Plant Physiol. (1980) 65, 478–479
0032-0889/80/65/0478/02/$00.50/0

Chlorophyll Determination in Intact Tissues Using *N,N*-Dimethylformamide

Received for publication June 15, 1979 and in revised form October 8, 1979

RAMI MORAN AND DAN PORATH
Department of Botany, The George S. Wise Faculty for Life Sciences, Tel Aviv University, Ramat Aviv, Israel

ABSTRACT

Photosynthetic pigments from etiolated cucumber (*Cucumis sativus* var. Beit Alpha improved, Hazera Co., Gedera) cotyledons were extracted by direct immersion of the intact cotyledons into the solvent *N,N*-dimethylformamide (DMF). The solvent is especially efficient when pigment concentration is low; time and tools are saved and the loss of pigment that usually occurs in more complicated extraction procedures is prevented. The specific absorption coefficient of chlorophyll *a* in DMF was also determined.

Problems in extracting sufficient pigment are often encountered when working with etiolated plants where Chl concentration is low, especially if material available for sampling is limited.

In studies on Chl determination (1, 3, 4) extraction of pigments usually involves a number of procedural steps which inevitably results in loss of material. Furthermore, grinding and centrifugation of tissues require a relatively high minimum volume of solvent which in effect lowers the concentration of pigment in the final solution. DMF[1], an organic solvent in which Chl is soluble (6), was used to extract Chl from an alga (8) and from leaves of higher plants without the benefit of grinding (2). In the present work, DMF was used to extract photosynthetic pigments from intact etiolated cucumber cotyledons. Extraction with DMF is simpler than with acetone and the results are comparable.

MATERIALS AND METHODS

Cotyledons were excised from dark-grown 3- to 4-day-old cucumber seedlings (*Cucumis sativus* var. Beit Alpha improved, Hazera Co., Gedera). Both DMF and 80% acetone extracts were prepared for Pchl determination by direct immersion of the cotyledons in the solvents. The ratio for the extraction was 5–10% (w/v). Working volumes ranged between 2 and 10 ml. Extracts for Chl *a* determination were prepared in the same manner from seedlings which had been exposed to 10 min white light prior to removing their cotyledons. Chl *b* is not present in detectable amounts in etiolated seedlings within the first hours following light exposure (7). Extracts were also prepared by grinding 10 pairs of cotyledons whose approximate weight was 500 mg in a Sorvall Omni-Mix for 3 min and then centrifuging at $27 \times 10^3 g$ for 10 min without repetition. All operations were carried out under a green safelight. The extracts were stored in the dark for 24–48 h at 4 C prior to spectroscopic examination.

To compare absorption spectra of Chl *a* in the two solvents, approximately 1.0 mg of pure Chl *a* (Sigma) was first dissolved in 2 ml of 100% acetone. Aliquots of 0.1 ml were transferred to both 6 ml of 80% acetone and 6 ml of DMF so that the final absorbance of these two solutions ranged between 0.1 and 1.0. The concentration of 100% acetone in the solutions was only 1.6% and therefore its effect on the spectrum was considered negligible.

The various preparations were examined by means of a Varian Techtron U.V.-V.S. model 635 scanning spectrophotometer using the 0.2 nm bandwidth-measuring beam and a 1-ml cuvette having a path length of 10 mm. For plant extracts, the extent of absorption at the maxima was determined as the height of the peak above the corresponding base line (5). For the pure Chl *a* solution, absolute absorption was recorded.

RESULTS

DMF was more efficient in extracting Pchl and Chl *a* from intact etiolated cotyledons than acetone (Fig. 1). Even when both extracts were prepared by first grinding the plant material, higher pigment yield was obtained with DMF than with acetone; however, had the extraction procedure been repeated, the difference in pigment concentration between DMF and acetone possibly could have been minimized. No differences in pigment concentration were noted in DMF extracts prepared by grinding compared to those prepared by direct immersion (Table I).

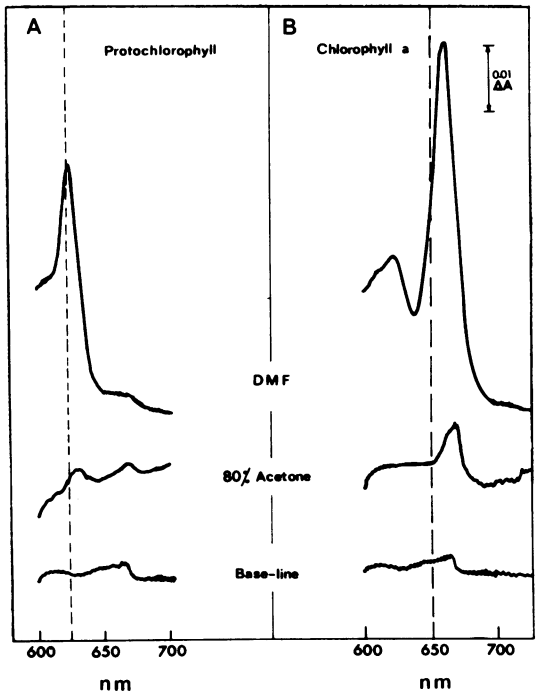

FIG. 1. Absorption spectra of extracts prepared by direct immersion of intact cotyledons into solvent. A: dark-grown; B: illuminated for 10 min just before fixation.

[1] Abbreviation: DMF: *N,N*-dimethylformamide.

Table I. *Absorption in Extracts Prepared by (A) Grinding and Centrifugating, and (B) Direct Immersion of Intact Cotyledons*

	Absorbance g^{-1} ml^{-1}	
	Pchl λ 626 nm	Chl λ 664 nm
A. DMF	0.22	0.54
Acetone 80%	0.20	0.35
B. DMF	0.23	0.53
Acetone 80%	0.03	0.07

Table II. *Absorbance of DMF Extracts Stored in the Dark at 4 C*

	Days after Fixation			
	1	13	21	38
			%	
Pchl	100	75.4 ± 0.6	68.9 ± 0.7	64.4 ± 7.9
Chl	100	100.7 ± 2.5	98.1 ± 2.6	92.7 ± 3.2

Table III. *Absorption Maxima of Pure Chl a in DMF and in Acetone*

	DMF	Acetone 100%	Acetone 80%
		nm	
Red region	664.6 ± 0.1	662.8 ± 0.2	664.5 ± 0.3
Blue region	433.0 ± 0.2	431.5 ± 0.3	432.4 ± 0.3

Both Pchl and Chl extracts in DMF remained quite stable during storage (Table II).

The position of the maxima of Pchl at the red band in the DMF extracts was located at 626.0 nm.

The position of the maxima of pure Chl *a* in DMF was compared to those of Chl *a* in acetone (Table III). The location of the maxima shifted toward longer wavelengths as water percentage was raised. An increase from 0 to 20% displaced the maxima in both solvents by 1.6 nm.

Absorptions at the maxima in the red region of the spectrum of equal concentrations of pure Chl *a* in DMF and in 80% acetone were recorded and the corresponding values obtained were 0.772 ± 0.013 and 0.755 ± 0.024. By comparing with the specific absorption coefficient determined by MacKinney (4), the value of the specific absorption coefficient of Chl *a* in DMF at 664.5 nm was calculated to be 83.89 g^{-1} l^{-1}.

DISCUSSION

The positions of the red bands of Pchl and Chl in DMF and in 80% acetone are quite similar (Fig. 1) which can probably be related to the similarity in the general chemical structure of the two solvents (6). The absorption spectra of pure Chl *a* in DMF and in acetone show relatively slight differences in the location of the maxima (Table III) and in intensity of absorption at the maxima of the red band. Dilution of both extracts with water shifted the maxima toward the longer wavelengths. However, DMF is capable of extracting pigments from intact tissues, whereas 80% acetone is not (Table I). This fact greatly simplifies and expedites the extraction procedure with DMF. The loss of plant pigment which inevitably occurs during grinding and centrifuging is avoided, which is especially important when photosynthetic pigments must be extracted from tissue in which pigment concentration is low. In addition, with DMF the time lapse between extraction and spectrophotometric examination appears not to be too critical (Table II).

Acknowledgment—The authors would like to thank Lila Cohen for the help she has so willingly given.

LITERATURE CITED

1. ARNON DI 1949 Copper enzymes in isolated chloroplasts. Polyphenol-oxidase in *Beta vulgaris*. Plant Physiol 24: 1–15
2. BACK A, A RICHMOND 1969 An interaction between the effect of kinetin and gibberellin in retardation leaf senescence. Physiol Plant 22: 1207–1216
3. HOLDEN M 1976 Chlorophylls. *In* TW Goodwin, ed. Chemistry and Biochemistry of Plant Pigments, Vol II Chap 1. Academic Press, New York, pp 1–37
4. MACKINNEY G 1941 Absorption of light by chlorophyll solutions. J Biol Chem 40: 315–322
5. SCHOPFER P, HW SIEGELMAN 1968 Purification of protochlorophyllide holochrome. Plant Physiol 43: 990–996
6. SEELY GR, RG JENSEN 1965 Effect of solvent on the spectrum of chlorophyll. Spectrochim Acta 21: 1835–1845
7. SUNDQUIST C 1974. The pool size of protochlorophyllide during different stages of greening of dark grown wheat leaves. Physiol Plant 30: 143–147
8. VOLLA SL, NI BISHOP 1968 Photosynthetic efficiency of a phycocyanine-less mutant of *Cyanidium*. Photochem Photobiol 8: 213–221