a number of characteristic derivatives with the retained macrocyclic ring. The simplest conversion is the loss of the central magnesium atom which gives the product known as pheophytin (Fig. 2). Hydrolysis of the ester bond between the macrocyclic ring and long chain alcohol results in the formation of chlorophyllide, while both above-mentioned reactions yield pheophorbide (Fig. 2, Svec 1978).

Fig. 2. Chlorophyll derivatives formed by removal of phytol side chain and/or Mg²⁺

All chlorophylls have characteristic UV and visible absorption spectra. They are important in identifying chlorophylls and the products of their breakdown. Chlorophylls, because of the delocalized electron system characteristic for all porphyrins, exhibit a strong Soret band at about 400 nm. However, there is also a strong light absorbance in the region of 650 nm responsible for their green pigmentation. The ratio of red to blue absorbance can be used to identify the pigment (Vernon 1960, Strain et al. 1963, Jones 1969, Wintermanns 1969, Svec 1978). The centrally located magnesium atom makes little contribution to the overall chromophore as does the phytol group that can be removed with no significant effect on the spectra (Hendry et al. 1987). Bacteriochlorophylls have different absorption spectra than chlorophylls because of the additional reduced pyrrole ring. Most characteristic is that they have no absorption band around 650 nm, but a strong peak at about 770 nm. The Soret band is blue-shifted towards 360 nm. For this reason bacteriochlorophyll *a* appears pink to our eyes.

A major problem in any investigations of the breakdown of chlorophyll is the extent to which degradation occurs biochemically (specific enzymes) or chemically without enzymic influence. In the latter case one may consider herbicidal damage or photodegradation of chlorophyll, which may occur during plant senescence. Chlorophylls possessing no polar or hydrophilic groups are soluble in lipids but almost totally insoluble in aqueous systems in the physiological pH range. This property can be used in the extraction and purification of chlorophylls with organic solvents, but it is also a major problem in experiments on chlorophyll breakdown. The ease with which the loss of the chelated magnesium atom may occur in diluted acids, or the loss of phytol in stronger acids or in the presence of oxidizing agents or alkali, is a potential cause of chlorophyll degradation artifacts easily produced in inadequately buffered solutions (Bacon and Holden 1967).

SOME GENERAL PROPERTIES OF CHLOROPHYLLASE

Chlorophyllase (chlorophyll-chlorophyllido-hydrolase, EC 3.1.1.14), known since 1913 (Willstätter and Stoll 1913), is widely distributed in higher plants and algae (Ardao and Vennesland 1960, Shimizu and Tamaki 1963, Berret and Jeffrey 1964, Böger 1965, Ziegler and Schanderl 1969, Aiga and Sasa 1970, Bacon and Holden 1970, McFeeters et al. 1971, Terpstra 1976, 1977, 1978, Moll and Stegwee 1978, Sabater and Rodriguez 1978, Shimokawa et al. 1978, Moll and De Witt 1979, Kuroki et al. 1981, Owens and Falkowski 1982, Amir-Shapira et al. 1986, Rodriguez et al. 1987, Zapata et al. 1987). A particularly rich source of this enzyme is the diatom *Phaeodactylum tricornutum* (Barret and Jeffrey 1964, Terpstra 1978, 1980). Acetone extracts of this alga provide a crude but

ready source of chlorophyllase (Terpstra and Goedheer 1979, Lambers et al. 1985). *In vitro* chlorophyllase catalyses esterification, transesterification and hydrolysis at the propionic side chain of chlorophyll (Shimizu and Tamaki 1963, Chiba et al. 1967, Wellburn 1970, Ellsworth 1971, 1972a, b, Ichinose and Sasa 1973, Holden 1976). The enzyme affects only chlorophylls, including bacteriochlorophylls and the corresponding pheophytins (Bogorad 1966), and esterifies chlorophyllides (Moll and De Witt 1979, Kuroki et al. 1981, Michalski et al. 1987). Chlorophyllase has been generally regarded as a membrane-bound enzyme intimately associated with stroma lamellae of chloroplasts (Terpstra 1976, Terpstra and Goedheer 1975, 1979, Moll and De Witt 1979, Owens and Falkowski 1982, Terpstra and Lambers 1983, Lambers et al. 1985, Schuch and Brown 1987). In contrast to the membrane-bound enzyme, Ogura (1969, 1972) was the first who demonstrated the presence of a soluble chlorophyllase in young tea leaf sprouts.

The apparent molecular weight of the enzyme purified from different plants was determined in the range of 110-350 kDa (Terpstra 1978, Kuroki et al. 1981, Shimokawa 1982, Lambers et al. 1985). It is suggested to be, at least in the case of *Phaeodactylum tricornutum*, a glycoprotein composed of several subunits of about 38 kDa each. This aggregation characteristic has been reported for chlorophyllases from sugar beet (Bacon and Holden 1970), wheat (Ellsworth 1971), *Phaseolus vulgaris* (Moll and Stegwee 1978), *Phaeodactylum tricornutum* (Terpstra 1978, 1981), *Chlorella* (Tamai et al. 1979), tea leaf sprouts (Kuroki et al. 1981) and *Citrus unshiu* fruits (Shimokawa 1982). The possibility of the existence of two isozymes, each forming a high molecular aggregate, has also been considered (Kuroki et al. 1981, Tarasenko and Khodasevich 1987).

Purified chlorophyllases are roughly classified by their optimum pH into two groups. The first group consists of chlorophyllases with an acidic optimum such as those from *Ailanthus altissima* (pH 4.5; McFeeters et al. 1971) or tea leaf sprouts (pH 5.5-5.8; Ogura 1972, Kuroki et al. 1981). Chlorophyllases with a neutral optimum belong to the second group. Such enzymes as those from *Chlorella vulgaris* (pH 7.2-7.3; Böger 1965, Ichinose and Sasa 1973), *Chlorella protothecoides* (pH 6.0-8.5; Tamai et al. 1979), sugar beet (pH 7.1; Bacon and Holden 1970), tobacco (pH 7.0-7.5; Shimizu and Tamaki 1963) or *Citrus unshiu* fruits (pH 7.0; Shimokawa 1982) are found here. Temperature dependence of chlorophyllase activity varies according to the source of the enzyme showing maximum activity in the range of 30-40°C (Ardao and Vennesland 1960, Chiba et al. 1967, McFeeters et al. 1971, Ichinose and Sasa 1973, Moll and Stegwee 1978, Terpstra 1978, Shioi et al. 1980, Kuroki et al. 1981) but an optimum of 20°C has also been reported for chlorophyllase isolated from *Citrus unshiu* fruits (Shimokawa 1982).

CHLOROPHYLLASE AND CHLOROPHYLL METABOLISM — WISHFUL THINKING AND REALITY

Though chlorophyllase is so widely distributed in the plant world, its physiological function is still largely unknown (Holden 1976, Terpstra 1981, Owens and Falkowski 1982, Schoch and Brown 1987). Chlorophyll breakdown should be considered as falling into two types (Hendry et al. 1987). The first type, called according to the nomenclature of Hendry et al. (1987), type I degradation, includes loss of magnesium, phytol and possible modifications of the side chains of the chlorophyll molecule to yield pheophytin and pheophorbide. The second — called type II degradation, involves cleavage of the macrocyclic ring system and its subsequent degradation to smaller fragments. If we assume that the function of chlorophyllase is degradative, then it is undoubtedly implicated in type I reactions. However, because this enzyme, under certain *in vitro* conditions, catalyzes the formation of the phytol ester bond, its role *in vivo* was for many years widely believed to be rather biosynthetic. It was Willstätter and Stoll (1913) who first reported that chlorophyll was formed from chlorophyllide and phytol after incubation with the air-dried meal of *Hordeum* leaves for more than 24 h. Although their direct followers (Holden 1961, Klein and Vishniac 1961) were unable to achieve this phytylation, experiments on the role of chlorophyllase in the phytylation of chlorophyllide were continued for nearly 20 years. In 1962-1963 Shimizu and Tamaki also claimed that their enzyme preparation from tobacco leaves esterifies both chlorophyllide and pheophorbide with phytol *in vitro*. Chiba et al. (1967) reported that a chlorophyllase preparation from *Chlorella* cells catalyzed *in vitro* formation of chlorophyll from methyl chlorophyllide and phytol. Also Hines and Ellsworth (1969) suggested the participation of methyl chlorophyllide, phytol and chlorophyllase in the final steps of chlorophyll *a* biogenesis. More recently, Ganoza and McFeeters (1976) observed a parallel increase in chlorophyll and chlorophyllase activity during greening of *Chlorella protothecoides*. On the other hand, high levels of chlorophyllase activity were also found by these authors during bleaching of the algal culture. Their results seemed to confirm the hypothesis of Ichinose and Sasa (1973) that chlorophyllase, at least that of *Chlorella protothecoides*, consists of two enzymes. One enzyme would catalyze chlorophyll *a* hydrolysis, whereas the other transphytylation of chlorophyllide *a*. A similar, but more precisely expressed suggestion, has also been put forward by Ellsworth et al. (1976). According to it, the "chlorophyllase" activities observed *in vitro* were most likely due to two enzymes:

1. a chlorophyll *a* (or pheophytin *a*) hydrolytic enzyme — chlorophyllase (E.C. 3.1.1.14), and
2. a chlorophyllide *a* phytylating enzyme (system or multienzyme complex?).

Finally, a year later Rüdiger et al. (1977) first detected the enzymic activity,

termed "chlorophyll synthetase". Chlorophyll synthetase was found in etioplasts (Rüdiger et al. 1980), in chloroplasts (Block et al. 1980, Soll and Schultz 1981, Soll et al. 1983), and in chlorophyll-free chromoplasts (Kreuz and Kleinig 1981). It is localized in prothylakoid fractions of oats and wheat etioplasts (Lütz et al. 1981, Lindsten et al. 1989), in the stromal fraction of chromoplasts (Kreuz and Kleinig 1981) and in the prolamellar bodies (Lindsten et al. 1989). It is probably a peripheral thylakoid membrane protein (Rüdiger and Benz 1984, Rüdiger 1987). Most of the properties of chlorophyll synthetase evidently differ from those of chlorophyllase (Table 1). Thus, it seems obvious now that we should concentrate our subsequent discussion on the degradative properties of chlorophyllase and its role in the catabolism of chlorophyll, i.e. participation in the so-called type I degradation mentioned at the beginning of this chapter.

Table 1

Chlorophyllase *versus* chlorophyll synthetase in the esterification of chlorophyllide (data based on Ellsworth 1971, 1972a, Rüdiger et al. 1980, Rüdiger and Benz 1984)

Properties	Chlorophyllase	Chlorophyll synthetase
Activity in phosphate buffer	tr	+
Activity in the presence of detergents or organic solvents	+	—
Diphosphate derivatives of isoprenoid alcohols	—	+
Free isoprenoid alcohols without ATP	+	—
Alcohols other than C ₂₀ isoprenoids	+	tr
Pheophorbide, bacteriochloride	+	—
Pchl _a	—	—
Chl _a , b	+	+
Yield (amount of esterified product)	1-15%	ca. 90%

(+) — activity; (tr) — trace of activity; (—) — no activity

Let's then return to the hydrolytic properties of chlorophyllase. Numerous studies have been carried out on this subject, mainly on chlorophyllase from *Phaeodactylum tricornutum* mentioned in the previous chapter. Although the activity of this enzyme could not be detected *in vivo* in unbroken cells, it was easily measured after disruption of thylakoid membranes (Terpstra and Goedheer 1975, Terpstra 1976, Owens and Falkowski 1982, Terpstra and Lambers 1983). A wide range of substances may effect the activity of chlorophyllase, among them cations, thiol reagents, fatty acids, lipids and detergents. Divalent cations Mg²⁺ and Ca²⁺ appeared to be more effective in stimulation of chlorophyllase activity than monovalent ones; while anion valency was of little importance (Terpstra and Goedheer 1975, Terpstra 1975, 1977, 1978, Owens and Falkowski 1982).

An inhibitor of sulfhydryl residues — p-mercurobenzoate (PCMB) was reported to have a more diverse effect. It inhibited the activity of chlorophyllases from *Euglena gracilis* and *Skeletonema costatum* (Terpstra 1977, Owens and Falkowski 1982), but was ineffective on the *Phaeodactylum tricornutum* enzyme (Terpstra 1977), which suggested the lack or masking of free sulfhydryl groups in the active site of this particular chlorophyllase. Oleic acid, at a concentration of 1-8 times that of chlorophyll, increased internal chlorophyll to chlorophyllide conversion in osmotically shocked *Phaeodactylum* cells (Terpstra and Goedheer 1975). Unsaturated fatty acids, including oleic acid, may be considered to act as detergents, especially when used at such high concentrations. Their positive effect on the activity of chlorophyllase should then be ascribed to the disintegration of the lamellar structure of chloroplasts with further decomposition of chlorophyll-protein complexes, thus allowing contact between the substrate and the enzyme. Ionic as well as nonionic synthetic detergents are very often used in *in vitro* studies on chlorophyllase. Low concentrations of detergents such as sodium deoxycholate, lauryl diamine oxide or Triton X-100 markedly enhanced the hydrolytic activity of the enzyme (Schoch and Brown 1987, Tarasenko and Khodasevich 1987), whereas sodium dodecyl sulfate showed the opposite effect (Tarasenko and Khodasevich 1987). A similar inhibitory effect was observed while using high concentrations of Triton X-100 (Terpstra 1980). This effect could possibly be ascribed to the formation of micelles blocking the access of the enzyme to the substrate, or even to denaturation of the enzymic protein (Tarasenko and Khodasevich 1987). There also exists the possibility of replacing a natural esterifying group like phytol with Triton X-100 to produce tritonyl esters of chlorophyll competitive with the natural substrate (Michalski et al. 1987). The existence of at least major amounts of the whole cellular pool of chlorophyllase as more or less tightly bound to the thylakoid membranes inspired some researchers to investigate the effect of chloroplast acyl lipids on the activity of chlorophyllase. Most data on this subject came from Terpstra's laboratory (Terpstra 1980, Terpstra 1982, Terpstra and Lambers 1983, Lambers and Terpstra 1985). Phosphatidylcholine (lecithin) showed some positive effect on chlorophyllase activity (Terpstra 1980), which was later interpreted as rather indirect. The enhancement of hydrolysis of chlorophyll incorporated in phosphatidylcholine liposomes was brought about by removal of chlorophyllide due to the incorporation of this product into liposomes, thus preventing feedback inhibition (Terpstra 1982). Monogalactolipids and digalactolipids which are major lipid constituents of thylakoid membranes showed opposite effects on the activity of chlorophyllase. Monogalactolipid, stimulating the monomerization of chlorophyll, enhanced the enzymic activity, whilst digalactolipid caused crystallization of chlorophyll molecules and loss of contact between the enzyme and substrate (Lambers et al. 1984, Lambers and Terpstra 1985).

A similar effect was also observed for sulfolipid and phosphatidylglycerol, both being strongly anionic chloroplast lipids (Terpstra and Lambers 1983). It was also shown that these anionic lipids may fuse with chlorophyllase and inactivate the enzyme. Mg^{2+} ions, linking to the negatively charged head groups of these lipids, might cause dissociation of the lipids from the active site of the enzyme allowing it to function properly (Lambers and Terpstra 1985). Local changes in the concentrations of divalent cations such as Mg^{2+} together with strong enzyme-anionic lipid interactions may be a kind of regulatory mechanism of the activity of intramembraneous chlorophyllase (Terpstra and Goedheer 1975, Lambers and Terpstra 1985). It should be noted here that, although many attempts have been made to explain the mechanism of activation of chlorophyllase *in vivo*, the problem itself still awaits clarification.

Another intriguing and important problem is the way in which chlorophyllase binds chlorophyll to its active site. Obtaining definitive experimental data for this enzyme has been hindered because both the enzyme and its substrate are insoluble in aqueous buffers. Many procedures have been developed to solubilize chlorophyllase from a number of plant tissues and the solubilization of both the enzyme and its substrate has usually been accomplished by using buffers containing high concentrations of acetone or methanol, and/or low concentrations of detergents like Triton X-100. It is difficult to interpret the reaction kinetics of the enzyme under such different conditions. Moreover, the substrate is only partially soluble even at high — 45-66% concentrations of acetone (Ardao and Vennesland 1960, Bacon and Holden 1970). Nevertheless, some conclusions have been drawn. Seiler and Grob (1969) proposed that the substrate is held in the active site of chlorophyllase by hydrogen bonding of the 7 and 8 hydrogens to the sulfur atoms of a disulfide bond, and by the coordination bond between the keto group at C-9 and a metal ion in the enzyme. Since Fleming (1967) and Brockmann (1968) established the absolute configuration of chlorophyll which places the 7 and 8 hydrogens on the opposite side of the plane of the porphyrin ring and because sulfur atoms are not likely to form hydrogen bonds, this type of bonding seemed to be rather impossible. More recent data, however, point out that ring V of the chlorophyll molecule with its keto group at C-9 is essential for substrate-enzyme binding. Compounds without ring V or, for some reasons, lacking keto group in this ring, do not bind well to the enzyme (McFeeters 1975, Michalski et al. 1987).

TO BE OR NOT TO BE INVOLVED IN CHLOROPHYLL CATABOLISM?

“... according to present ignorance, the chlorophyll simply disappears” — Matile et al. (1988). This sentence fits perfectly the beginning as well as the end of any review article considering the biochemical mechanism of chloro-

phyll degradation. Our present knowledge about this phenomenon consists mostly of question marks, and incomplete and random biochemical reactions leading to nowhere. The intermediates of the chlorophyll breakdown *in vivo* are normally not accumulated and, therefore, almost undetected. The final products of degradation are not known, nor has the mechanism of Mg-porphyrin catabolism been elucidated. The fate of the four nitrogen atoms of the chlorophyll molecule also remains unknown. The gap of knowledge is also largely due to the fact that intermediary non-green products of chlorophyll breakdown have yet not been indentified well. So far mainly derivatives having an intact porphyrin ring have been claimed to be the possible first natural products of chlorophyll *a* degradation *in vivo*. This is surprising in view of the well-known breakdown of other porphyrins such as, for instance, hemoglobin. The elucidation of chlorophyll catabolism is a challenge because the current data about yellowing of leaves and color changes of fruits suggest that it is an orderly process (Goldschmidt 1980). In the functional state, the chlorophylls are associated with the apoproteins of light-harvesting and reaction center complexes of photosystems. Thus, it must be assumed that disassembly of thylakoids and dissociation of protein-pigment complexes must precede or initiate the first steps of chlorophyll catabolism. It still remains unknown whether dephytylation by the action of chlorophyllase is the first step of this process necessary for further breakdown.

Several recent reports indicate that the removal of the phytol chain by chlorophyllase might be one of the initial steps of the natural breakdown of chlorophyll during senescence (Sabater and Rodriguez 1978, Purvis and Barmore 1981, Owens and Falkowski 1982, Hirschfeld and Goldschmidt 1983, Ziegler et al. 1988). Chlorophyllase appears to be a component of the thylakoid membranes but it does not normally interact with its substrate situated in the same membrane (Terpstra and Weijman 1972, Terpstra and Goedheer 1975, Terpstra 1975, 1976, 1977, 1978, 1980, Hirschfeld and Goldschmidt 1983, Schoch and Brown 1987). A kind of structural separation should then exist which does not permit the hydrolysis of chlorophyll by the enzyme. Disruption of the thylakoid membrane during senescence should bring the enzyme and substrate into contact and start the degradation of the pigment. However, doubts regarding the physiological significance of chlorophyllase in the natural breakdown of chlorophyll still prevail. Chlorophyllase is present in green plant tissue before the onset of senescence and the problem of how the destruction of chlorophyll is really triggered is far from clear (Phillips et al. 1969, Thomas and Stoddart 1975). Detergents and other membrane-disrupting treatments did set the hydrolysis of chlorophyll into motion in chloroplast fragments (Amir-Shapira et al. 1986). It is, however, not clear whether *in vivo* the increase in chlorophyllase activity arises by *de novo* synthesis of the enzyme protein or by some other forms of activation.

Recently, Amir-Shapira et al. (1987) demonstrated the *in vivo* accumulation of large amounts of chlorophyllide *a* in senescing *Citrus reticulata* peel and the accumulation of pheophytin *a* and other phytylated derivatives in dark-senescing parsley leaves. The results of these experiments suggest that different initial steps of the degradative pathways for chlorophyll might exist, as has been frequently reported (Shimizu et al. 1966, Mukherji and Biswas 1981, Schoch et al. 1981, Owens and Falkowski 1982, Maunders et al. 1983, Schoch and Vielwerth 1983, Amir-Shapira et al. 1986, Ziegler et al. 1988, Thomas et al. 1989). Thus, the possible existence of "tangerine-like" (accumulation of chlorophyllide- and pheophorbide-like materials) and "parsley-like" (phytylated derivatives) initial routes of chlorophyll catabolism *in vivo* should be considered.

Matile et al. (1988) have recently reported that although a small but significant proportion of pink pigments with the chemical properties of chlorophyll catabolites were recovered from the plastid fraction in senescing barley leaves, the bulk of these compounds can be assigned to the vacuole. The catabolites were already dephytylated, which indicates the importance of the removal of the phytol side chain for the first steps of chlorophyll breakdown. Another very important aspect of this finding is that we should look for the later steps of the chlorophyll degradation pathway outside rather than inside the chloroplasts.

We started our considerations from the point where the loss of chlorophyll in the photosynthetic tissues is inevitable and can be easily recognized — from senescence of plants in general and that of photosynthetic apparatus in particular. However, we should remember that the turnover of chlorophylls also occurs in healthy tissues during their whole lifetime. This turnover is well documented and the process has been monitored in photosynthetic bacteria (Haidl et al. 1985), in algae (Grombach et al. 1978, Riper et al. 1979) and in higher plants (Perkins and Roberts 1963, Stobart and Hendry 1984, Gaponenko et al. 1986, Hendry and Stobart 1986). The recent precise data on the constants and half-lives for turnover indicate that the destruction of chlorophyll occurs not only in the premature and senescent steps but throughout the entire plant life. The observed rates of chlorophyll turnover, i.e. half-lives of several hours to few days imply that a significant amount of chlorophyll is destroyed during the pre-senescent stage of growth (Grombach et al. 1978, Riper et al. 1979, Stobart and Hendry 1984, Hendry and Stobart 1986). And again, the extent to which chlorophyllase participates in this phenomenon remains obscure. On nearing the conclusion of this review we have found ourselves back at the starting point of this chapter where the question was put about the participation of this specific enzyme in the catabolism of chlorophyll *in vivo*. Chlorophyllase is the only well-defined enzyme system that has so far been shown to be involved in one way or another in the degradation of chlorophyll. Since other enzyme systems

of this process await characterization or are still to be discovered, only a complete elucidation of the initial reactions occurring within the chloroplasts and yielding the first breakdown products of Mg-porphyrins will answer this question.

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Udział chlorofilazy w przemianach chlorofilu

Streszczenie

Zjawisko degradacji chlorofilu jest łatwe do zauważenia, jednakże los tych barwników fotosyntetycznych i ich katabolizm są dotychczas mało poznane. Enzym zwany "chlorofilazą" (hydrolaza chlorofil-chlorofilid, EC 3.1.1.14) przez wiele lat uważany był za enzym uczestniczący zarówno w biosyntezie, jak i w degradacji chlorofilu. Obecnie wydaje się być faktem bezspornym, iż chlorofilaza *in vivo* uczestniczy w pierwszych etapach degradacji chlorofilu.