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Chlorophyll *a* epimer and pheophytin *a* in green leaves

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The composition of chlorophyll- (Chl-) type pigments, particularly the contents of C10-epichlorophyll *a* (Chl *a'*) and pheophytin (Ph) *a*, in leaves of various plants has been determined by an improved HPLC procedure. It is demonstrated that one Chl *a'* molecule is present per 305 ± 20 (mean ± S.D.) molecules of Chl *a* in leaves of fifteen out of eighteen plants submitted to analysis. The possibility of artifact formation in the extraction, sample conditioning and analytical stages has been excluded rigorously. For three plants, a very rapid pheophytinization in the extract solution interfered with reliable determination of the Chl *a'* content. The Ph *a* content has been found to be one Ph *a* molecule per 58 ± 5 molecules of Chl *a* in the fifteen plants. These results are briefly discussed in the context of the construction of the photosynthetic apparatus.

Introduction

In 1942 Strain and Manning [1] discovered, during sucrose column chromatography of plant extracts, that chlorophyll (Chl) *a* was accompanied by a minor component, named Chl *a'*, showing an absorption spectrum very similar to that of Chl *a*. A few decades later Chl *a'* was confirmed to be the C10-epimer of Chl *a* [2–4] (cf. Fig. 1). Except for the ultraviolet-visible absorption characteristics, Chl *a* and Chl *a'* in vitro behave quite differently in many respects; NMR [2–4], circular dichroism [5–7], solubility in organic solvents [8,9], rate of pheophytinization (Mg^{2+} elimination) [3,8,10], reducibility of the C 9 ketocarbonyl moiety [10], and intermolecular aggregation [2,3,9,11,12].

In view of the relatively facile epimeric interconversion observed in vitro [1–3,11–19], the question arises whether or not Chl *a'* is present in vivo. To our knowledge, only one paper, published in 1965 by Michel-Wolwertz and Sironval [13], argued for the presence of Chl *a'* in vivo based on analytical results. Strain [20] and many other

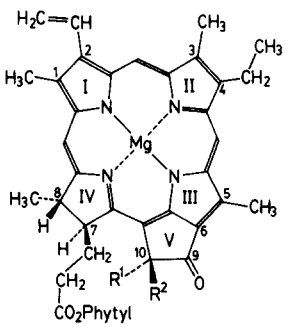

Fig. 1. Structure formula for chlorophyll *a* ($R^1 = COOCH_3$, $R^2 = H$) and *a'* ($R^1 = H$, $R^2 = COOCH_3$). Replacement of the central Mg with 2H gives the corresponding pheophytins. Phytyl = $C_{20}H_{39}$.

Abbreviations: Chl, chlorophyll; Ph, pheophytin.

workers [2,14,16–18], especially after a criticism addressed by Bacon [21] on Michel-Wolwertz and Sironval's result, considered that Chl *a'* is merely an artifact produced in the course of handling of plant extracts. It appeared to us, however, that both the positive and negative arguments are not fully supported by persuasive experimental evidence. The present work has been undertaken to unravel this question, which might have some bearing on the construction of the photosynthetic apparatus in plants.

An experiment designed to answer the above question must fulfill the following five requirements. (1) A solvent having a sufficient affinity to both Chl *a* and Chl *a'* should be used for extraction. This point is not trivial in view of the significantly lower polarity of Chl *a'* as compared to Chl *a* [8,9,12]. (2) The analytical method should be capable of detecting plant pigments with high enough sensitivity and resolution. (3) The possibility of artifact formation during extraction should be checked rigorously. To comply with this requirement, it is indispensable to prepare a standard Chl *a* sample with an epimeric purity much higher than that of Chl *a* in vivo, and to examine quantitatively to what extent the standard sample undergoes epimerization during the same treatment as that applied to the leaf tissue. (4) After the preparation of an extract solution for the analysis, the temporal evolution of its composition should be followed to obtain the initial composition by extrapolation to time zero. (5) A series of different plants should be analyzed to examine the generality of the result.

By establishing an analytical procedure fulfilling all of these requirements, we have attempted to determine the Chl *a'* content in leaves of green plants. It was possible, at the same time, to determine the content of Ph *a*, whose presence and function in chloroplasts are currently of much concern in photosynthetic research [22,23].

Methods and Materials

Leaves were analyzed through the following three steps, keeping the operational time as short as possible.

Step 1 (usually 2–5 min). Third or fourth youngest green leaves, apparently healthy and active in photosynthesis, of a cultured or wild plant were harvested, rinsed with distilled water, and blotted with tissue paper.

Step 2 (usually 8–13 min). 3–5 g of the leaf tissue was ground with 20–30 g of anhydrous Na_2SO_4 in a glass mortar. After grinding for 1–2 min, roughly 50 ml of chloroform was added, and the mixture was transferred into a glass beaker. After sonication for 15–30 s, the mixture was filtered, and the initial 10–20 ml portion of the filtrate was immediately evaporated to dryness in a rotary evaporator under reduced pressure. The solid material was then redissolved in 2 ml of chloroform to obtain a sample solution for analysis (roughly 2–5 mM in Chl *a*). The chloroform used was of reagent grade containing approx. 0.8% of ethanol as a stabilizer. The coexistence of such a nucleophile did not seriously influence the final results, as described later.

Step 3. The chloroform solution was analyzed by isocratic high-performance liquid chromatography (HPLC) on a silica-packed column cooled to around 7°C, with hexane/2-propanol (98.6 : 1.4, v/v) as an eluent. The details of HPLC operation are given elsewhere [7]. The HPLC analyses were carried out immediately after completion of step 2 and also at appropriate time intervals thereafter.

The glasswares, Na_2SO_4, and chloroform had been cooled in a refrigerator prior to use. All the operations were conducted under dim light. The ambient temperature was 17–18°C, unless otherwise noted.

Standard samples of Chl *a* (99.97%), Chl *a'* (approx. 99%), and Ph *a* (approx. 98%) were prepared by means of preparative-scale HPLC [7]. Each sample, characterized by a series of analytical techniques [7], contained the corresponding epimer as practically the sole impurity.

Results

Chl a' content in leaves

About 75% of the total colored compounds were extracted by sonication for 15–30 s at step 2, as estimated by exhaustive post-extraction of the residue with acetone. The chloroform and acetone extracts gave essentially the same visible absorption spectra. Sonication for 30 min with chloroform resulted in extraction of approx. 90% of the

pigments. Although no significant difference was noted for the Chl *a'* content in the chloroform solutions obtained by 15–30 s and 30 min sonication treatments, we limited the sonication period to 30 s throughout the present experiments; this was done for the purpose of determining simultaneously the Ph *a* content, which occasionally exhibits a rapid grow-in with time after extraction (see below).

Typical HPLC charts are displayed in Fig. 2. Traces a, b and c are for the authentic samples of Chl *a'*, Ph *a*, and Chl *a*, respectively. The minor

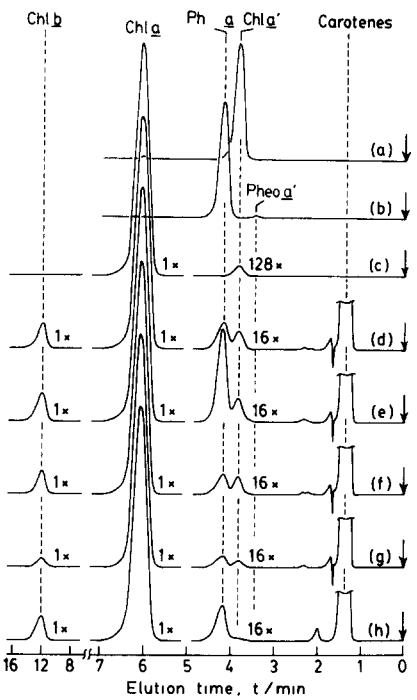

Fig. 2. HPLC charts for (a) 99% Chl *a'* (+1% Chl *a*), (b) 98% Ph *a* (+2% Ph *a'*), (c) 99.97% Chl *a* (+0.03% Chl *a'*), (d) chloroform extract of *H. macrophylla* at 14 min from harvest, (e) same as (d) but at 140 min from harvest (136 min from contact with chloroform), (f) chloroform extract of *C. album* at 15 min from harvest, (g) mixture of 5 mg of 99.97% Chl *a* + 4 g of *H. macrophylla* leaf tissue submitted to steps 2 and 3 (see text), and (h) acetone extract of *H macrophylla* at 14 min from harvest. The Chl *a* peaks are normalized to a common intensity in traces c–h. Intensities at $t < 5$ min are magnified in traces c–h. The tracing speed at $t > 7$ min is reduced 6-fold Analytical conditions: column, Senshupack 50-5 (150 mm × 4.6 mm, i.d.), column temperature, 7°C; eluent, hexane/2-propanol (98.6/1.4, v/v); flow rate, 1 ml/min; sample injector, Rheodyne 7125; sample size, 0.4–1 μl, ambient temperature, 17–18°C; detection wavelength, 430 nm

peak seen on each chromatogram represents the corresponding epimer as an impurity. Trace d is the chromatogram for a chloroform extract of *Hydrangea macrophylla* at 14 min from harvest. Carotenes are eluted first, followed by two slightly overlapping peaks at around $t = 4$ min. The two peaks are assigned, in the order of elution, to Chl *a'* and Ph *a*, respectively, by observing (i) the dependence of the peak intensity on the detection wavelength and (ii) the coincidence of their retention times with those of the authentic samples, in cases where the latter were used either as internal or as external standards. The column temperature, 7°C for Fig. 2, was found to be a critical factor for the separation feature of Chl *a'* and Ph *a*. Thus, the two components were hardly separated at 15–20°C, and the order of elution was reversed at temperatures above 22°C. By regulating the elution conditions with the column temperature kept at 7°C, it was possible to elute the two components as two completely isolated peaks separated by about 2 min on the chromatogram. Again in this case, we confirmed the perfect coincidence of the retention times of the two peaks with those of authentic samples. The Chl *a'* and Ph *a* peaks are followed by major peaks of Chl *a* and Chl *b*. After standing for approx. 2 h, the chloroform extract of *H. macrophylla* gave chromatogram e. In comparison with trace d, a slight increase in the Chl *a'* content and a substantial increase in the Ph *a* content are noted in e. The latter finding will be discussed later. Trace f shows the result of the analysis of another plant, *Chenopodium album*. It can be seen that, except for a small difference in the Ph *a* content, traces d and f are nearly identical to one another. Finally we note that Ph *a'* (C10-epimer of Ph *a*) is barely detectable in chromatograms of plants extracts. The HPLC conditions for the recording of these chromatograms have been optimized such that the total elution time is short enough and that, at the same time, all the Chl-type pigments (Ph *a'*, Chl *a'*, Ph *a*, Chl *a*, and Chl b) are separated to a sufficient extent.

Based on these observations we evaluated the Chl *a'* content in the chloroform extracts of leaves. A typical result, obtained for six leaf samples *H. macrophylla* harvested on three separate days and at various times of the day, is given in Fig. 3. By extrapolation to $t = 4$ min (instant of contact with

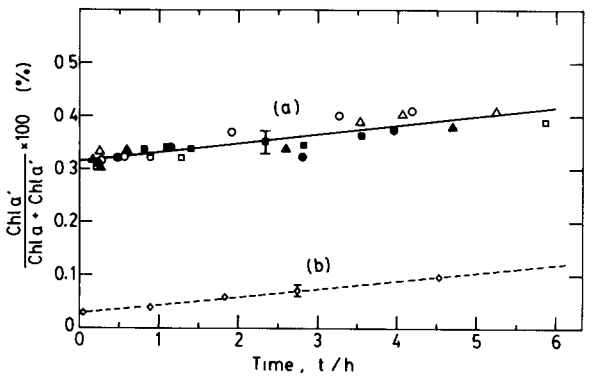

Fig 3 Temporal evolution of the Chl *a'* content in (a) chloroform extracts of six leaf samples of *H macrophylla* and (b) a chloroform solution of 99.97% pure Chl *a* Time is measured from the instant of harvest for (a) and from the instant of dissolution for (b). Storage temperature, 17–18°C.

chloroform), a value of 0.0032 ($\pm$0.0002) is obtained as the initial Chl *a'* fraction in Chl *a* + Chl *a'*. Similar measurements have been carried out on leaves of eighteen different plants, of which fifteen gave a remarkably unified result, 0.0033 ± 0.0002 (mean $\pm$ S.D.) as the initial Chl *a'* fraction (Table I). For the remaining three plants (*Ginkgo biloba*, *Houttuynia cordata* and *Rosa hybrida*), an extremely rapid Chl *a* $\rightarrow$ Ph *a* conversion during steps 2 and 3 interfered with a reliable determination of the Chl *a'* content.

From Fig. 3 it is seen that the Chl *a'* content gradually increases in the chloroform solution during storage. This reflects the occurrence of epimerization toward an equilibrium state, in which the ratio [Chl *a'*]/([Chl *a*] + [Chl *a'*]) ranges from 0.16 to 0.25, depending on the solvent and temperature (Watanabe et al., unpublished result). As we have verified recently [19], an off-equilibrium composition approaches the equilibrium one with a rate constant $k_0 = k + k'$, where k and k' are, respectively, the forward and backward rate constant for the process:

$$\text{Chl } a \underset{k'}{\overset{k}{\rightleftarrows}} \text{Chl } a' \qquad (1)$$

The value of k_0 for the leaf extract (curve a in Fig. 3) is calculated to be $(2.7 \pm 0.8) \cdot 10^{-7}$ s^{-1}, while that for the 99.97% Chl *a* sample (curve b) is

TABLE I

CONTENTS OF Chl *a'* AND Ph *a* IN THE CHLOROFORM EXTRACTS OF DIFFERENT PLANTS (15–20 MIN FROM HARVEST)

Experimental errors are $\pm$0.0002 for Chl *a'* content and $\pm$0.001 for Ph *a* content. The scatter in the Ph *a* content reflects the plant-dependent rate of pheophytinization after extraction (see text).

Plant	Chl *a'* / Chl *a* + Chl *a'*	Ph *a* / Chl *a* + Ph *a*
Hydrangea macrophylla (hydrangea)	0.0032	0.020
Rubus palmatus (bramble)	0.0027	0.038
Ligustrum japonicum (Japanese privet)	0.0033	0.045
Fatsia japonica (an evergreen shrub)	0 0033	0.032
Citrus natsudaidai (orange)	0.0036	0.015
Aucuba japonica (Japanese aucuba)	0 0036	0.017
Commelina communis (dayflower)	0 0031	0.017
Chenopodium album (goosefoot)	0.0033	0.017
Taraxacum platycarpum (dandelion)	0.0033	0.018
Paederia scandens (a creeping herb)	0 0035	0.023
Artemisia vulgaris (mugwort)	0.0031	0 021
Phytolacca americana (pokeweed)	0.0031	0.015
Erigeron sumatrensis (fleabane)	0.0034	0.018
Mirabilis jalapa (marvel-of-Peru)	0.0032	0.016
Youngia japonica (snow thistle)	0.0036	0.015

$2.2 \cdot 10^{-7}$ s^{-1}. The similarity of these two values suggests that the Chl *a* and *a'* in the extract solution exist as sufficiently isolated molecules. This (slow) epimerization probably results from a nucleophilic action of ethanol present at a 0.8% level in chloroform. In a separate experiment we determined the k_0 value in absolute ethanol to be $1.6 \cdot 10^{-6}$ s^{-1} at 18°C (unpublished result). This rate constant would, though it is 10-fold higher than in chloroform, lead to the formation of only 0.03% Chl *a*, in Chl *a* if the latter was kept in

contact with ethanol for a period of approx. 15 min (as in the present extraction procedure). For a 0.3% level Chl $a \rightarrow a'$ conversion, a contact period of 2.6 h would be necessary at 18°C, even in absolute ethanol. (We must admit, in the light of these findings, that a fairly rapid epimerization of Chl *a* and Chl *b*, observed in a previous work [7] during 2-h storage in chloroform, might have been due to the presence of small amount of extraneous nucleophiles other than ethanol in the solvent.)

The possibility that the Chl *a'* thus detected is an experimental artifact is excluded on the basis of the following observations. First, the Chl $a \rightarrow a'$ conversion at step 3 (HPLC) is negligible because a chromatogram of 99.97% Chl *a*, much purer than the Chl *a* in leaves, can be actually recorded (trace c of Fig. 2). Further purification by means of the preparative HPLC [7] led occasionally to a Chl *a* sample with even 99.99% or higher purity. Second, variation of the time spent in step 1 (from harvest to grinding) in a range from 1 to 30 min for *H. macrophylla* and from 2 to 60 min for *Commelina communis* did not cause any noticeable change in the resulting analytical value of the Chl *a'* content. Thus the Chl *a'* formation during step 1 is also negligible. Finally the possible artifact production during step 2 was checked as follows. 3–5 grams of the leaf tissue of *H. macrophylla* were mixed with 1–10 mg of the 99.97% Chl *a* sample, and such mixtures were submitted to the same treatment (steps 2 and 3) as in the ordinary extraction procedure. An example of the resulting HPLC trace (5 mg of the 99.97% Chl *a* added to 4 g of leaf tissue) is given as trace g of Fig. 2. As can be seen, the peak intensity of Chl *a'*, as well as those of Ph *a* and Chl *b*, has significantly decreased relative to Chl *a* in going from trace d (leaf tissue alone) to trace g. Using the molar ratio Chl *a*/Chl *b* (roughly 3.0 for leaf tissue alone) as a measure for admixing of the 99.97% Chl *a*, one can predict the change in the ratio Chl *a'*/(Chl *a* + Chl *a'*) as a function of Chl *a*/Chl *b* for the two circumstances: (a) the 99.97% Chl *a* remains intact and (b) the 99.97% Chl *a* undergoes approx. 0.3% transformation to Chl *a'*, during steps 2 and 3. The two working curves and the experimental points are displayed in Fig. 4. This result provides conclusive evidence that a 0.3% level of Chl *a'* was present already in the initial leaves. It is worth

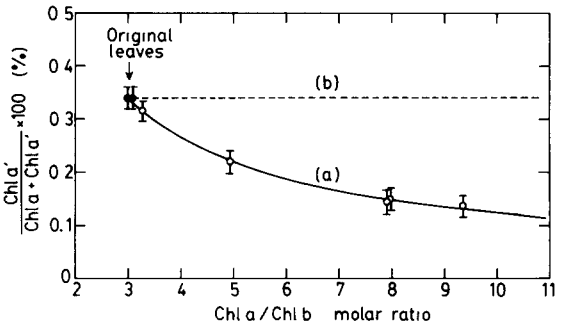

Fig. 4. Dependence of the Chl *a'* content on the Chl *a*/Chl *b* molar ratio Full circles represent the composition of chloroform extracts of *H macrophylla* leaf samples. Open circles are the analytical results for 1–10 mg of 99.97% Chl *a* + 3–5 g of *H macrophylla* leaf tissue mixtures submitted to the same treatments as in extraction (steps 2 and 3) Working curves: (a) in case where the 99 97% Chl *a* remains intact during the treatments; (b) in case where the 99 97% Chl *a* attains, during the treatments, the same Chl *a*/*a'* ratio as that found for the leaf extract

emphasizing here that the commercial Chl *a* samples, containing about 1% of Chl *a'* [7,15], cannot serve as standard materials for this type of investigation, since they merely 'contaminate' the leaves.

The choice of the extraction solvent was found to be crucial in the present work. As mentioned above, a highly unified value of the Chl *a'* content was obtained for the chloroform extract, depending little on the sonication period and the nature of the plant. The ambient temperature and the degree of grinding also did not influence the final result to a measurable extent. In contrast, the use of acetone, which is widely employed for extraction of plant pigments [13,14,16–18,20], led to somewhat complicated results with respect to the Chl *a'* content. As an example, the chromatogram of an acetone extract (prepared at 18°C) of *H. macrophylla* is shown as trace h in Fig. 2. Here the Chl *a'* content is apparently 10-fold lower than that in the chloroform extract, trace d. At a higer ambient temperature (28°C), the acetone extract of the same plant gave a Chl *a'* content of approx. 0.1% based on Chl *a*. Prolonged grinding and sonication in acetone tended to increase the apparent Chl *a'* content, but it was always below 0.3%. The efficiency of Chl *a'* extraction with acetone depended also on the nature of the plant.

For instance, sonication of the leaf tissue of *C. communis* for 30 s in acetone led to a Chl *a'* content ranging from 0.1% (20°C) to 0.2% (28°C), which is substantially higher than in the case of *H. macrophylla* stated above, but is still lower than the Chl *a'* content found for the chloroform extracts. A prolonged (10 min) sonication of the leaf tissue of *C. communis* in acetone at 28°C gave a final Chl *a'* content of 0.3–0.35%. These results could be accounted for by invoking a much lower solubility of Chl *a'*, having a reduced polarity [8,9,12], in a polar solvent (acetone) than in chloroform. Another possibility may be that the Chl *a'* molecules are situated in chloroplasts at a site difficult to attack by polar molecules.

Ph a content in leaves

Ph *a* is also clearly detected in the HPLC charts of plant extracts (Fig. 2). By examining trace g (leaf tissue + 99.97% Chl *a*) in comparison with trace d (leaf tissue alone) in Fig. 2, it is evident that the Ph *a* is not an artifact derived from Chl *a* during the treatments. By calibrating the apparatus sensitivity for Chl *a* and Ph *a* at the detection wavelength (430 nm) with a chloroform solution containing known amounts of the two pigments, the Ph *a* content was determined as the ratio Ph *a*/(Chl *a* + Ph *a*).

In sharp contrast to what was found for Chl *a'*, the Ph *a* content at the completion of step 2 (Table I) exhibits a remarkable scatter from one plant to another. This scatter, however, does not represent an intrinsic scatter in the Ph *a* content at the instant of harvest, but results from a difference in the rate of pheophytinization (Chl *a* → Ph *a* conversion) after extraction. Such a difference arises most probably from a difference in acidity, depending on the extraction efficiency of acidic components with chloroform, of the final extract solution. As typical examples, the temporal evolution behaviors of the Ph *a* content in chloroform extracts of *C. communis* and of four leaf samples of *H. macrophylla* are depicted in Fig. 5. The different rates of pheophytinization among leaf samples of the latter plant may be due to the difference in the leaf weight/solvent volume ratio, which in turn would alter the effective concentration of acidic components in the final extract.

Leaves of nine plants (*Citrus natsudaidai*, *Aucuba japonica*, *Chenopodium album*, *Taraxacum platycarpum*, *Phytolacca americana*, *Erigeron sumatrensis*, *Mirabilis jalapa*, *Youngia japonica*, and *C. communis*), giving relatively low values of the Ph *a* content at the end of step 2, showed thereafter pheophytinization slow enough to allow reliable extrapolation to $t = 2$–5 min (instant of contact with chloroform) to obtain Ph *a*/(Chl *a* + Ph *a*) = 0.017 ± 0.001 (mean ± S.D.). Other six plants gave a Ph *a* content ranging from 0.020 to 0.045 at 15–20 min from harvest (Table I). For these plants, however, extrapolation to $t = 2$–5 min led also to a value of 0.016–0.019 as the initial Ph *a* content (see the curves for *H. macrophylla* in Fig. 5).

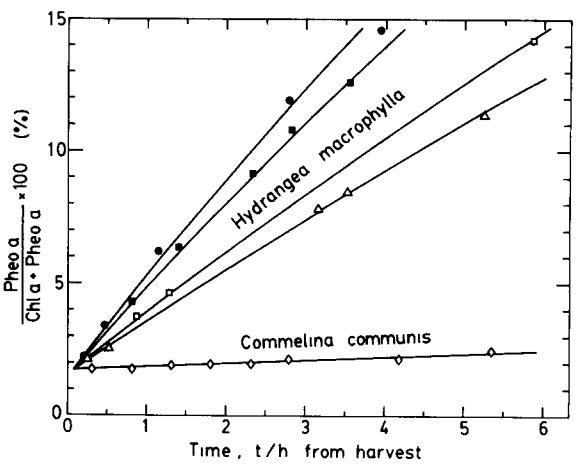

Fig 5. Temporal evolution of the Ph *a* content in chloroform extracts of *H macrophylla* (four samples) and *C communis*. Curves are drawn by assuming a first-order scheme for pheophytinization.

On the other hand, chloroform extracts of three plants (*G. biloba*, *H. cordata*, and *R. hybrida*) showed extremely high rates of pheophytinization, resulting in nearly complete Chl *a* → Ph *a* conversion in 1–2 h. This would reflect a higher content of (unidentified) acidic substances, extractable with chloroform, in these leaves. In such cases reliable determination of the Chl *a'* content also became impossible.

As in the determination of the Chl *a'* content (see above), the use of acetone as an extraction solvent again gave less satisfactory results in the determination of the Ph *a* content, in that the pheophytinization rate was considerably higher in

acetone than in chloroform extracts. Comparison of traces d and h in Fig. 2 (higher apparent Ph *a* content in the latter) exemplifies this situation. This may reflect the stronger affinity of (unknown) acidic compounds to a polar solvent.

Discussion

The composition of the Chl-type pigments in green leaves, averaged over fifteen different plants examined in the present work, is summarized in Table II. These figures have been obtained after correction for the alterations (epimerization and pheophytinization) after extraction, and therefore apply to leaf tissues at the instant of harvest. As already mentioned Ph *a'* (C10-epimer of Ph *a*) is barely detectable in the chromatograms of leaf extracts. Taking the apparatus sensitivity into account, we estimate that the Ph *a'* content is less than 1% of the Ph *a* content. This in turn corresponds to Chl *a*/Ph *a'* > 6000, which is evidently too large a figure to regard Ph *a'* as one of the ingredients in the photosynthetic apparatus.

Of primary interest is the presence of Chl *a'* in vivo at a level of Chl *a'*/Chl *a* ≈ 1/300. In so far as we are aware, this is the first case where Chl *a'* has been unambiguously detected in plants. The surprising uniformity of the Chl *a'* content among various plants (Table I) makes us suppose that this compound is an integral component of photosynthetic apparatus, rather than a metabolic waste material. (Recently, Lötjönen and Hynninen [4] speculated, though without any experimental support, that Chl *a'* might function in plant photosystems.) According to Melis and Anderson [24], the size of one photosynthetic unit (PSU), composed of reaction-center complexes and antenna pigments, in spinach chloroplasts is estimated as follows: 180 Chl *a* + 30 Chl *b* in photosystem (PS)I; 146 Chl *a* + 84 Chl *b* in PS II_α; and 85 Chl *a* + 15 Chl *b* in PS II_β. It follows that about 540 Chl molecules (410 Chl *a* + 130 Chl *b*) constitute one PSU. Although these figures may still be controversial and the molecular composition may be somewhat plant-dependent, our result of analysis indicates that 1 or 2 molecules of Chl *a'* are present per 1 PSU. This 'concentration' is roughly the concentration of reaction centers. As a consequence, there is a possibility that Chl *a'* is a necessary constituent of, or is at least associated with, the as of yet unidentified reaction center(s). In view of the generally accepted hypothesis that a Chl 'special pair' might function as reaction center(s) [25], we are currently studying in detail the intermolecular aggregation behaviors of Chl *a*, Chl *a'*, and their mixtures in vitro. A preliminary experiment shows that Chl *a*-*a* and Chl *a*-*a'* pairs exhibit quite different visible absorption spectra upon aggregation, and that the latter pair possesses a higher aggregation tendency than the former (unpublished results).

The analytical result of the Ph *a* content (Table II) suggests the presence of about 6 Ph *a* molecules in 1 PSU. The involvement of Ph *a* as a primary electron acceptor in PS II has been demonstrated by Klimov et al. [22] by means of ESR spectroscopy. A recent work by Omata et al. [23] shows that two Ph *a* molecules are present per one PS II complex in spinach chloroplasts. If 1 PSU is composed of three photosystems (PS I, II_α, and II_β), as was supposed by Melis and Anderson [24], the present experimental data would indicate that each photosystem contains two Ph *a* molecules. This remains, however, still a speculation and shall be elucidated in future investigations.

TABLE II

AVERAGE COMPOSITION OF CHLOROPHYLL-TYPE PIGMENTS IN THE CHLOROFORM EXTRACTS OF LEAVES OF FIFTEEN DIFFERENT PLANTS

Values have been obtained by extrapolation to the instant of contact with chloroform (2–5 min from harvest)

Pigment	Molar abundance (mean ± S.D.)
Chlorophyll *a*	305
Chlorophyll *b*	89 ±12
Pheophytin *a*	5.3 ± 0.4
Chlorophyll *a'*	1.0 ± 0.07

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