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Identification of Catabolites of Chlorophyll-Porphyrin in Senescent Rape Cotyledons[1]

Samuel Ginsburg and Philippe Matile*

Department of Plant Biology, University of Zurich, Zollikerstrasse 107, CH-8008 Zurich, Switzerland

Developing shoots of rape seedlings (*Brassica napus* L.) were excised and fed with 4-[^{14}C]5-aminolevulinic acid to label the pyrroles in chlorophyll (Chl) synthesized during the final phase of expansion and greening of the cotyledons. About 80% of ^{14}C taken up into the cotyledons was incorporated into Chl. The subsequent incubation of labeled shoots in permanent darkness caused the rapid loss of labeled Chl while increasing proportions of ^{14}C appeared in the fraction of water-soluble compounds. Reversed-phase high performance liquid chromatography resolved three nonfluorescent polar catabolites of Chl-porphyrin that were progressively accumulated as senescence advanced. At intermediate stages of senescence, the cotyledons contained a fluorescent radioactive derivative of Chl that was also detectable, together with traces of other putative fluorescent catabolites, in isolated senescent chloroplasts. The nonfluorescent catabolites, identified by means of radiolabeling, were also found to accumulate in attached cotyledons senescing under photoperiod; under these conditions, one of the compounds, NCC-1, was particularly abundant. The catabolites of rape exhibited the same ultraviolet spectra, characterized by a maximum at 320 nm, as a previously reported seco-porphinoid catabolite from barley (B. Kräutler, B. Jaun, W. Amrein, K. Bortlik, M. Schellenberg, P. Matile [1992] Plant Physiol Biochem 30: 333–346). Different polarities suggest, however, that the structures may be different. A terminology for Chl catabolites is proposed because present knowledge suggests that a large number of different structures results from species-specific processing of breakdown products and may require a suitable nomenclature.

The yellowing of senescent leaves is a natural phenomenon as conspicuous as the greening of sprouting shoots, but the interest of researchers has largely been, and still is, in the biosynthesis of Chl. Whereas abundant knowledge is available about the biosynthetic pathway (see Castelfranco and Beale, 1983, and recent reviews in Scheer, 1991), the biochemistry of Chl breakdown is still largely a matter of precarious fumbling (see Brown et al., 1991, for the most recent review).

A serious impediment to the elucidation of Chl catabolism exists in the scarcity of information about the fate of the porphyrin moiety. Until recently, it was supposed that Chl disappeared from senescent leaves, leaving behind only phytol (Peisker et al., 1989). The reason for the late discovery of derivatives of the porphyrin moiety is probably due to the fact that in their native form they are colorless. Thus, colored putative catabolites that originally had been extracted from senescent leaves of barley and meadow fescue (Matile et al., 1987) turned out to be colorless blue fluorescent compounds, which, in the presence of an acidic solvent, are readily converted into pink pigments (Matile et al., 1989). Another group of colorless putative catabolites attracted attention because after TLC on silica gel plates, they assumed a rust-like color when exposed to air and light (Bortlik et al., 1990). The origin of one of these "rusty pigments," RP 14, from Chl-porphyrin has been demonstrated through radiolabeling of Chl in etiolated, greening, primary leaves of barley (Peisker et al., 1990).

Meanwhile, RP 14 has been purified and assigned the constitution of a secoporphinoid derivative of pheophorbide *a* (Kräutler et al., 1991, 1992). This constitution reveals that the oxidative cleavage of the macrocycle occurs in the α-position, producing a lactam-carbonyl in pyrrole A while carbon atom 5 of the methine bridge is converted into a formyl group attached to pyrrole B. Unexpected features of RP 14 include the hydroxylations in both vinyl and ethyl side chains of pyrroles A and B as well as the preservation of the carboxy-methylester in the isocyclic ring. The constitution of a similar linear tetrapyrrole isolated from *Chlorella prototheocoides* (Engel et al., 1991) suggests that ring cleavage in the α-position also occurs in green algae. In contrast, a catabolite of Chl that acts as light emitter in a bioluminescent dinoflagellate is cleaved in the δ-position between pyrroles A and D, whereby the carbon atom of the methine bridge is lost (Nakamura et al., 1988, 1989).

Practically all the information available on nongreen catabolites of Chl from senescent leaves concerns grasses. A survey has shown that compounds resembling established catabolites of barley occur in yellowing leaves of a large number of plant species (P. Matile, unpublished results). It appeared, however, that none of those putative catabolites was identical with established ones, as judged by retention times on HPLC. To thoroughly investigate the products of Chl breakdown in a dicot, we have chosen the cotyledons of rape (*Brassica napus*) seedlings because the radiolabeling of Chl-porphyrin is efficient and specific. Because foliar senescence was also readily achieved upon exposure of excised cotyledons to darkness as well as during natural development, the system seemed well suited for the purpose.

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* Corresponding author; fax 41–1–385–42–04.

Abbreviations: ALA, 5-aminolevulinic acid; FCC, fluorescent Chl catabolite; NCC, nonfluorescent Chl catabolite.

MATERIALS AND METHODS

Cultivation

Rape seeds (*Brassica napus* L. cv Arabella, supplied by VOLG Agricultural Cooperative, Winterthur, Switzerland) were germinated in pots (12 cm in diameter, 50 seeds per pot) filled with compost over a bottom layer of vermiculite. Conditions of cultivation in the growth cabinet were 12/12-h photoperiod, 21/16°C thermoperiod, 70% RH. Irradiance was varied between 22 W m^{-2} (6:00–7:30 AM and 4:30–6:00 PM), 60 W m^{-2} (7:30–9:00 AM and 3:00–4:30 PM), and 120 W m^{-2} (9:00 AM–3:00 PM). Under these conditions, expansion and greening of the cotyledons was completed 11 to 12 d after sowing, and after 25 d, the cotyledons reached the end of the senescence process.

Radiolabeling

Shoots were excised on day 10 after sowing when the cotyledons had accumulated about 80% of the Chl present at the end of the greening period. Groups of five shoots were placed in narrow plastic vials, and the hypocotyls were immersed in 2 mL of tap water containing 1.6×10^5 Bq carrier-free 4-[^{14}C]ALA (specific activity 1.7×10^9 Bq mol^{-1}; Dositek, Orsay, France). Four vials were run in parallel. The initial concentration of ALA was 47 mM. The vials were placed at 10 AM in the growth cabinet and remained there throughout the 48-h labeling period. The water that was consumed through transpiration and expansion of the cotyledons was replaced and recorded at regular intervals. During the initial 8 h, replacements were made every 2 h and thereafter every 4 h. The initial rate of water consumption was as high as 0.4 mL h^{-1} for five shoots, so that [^{14}C]ALA was rapidly diluted upon replacements of water. After each replacement, 20-μL aliquots were withdrawn to determine residual ^{14}C. After 48 h, 98% of the initial [^{14}C]ALA had been transferred to the shoots.

At the end of the labeling period, the shoots were transferred to 25-mL conical flasks containing 10 mL of tap water and were allowed to senesce in permanent darkness and ambient room temperature. During the course of the senescence period, cotyledons were sampled, weighed, and eventually stored at −20°C.

Extraction of Cotyledons

Cotyledons (600–800 mg fresh weight) were ground in a glass homogenizer in the presence of 200-μL of 50-mM phosphate-K^+ buffer, pH 8.0. The homogenate was made up with buffer to 1.0 mL and mixed with 4.0 mL of acetone (precooled to −20°C). After vortexing, two aliquots of 500 μL were pipetted into 1.5-mL Eppendorf tubes. From these aliquots, 20 μL were transferred to scintillation-counting vials for determinations of total radioactivity. The remaining 480 μL were subjected to fractionation into preparations H (hexane solubles), W (water-soluble fraction of 80% acetone-soluble materials), and S (sediment of 80% acetone insolubles).

These preparations were obtained as follows: After exposure to −20°C for 15 min, the tubes were centrifuged for 5 min in a Beckman Microfuge and supernatants were transferred to a new set of tubes. The sediments were washed once with ice-cold 80% acetone. The combined 80% acetone solubles were extracted with 550-μL of hexane. The resulting preparation H was brought to dryness under a stream of N_2 and redissolved in 200 μL of 80% acetone. Aliquots of 50, 20, and 40 μL were withdrawn for determinations of Chl, radioactivity, and HPLC profiles, respectively. The fraction of hexane insolubles was freed from acetone under reduced pressure to yield preparation W, of which, after determination of volumes, 20 and 40 μL were withdrawn for counting of total ^{14}C and HPLC, respectively. Preparation S was obtained by adding 100 μL of buffer to the sediment of acetone insolubles; after vortexing and incubation in a sonicated water bath, 20-μL aliquots were withdrawn for determination of ^{14}C.

HPLC

The reversed-phase system consisted of a C18 Hypersil ODS column (25 × 0.8 cm, 5 μm) and buffered solvents A (100 mM phosphate-K^+, pH 7.0) and B (60% methanol, 40% A; v/v). The elution program consisted of a linear gradient from A to B in 27 min, an isocratic run with B for 6 min, a return to A in 2 min, and equilibration with A for 5 min. The flow rate was 1 mL min^{-1}. Sequential monitoring was with a multiwavelength detector Linear 206 PHD (scanning: 200–365 nm, recording: 320 nm), a Linear Fluor LC 304 (Linear Instruments Corp., Reno, NV), and a Radiomatic FLO-ONE/β radiodetector A-250 (Canberra Packard, Zurich, Switzerland) using a 250-μL flow cell and a flow rate of Pico-Aqua (Packard) scintillation cocktail of 2 mL min^{-1}. The fluorescence detector was bypassed whenever only UV and radioactivity signals were desired.

Determination of Radioactivity

Preparations containing pigments were bleached according to Smith and Lang (1987) before mixing with 5 mL of Safetron 150 cocktail and counting in a Beckman LS 7800 liquid scintillation counter.

Determination of Chls

Fifty microliters of preparation H were diluted with 1950 μL of 80% acetone and analyzed spectrophotometrically using the coefficients provided by Lichtenthaler and Wellburn (1983).

RESULTS

Radiolabeling of Chl

The unambiguous identification of Chl catabolites in senescent leaves requires an exclusive incorporation of ^{14}C into Chl-porphyrin of the presenescent tissue. On one hand, this can be achieved by using the precursor of porphyrin biosynthesis, ALA, labeled in carbon atom 4, which, upon the formation of porphobilinogen, is locked in the pyrrole unit. On the other hand, efficiency and specificity of labeling depended upon the strategy of feeding [^{14}C]ALA to detached rape shoots during the final phase of expansion and greening

of cotyledons. Thus, the continuous feeding of [^{14}C]ALA throughout the labeling period resulted in incorporation of only 40% of total ^{14}C into Chl. Unfortunately, a pulse-chase strategy was not feasible because ALA supplied at concentrations of 1 mM and higher caused severe photodynamic damage in the cotyledons. As a result, the short-term feeding of the desired radioactivity at the beginning of the labeling period, followed by repeated replacements of the water consumed for transpiration and expansion of leaf tissue, resulted not only in the almost quantitative transfer of [^{14}C]ALA to the shoots (see "Materials and Methods"), but also in a remarkably specific labeling of Chl-porphyrin in the cotyledons. Reproducibly, about 80% of the total ^{14}C incorporated into the cotyledons was recovered in the fraction of hexane-soluble compounds (H) in which the label was present in Chls *a* and *b*, exclusively (Figs. 1 and 2). An interesting detail concerns the Chl *a*/*b* ratio at the end of the labeling period: the ratio was much higher for ^{14}C (7) than for the spectrophotometrically determined pigments (3.3). Apparently, Chl synthesized during the final period of greening of rape cotyledons is accumulated largely as Chl *a*.

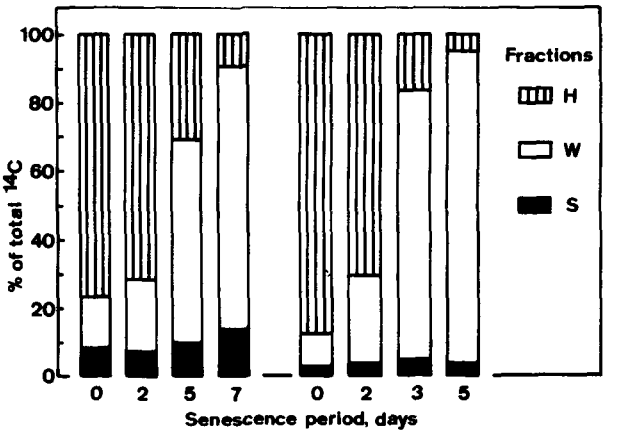

Figure 2. Effect of foliar senescence on the distribution of ^{14}C among fractions obtained from homogenates in 80% acetone. H, Fraction of hexane solubles; W, water-soluble compounds (soluble in 80% acetone, insoluble in hexane); S, sediment of 80% acetone insolubles. Data are from two independent experiments. Note that rate of Chl loss between the two experiments was considerably different.

Identification of Chl Catabolites

In the course of dark-induced senescence, ^{14}C was progressively transferred from the Chl-containing hexane phase (H) to the fraction (W) of water-soluble compounds (Fig. 2). The total radioactivity was preserved. Therefore, it can be concluded that the porphyrin moiety of Chl had been converted into polar catabolites. Green products such as Chlides and pheophorbides could not be detected radiochromatographically (data not shown).

In the presenescent cotyledons, a small proportion of ^{14}C was insoluble in 80% acetone. This fraction (S) accounted for less than 10% of total ^{14}C. It probably contained radioactivity in heme proteins and did not change significantly during yellowing of cotyledons. About 10 to 20% of ^{14}C present in the presenescent cotyledons was recovered in the aqueous phase (W). This fraction contained free ALA and several unidentified compounds that, upon HPLC, were eluted together with the front (Fig. 3). Because the proportion of these compounds was small and the properties were clearly different from those of Chl catabolites, they were not considered as a possible source of erroneous results.

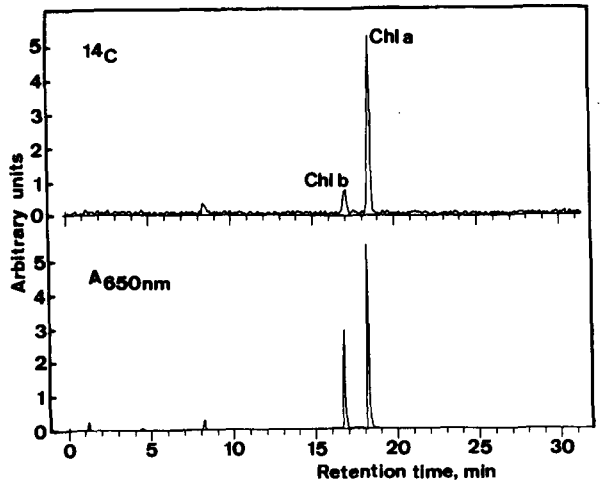

Figure 1. HPLC chromatogram of hexane-soluble compounds present in presenescent radiolabeled cotyledons of rape. A_{650} and ^{14}C were monitored sequentially in the same run.

As senescence was induced upon the incubation of labeled shoots in permanent darkness, a group of three radioactive compounds began to appear and progressively accumulated concomitant with the loss of Chl. Figure 3 illustrates that the most abundant compound, NCC-1, could already be detected after 1 d of dark-induced senescence, whereas NCC-2 and NCC-3 began to accumulate with some delay (Fig. 4). These three catabolites were nonfluorescent as demonstrated by the absence of corresponding signals recorded by the fluorescence monitor (Fig. 5). They absorbed UV at 320 nm but were distinguished by their radioactivity from other UV-absorbing compounds present at all stages of foliar development (Fig. 3).

Whereas the kinetics of accumulation of the three NCCs (Fig. 4) suggested that they represent final products, another ^{14}C-labeled compound could be detected only when rates of Chl breakdown in the cotyledons were high. As shown in Figure 5, this compound (marked with an asterisk in Fig. 3) displayed fluorescence if excited at 320 nm. It was designated FCC-1. A few minor FCCs that were less polar than FCC-1 could also be detected in fraction W from rapidly yellowing cotyledons; however, the putative radioactivity of these scarce compounds was below the detection limit, and, hence, the identification as Chl catabolites was ambiguous. In contrast to the NCCs, FCC-1 and the minor fluorescent compounds could be detected not only in fraction W but also in a 50% methanolic extract from isolated senescent chloroplasts (Fig. 5).

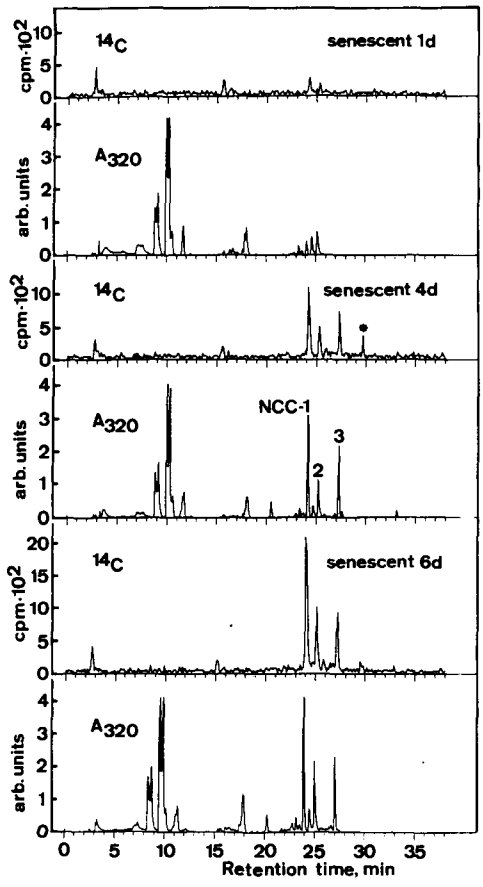

Figure 3. HPLC chromatograms demonstrating the progressive appearance during yellowing of cotyledons of radiolabeled water-soluble compounds. Monitoring at 320 nm revealed the presence of a number of unlabeled compounds that are unrelated to the breakdown of Chl-porphyrin. Three nonfluorescent catabolites, NCC-1 to -3, also absorb at 320 nm, as does a labeled compound (marked with an asterisk and further identified as a fluorescent catabolite in Fig. 5).

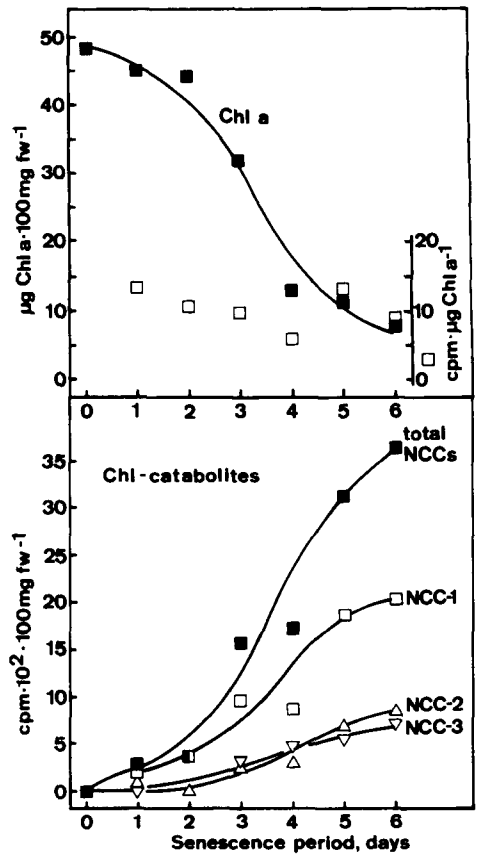

Figure 4. Kinetics of accumulation of radiolabeled nonfluorescent catabolites concomitant with the loss of Chl *a* during senescence of rape cotyledons. Fluctuations of specific radioactivities suggest that the Chl *a* incorporated during the final phase of greening and the bulk of unlabeled Chl *a* may not be catabolized at random.

Spectral Optical Properties of Catabolites

By employing a multiwavelength monitor, the spectra of radioactive catabolites were identified in senescent rape cotyledons and were compared with those of known catabolites occurring in senescent primary leaves of barley. It appeared that all the spectra of NCCs from rape are characterized by the same absorption maximum at 320 nm (Fig. 6), which has been reported previously for RP-14, a nonfluorescent seco-porphinoid catabolite from barley (Bortlik et al., 1990; Kräutler et al., 1992). Incidentally, in the HPLC system developed for the separation of rape catabolites, RP-14 has a retention time similar, but not identical to, that of NCC-1. The fluorescent catabolites FCC-1 from rape (Fig. 6) and FC-2, an established fluorescent derivative of Chl-porphyrin in barley (Schellenberg et al., 1990; Matile et al., 1992; spectrum not shown), were also characterized by a conspicuous maximum at 320 nm; in addition, they exhibited two minor maxima at 270 and 360 nm, respectively.

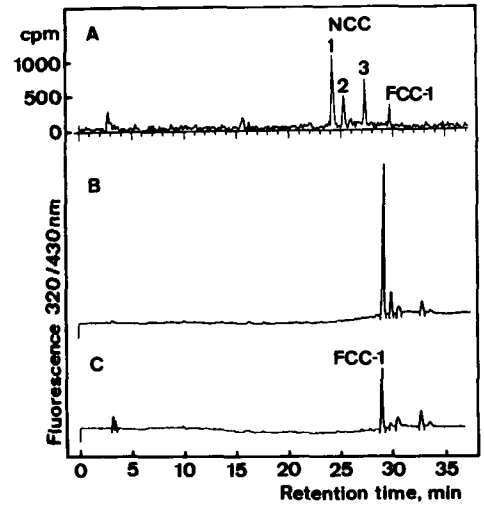

Figure 5. Identification of a fluorescent Chl catabolite, FCC-1, in cotyledons kept in darkness for 4 d. A and B, HPLC chromatograms monitored for ^{14}C and fluorescence, respectively. C, Chromatogram demonstrating the presence of FCC-1 in isolated intact chloroplasts.

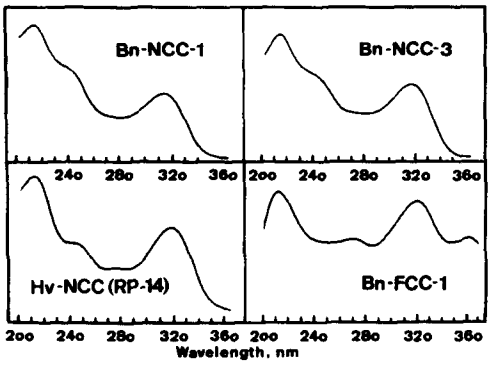

Figure 6. Absorption spectra of selected catabolites of Chl-porphyrin from rape cotyledons (prefix Bn) as compared with the spectrum of a nonfluorescent catabolite from barley (prefix Hv). The catabolites are designated according to the nomenclature proposed in the discussion.

Catabolites in Naturally Senescing Cotyledons

Following the identification of Chl catabolites produced under such unnatural conditions as continuous darkness, it was interesting to investigate whether the same catabolites can be detected in attached cotyledons that are allowed to senesce under photoperiod. Indeed, the three NCCs identified by means of radiolabeling were found to accumulate also in the naturally senescing cotyledons. Figure 7 illustrates the kinetics of appearance of NCCs concomitant with the loss of Chl.

There was, however, a significant quantitative difference between the patterns of NCCs in the two senescence systems. In the case of dark-induced senescence, NCC-1 accounted for about 55% of the total NCCs accumulated at the end of foliar yellowing. In the naturally degreening cotyledons, NCC-1 was the predominant catabolite and, by the end of the senescence period, accounted for as much as 90% of the total pool of NCCs. For obvious reasons it was impossible to employ radiolabeling for the identification of catabolites in the attached cotyledons. Thus, the identity of the three NCCs was inferred from retention times and spectra that were indistinguishable from those of the radiolabeled compounds.

It was tempting to estimate the molar contents of NCCs and compare values with molar losses of Chl. Because the absorption maximum at 320 nm is a common property of NCCs, the molar absorption coefficient of the purified barley NCC, RP 14 (1.5×10^4), determined by Kräutler et al. (1992), was employed for the conversion of absorption values integrated during HPLC runs. It appeared that nmol of accumulated NCCs corresponded with nmol of Chl *a* that was lost by the end of the senescence period.

DISCUSSION

A Nomenclature for Chl Catabolites

Current work with several senescence systems (barley, meadow fescue, rape, French bean) suggests the existence of a large number of species-specific breakdown products of Chl-porphyrin. Although the compounds identified so far have some properties in common, they also differ from one species to another with regard to retention times on reversed-phase HPLC. The detailed investigation of catabolites occurring in senescent primary leaves of barley has generated a rather irregular nomenclature that will not be practicable when this work is extended to other plant species. Thus, one of the predominant, final catabolites of barley has been designated as RP 14 (Bortlik et al., 1990) because it was eluted, when used with a particular solvent system, after 14 min, with RP referring to the artifactual oxidation of the native colorless form to a rust-like pigment.

In any case, it appears to be timely to propose a nomenclature that can eventually facilitate the exact designation of catabolites identified in a given species. This nomenclature is based on the observations that catabolites fall into two categories, fluorescent and nonfluorescent, and that they always occur in groups of individual compounds differing in polarity. We propose the following terminology and nomenclature.

1. Gerontophylls: generic term for all secoporphinoid catabolites of Chl-porphyrin.
2. NCC: abbreviation for nonfluorescent Chl catabolite.
3. FCC: abbreviation for fluorescent Chl catabolite.
4. NCCs and FCCs should be numbered by decreasing polarity within the respective group as judged by retention times on reversed-phase HPLC.
5. Use a prefix to identify the plant species: i.e. Hv (*Hordeum vulgare*), Bn (*Brassica napus*), etc.

According to these terminological rules, Hv-FCC-2 would

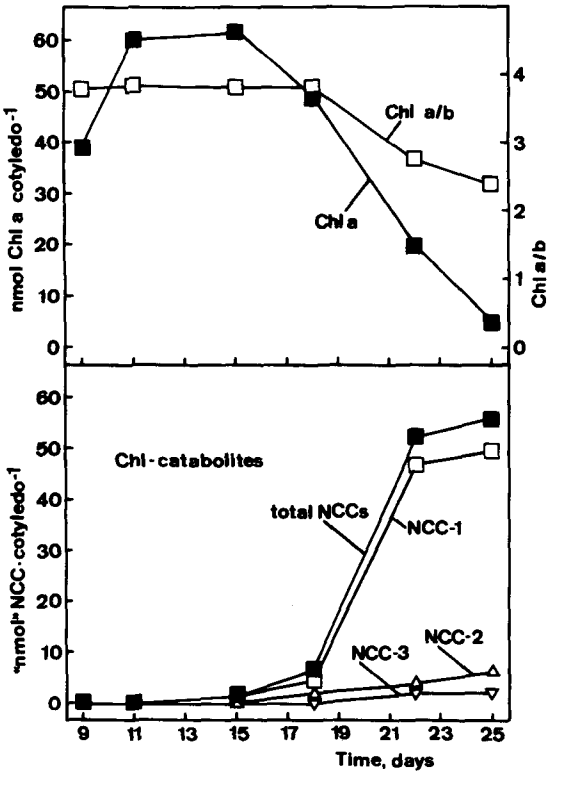

Figure 7. Breakdown of Chl and accumulation of NCCs in attached cotyledons allowed to senesce under photoperiod.

designate a fluorescent gerontophyll of barley having a lower polarity than Hv-FCC-1. This designation replaces a previous one, FC-2, an established derivative of Chl-porphyrin that, under specific conditions, is produced in senescent chloroplasts isolated from barley mesophyll protoplasts (Schellenberg et al., 1990; Matile et al., 1992).

Properties of Chl Catabolites

The phytol ester linkage has been claimed to be highly stable during foliar yellowing (Park et al., 1973), but this view is certainly wrong. The radioactive derivatives of Chl-porphyrin identified in rape cotyledons did not accumulate in the fraction of hexane-soluble apolar compounds; they were water soluble and, hence, undoubtedly represent dephytylated catabolites. The same observation has been reported for Chl catabolites accumulated in yellowing primary leaves of barley (Peisker et al., 1990).

A common feature of all catabolites investigated so far is the maximum of UV absorption at 320 nm, which has been assigned to the formyl-substituted pyrrole B of the secoporphinoid catabolites (Kräutler et al., 1992) that results from the oxidative cleavage of Chl-porphyrin in the α-position. The occurrence of this type of catabolite has been associated with Chl breakdown in higher plants (Bortlik et al., 1990; Kräutler et al., 1991) and in a green alga (Engel et al., 1991). This may indicate the existence of a common enzymic mechanism of Chl breakdown.

It is puzzling, however, that catabolites discovered so far in senescent tissues of higher plants have remarkably different polarities, as judged by retention times on reversed-phase HPLC. For example, in the HPLC system employed in the present study, the retention times of Hv-FCC-2 is close but clearly different from those of Hv-NCC (RP 14) and Bn-NCC-1, but the predominant fluorescent catabolite of rape, Bn-FCC-1, which has identical spectral properties to Hv-FCC-2, is markedly less polar.

Another puzzling observation concerns the occurrence in one species of both NCCs and FCCs in groups with graduated polarities. Thus, in barley we have identified so far four FCCs and a large group of NCCs. One might have expected a final accumulation of two catabolites, a predominant one originating from Chl *a* and a minor one from Chl *b*. Corresponding observations have not been made, however, nor was there any indication of types of catabolites occurring in couples at proportions of the two Chls.

As long as catabolites originating from Chl *b* have not been identified through structural analysis, the question of whether the two Chls are degraded by the same mechanism remains unanswered. Yet the only constitution known so far, the one of Hv-NCC (RP 14), can provide a guideline to the interpretion of differing polarities of chemically related catabolites. This derivative of Chl *a* is hydroxylated in the vinyl side chain of pyrrole A as well as in the ethyl group of pyrrole B (Kräutler et al., 1991, 1992). Such modifications will certainly increase the polarity without affecting the spectral properties, which are determined by the pyrroles. Hence, modifications in the side chains of a primary catabolite may result in the formation of several similar compounds differing in polarity.

In Hv-NCC (RP 14), the carboxymethyl group in the isocyclic ring is intact, but in other species modifications such as demethylation and decarboxylation may take place. Even conjugations in hydroxyl and carboxyl groups analogous to the modifications in many secondary compounds are feasible and would result in altered polarities. Indeed, the active excretion of an early fluorescent catabolite in senescent chloroplast of barley (Matile et al., 1992) and the final deposition of catabolites in the vacuoles of senescent mesophyll cells (Düggelin et al., 1988; Matile et al., 1988; Bortlik et al., 1990) point to similarities of processing of secondary compounds and Chl catabolites.

Whether or not Chl catabolites are fluorescent is likely to be due to the existence of a delocalized system of double bonds in at least two adjacent pyrroles. Such a system comprises a fluorescent Schiff-base sequence N=C—C=C—N originating from the methine bridge carbon attached to two adjacent pyrroles of the secoporphonoid catabolite. So far, FCCs have been identified as early or intermediary catabolites, whereas the NCCs turned out to represent a final product of the catabolic pathway. Therefore, the processing appears also to concern reductions in the system of pyrroles that result in the abolishment of delocalized double bonds and, hence, of fluorescence.

Meanwhile, Bn-NCC-1 has been purified and its constitution has been analyzed by W. Mühlecker in the laboratory of B. Kräutler (unpublished results). Regarding the oxidative cleavage of Chl-porphyrin, Bn-NCC-1 is identical with the secoporphinoid tetrapyrrole RP 14 from barley (Kräutler et al., 1992), but there are differences in the side chains of pyrroles A and B. Thus, the vinyl in A is not hydroxylated, and in the ethyl of B the hydroxyl group is esterified with malonic acid.

Fate of Chl Catabolites in Senescent Leaves

The differentiation of chloroplasts into gerontoplasts is associated with wholesale degradation of protein. Developing gerontoplasts represent the main source of nitrogen, which eventually is exported from senescent leaves. Of the total nitrogen contained in mature chloroplasts, only about 3% is incorporated in Chl-porphyrin (Dalling, 1987). Degradation of Chl is unlikely to take place for the benefit of reutilization of such a small amount of nitrogen. However, in the thylakoids, Chl is complexed with more than 30% of the total protein of chloroplasts.

In a mutant genotype of *Festuca pratensis* that is deficient in the breakdown of chloroplastic porphyrins, Chl is retained along with the apoproteins of Chl (Thomas and Hilditch, 1987). This finding suggests that Chl protects the apoproteins from proteolytic degradation, and that plants degrade Chl to accomplish the reutilization of nitrogen incorporated in the apoproteins. In any case, it would be difficult to think of enzymic mechanisms aimed at the conversion of linear tetrapyrroles into transportable forms of nitrogen. Therefore, it was not surprising to establish in senescent primary leaves of barley that Chl catabolites are retained upon development of secondary leaves (Peisker, 1991), and the present data obtained with rape cotyledons led to the same conclusion. It may be predicted, therefore, that the ultimate recycling is

achieved by soil microorganisms that are able to utilize Chl catabolites present in shed leaves as a source of nitrogen.

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