Phytochemistry, 1978, Vol 17, pp. 73–77 Pergamon Press Printed in England

THE EFFECT OF HAEM ON CHLOROPHYLL SYNTHESIS IN BARLEY LEAVES

GEORGE A. F. HENDRY* and ALLAN K. STOBART†

Plant Metabolism Laboratory, Department of Botany, The University, Bristol BS8 1UG, England

(*Revised received* 10 *June* 1977)

Key Word Index—*Hordeum vulgare*; Gramineae; barley; haem; chlorophyll; protochlorophyllide; glycine-[^{14}C]; glutamate-[^{14}C]; glycollate-[^{14}C]; δ-aminolaevulinic acid; haem turnover.

Abstract—Exogenously supplied bovine haemin, fed to etiolated barley leaves, inhibited chlorophyll synthesis in leaves exposed to light. Haemin inhibited the regeneration of protochlorophyllide (P650) and the conversion of exogenously supplied δ-aminolaevulinate (ALA) to protochlorophyll (P630). The effect of haemin on chlorophyll production was overcome by incubating the leaves in water in the dark before light treatment, suggesting the operation of a rapid haem destruction mechanism in leaves. Protohaem turnover in dark-grown leaves was between 8 and 9 hr, based on the rate of degradation of exogenous haemin and the rate of protohaem breakdown in laevulinic acid (LA) treated leaves. The rate constant for haem destruction was 85 pmol/nmol/hr in the dark and 45 pmol/nmol/hr after 4 hr light. There was no evidence that light affects the synthesis of protohaem. It appears that the regulation of endogenous levels of protohaem is by breakdown and it is this mechanism which is under light control. Haem considerably decreased the incorporation of radioactivity from glycollate-[^{14}C], glycine-[^{14}C] and glutamate-[^{14}C] into accumulated ALA in the presence of LA.

INTRODUCTION

The existence of 'free' haem (protohaem) pools in higher plants was recently described and functions sought for such pools in porphyrin synthesis. Duggan and Gassman [1] report that αα-dipyridyl induced an increase in δ-aminolaevulinate (ALA) formation and in P650 levels, together with a decrease in protohaem, in etiolated leaves. They suggest that the turnover of haem, in the absence of haem synthesis (i.e. in the presence of iron chelators), could account for the stimulation of ALA synthesis in a similar manner to that of dipyridyl-stimulated ALA-synthetase activity in avian hepatocytes. It has been suggested [2, 3] that while chlorophyll is being synthesised in greening barley, protohaem is turned over at such a rate that no net synthesis of haem or haem protein(s) occurs. It is also suggested that, in the dark, the pool of protohaem may function to suppress ALA-synthesis but on illumination haem breakdown may be stimulated and ALA synthesis for chlorophyll production permitted. In etiolated bean leaves protohaem levels are relatively constant [4], but in the presence of iron chelators there is a decrease in haem content. On illumination protohaem levels increase [4] in contrast to the absence of any change reported for illuminated barley seedlings [2]. Hendry and Stobart [5], however, have found changes in protohaem levels in etiolated barley seedlings of varying age exposed to light. In fact protohaem levels change within 50 min of light exposure.

If barley leaves have a similar enzyme system for ALA formation as animals, then, in the dark, ALA synthesis may be suppressed by protohaem as suggested by Castelfranco and Jones [2].

This report deals with the effect of exogenous haem on porphyrin synthesis and on the turnover of protohaem in barley leaves.

RESULTS

Haemin uptake

Little information was available on the uptake of haem by higher plant tissues. Since the MW of crystalline bovine haemin is large it seemed probable it would not enter plant cells. Several experiments were therefore set up to investigate its uptake by barley leaves. Seven-day-old dark-grown barley primary leaves were cut 7 cm below the leaf tip and fed haemin (1 mM) for 16 hr in the dark. After discarding the basal 1 cm the remaining 6 cm were cut into 3 equal lengths. Total haem (haemin + protohaem) was estimated in ammoniacal acetone extracts. The results (Table 1) show that the total haem

Table 1. The distribution of haem in barley leaves after haemin feeding

Leaf section	Haemin distribution nmol/50 segments	%
Top third	10.44	9.5
Middle	46.49	42.5
Bottom third	52.64	48.0
Total	109.57	

Seven-day-old dark-grown primary leaves were cut 7 cm below the tips and fed haemin (1 mM, in 50 mM K–Pi buffer, pH 7.6) for 16 hr in the dark. The leaves were rinsed in tap water, the basal 1 cm discarded, and the remaining tissue cut into three equal lengths. Haem was determined in each section.

*Present address; Biochemistry Department, The Medical School, University of Bristol.

†Please send reprint requests to AKS.

Table 2. The effect of haemin on chlorophyll synthesis in barley leaves

Haemin concentration (mM)	Total chlorophyll nmol/g fr. wt	%	Leaf fr. wt mg	%
10	81.63	63.4	27.9	97.9
1	91.68	71.6	29.1	102.1
0.1	113.54	89.1	28.5	100.0
0.01	123.98	96.9	29.6	103.9
0	128.0	100	28.5	100

Ten-day-old dark-grown barley leaves were cut 7 cm below the tip and fed haemin for 16 hr in the dark. After rinsing in tap water, the basal 1 cm was discarded, and the remaining leaf illuminated for 8 hr and chlorophyll determined

present in the 6 cm sections from 50 tops was 110 nmol of which 9.5% was present in the top 2 cm and 42.5% in the middle section. The total haemin taken up was about 5% of the total supplied.

The effect of haemin on chlorophyll production

Ten-day-old dark-grown leaves were cut 7 cm below the leaf tip and fed haemin in the dark for 16 hr. After discarding the bottom 1 cm the leaves were placed in distilled water and left in the dark for 1 hr before given 8 hr light. The effect of haem on chlorophyll synthesis is given in Table 2. Haem at 10 μM to 10 mM had no appreciable effect on leaf fr. wt. Chlorophyll, however, was inhibited *ca* 36% at 10 mM. Absorbance spectra (400–700 nm) of 80% Me_2CO extracts of haemin-fed and control leaves were similar.

The effect of haemin on protochlorophyllide regeneration

Seven-cm tops from 8.5-day-old dark-grown leaves were fed haemin (1 mM) for 16 hr in the dark. After washing, the leaves were packed in a 3 mm light path cuvette and the *in-vivo* absorbance spectrum measured before and after a 10 sec light treatment. The regeneration of protochlorophyllide (P650) was measured against time. Leaves fed haemin showed no appreciable P650 regeneration in 220 min against a regeneration time of 70–95 min in water controls.

To determine whether haem inhibited protochlorophyll synthesis at a point before or after ALA formation, leaves, after haemin treatment in the dark, were fed exogenous ALA and the production of protochlorophyll (P630) recorded. The results are given in Table 3. Haemin diminished P630 production from ALA by as much as 47% at 10 mM. The decrease in P630 correlates well with the concentration of haem in the tissue.

Table 3. The effect of haemin on protochlorophyll (P360) formation from exogenous ALA in etiolated leaves

Treatment ALA (mM)	Haemin (mM)	Protochlorophyll (P630) nmol/g fr. wt	%	Proto-haem (+ haemin) (nmol/g fr wt)
10	10	38.3	53.3	63.5
10	1	47.6	65.9	32.1
10	0.1	55.5	76.8	8.0
10	0	72.2	100	4.5
0	0	7.8		4.9

Eight-day-old dark-grown leaves were cut 7 cm below the tip and fed haemin for 2 hr in the dark before feeding ALA together with haemin for a further 14 hr in the dark. The leaves were rinsed in tap water, the basal 1 cm discarded, and protochlorophyll (P630) and haem determined in the remaining portion of the leaf.

Table 4. The effect of prolonged incubation on haemin inhibition of chlorophyll synthesis in barley leaves

Dark incubation (hr)	Light (hr)	Chlorophyll (nmol/g fr. wt) Control	Haemin-treated	% Control
0	3	16.70	9.02	54.0
0	5	28.48	20.11	70.6
0	7	62.75	48.17	76.8
24	3	4.88	5.59	114.5
24	5	9.78	13.59	139.0
24	7	17.09	21.25	124.3
48	3	4.01	—	—
48	5	8.28	11.10	134.1
48	7	7.64	12.06	157.8
48	14	102.83	130.62	127.0

Ten-day-old dark-grown barley leaves were cut 8 cm below the tips and fed haemin (10 mM, pH 7.5) or water, pH 7.5, for 16 hr in the dark. The leaves were rinsed in tap water, the basal 1 cm discarded, and the remaining portion incubated in distilled water for various times in the dark. Leaves were then illuminated for up to 48 hr and chlorophyll determined

Effect of dark-incubation on haemin inhibition of chlorophyll synthesis

Since haem turnover may be rapid in barley leaves [2] the effect of incubating the leaves in distilled water after haemin treatment was investigated. Ten-day-old dark-grown leaves were cut 8 cm below the tips and fed 10 mM haemin for 16 hr in the dark. After washing, the leaves were incubated in distilled water for various times before illumination. Chlorophyll was determined at regular intervals. The results (Table 4) show that when haemin-fed leaves were illuminated without dark incubation, chlorophyll synthesis was inhibited by 46, 39 and 23% after 3, 5 and 7 hr light respectively. However, when haemin-fed leaves were incubated for a further 24 hr in the dark and then illuminated there was no inhibition of chlorophyll formation. Chlorophyll synthesis was in fact enhanced in haem treated tissue given long incubation times in distilled water before illumination.

By decreasing the dark-incubation period to a few hours it was still possible to demonstrate a promotion of chlorophyll synthesis in haemin-fed leaves (Table 5).

Table 5. The effect of brief dark-incubation on haemin inhibition of chlorophyll synthesis

Dark incubation (hr)	Light (hr)	Chlorophyll (nmol/g fr. wt) Control	Haemin-treated	% Control
0	3	104.56	91.61	87.6
2	3	102.58	103.75	101.1
2.5	3	99.14	115.01	116.0
9	3	95.52	112.67	148.0

Seven-day-old dark-grown barley leaves were cut 6 cm below the tip and fed haemin (10 mM, pH 7.5) for 16 hr in the dark. After rinsing in tap water, the basal 1 cm was removed, and the remaining portion incubated in distilled water in the dark for varying periods before illumination for 3 hr. The leaves were then assayed for chlorophyll.

Table 6. The effect of haem on the incorporation of radioactive glutamate, glycine and glycollate into ALA in the presence of laevulinic acid

Haemin conc (mM)	Isotope uptake (pmol)	Specific radio activity ALA (dpm × 10^3/pmol)	% Control
L-glutamate-[U-^{14}C]			
10	205.1	1034	34.6
0	216.6	2987	100
glycine-[U-^{14}C]			
10	446.7	772	36.4
0	562.8	2123	100
glycollate-[1-^{14}C]			
10	5889.4	31	17.7
0	4371.3	175	100

Seven-day-old dark-grown leaves were cut 5 cm below the tip and fed haemin (10 mM, pH 7.5) or buffer only, for 16 hr in the dark. After rinsing in tap water the apical 1 cm and basal 2 cm were discarded. The remaining tissue was fed 2.5 μCi L-glutamate-[U-^{14}C] (275 mCi/mMol), glycine-[U-^{14}C] (114 mCi/mMol) or sodium glycollate-[1-^{14}C] (7.2 mCi/mMol) with laevulinic acid (10 mM, pH 7.5). After illumination at 26° for 3.75 hr, ALA was extracted and purified by high voltage electrophoresis before counting.

The effect of haem on the incorporation of radioactive glutamate, glycine and glycollate into ALA

Five-cm long tops from 7-day-old dark-grown barley leaves were incubated in 10 mM haemin for 16 hr in the dark, rinsed, and the apical 1 cm and basal 2 cm discarded. The remaining tissue was fed 2.5 μCi of L-glutamate-[U-^{14}C], glycine-[U-^{14}C] or glycollate-[1-^{14}C] with LA (10 mM). The leaves were illuminated in stoppered flasks at 26° for 4 hr after-which the leaves were extracted and ALA isolated by high-voltage electrophoresis.

Little difference was observed between uptake of isotope in all treated tissue and water controls (Table 6). δ-Aminolaevulinic acid labelling from all three isotopes was considerably decreased in all haem treated tissue (Table 6).

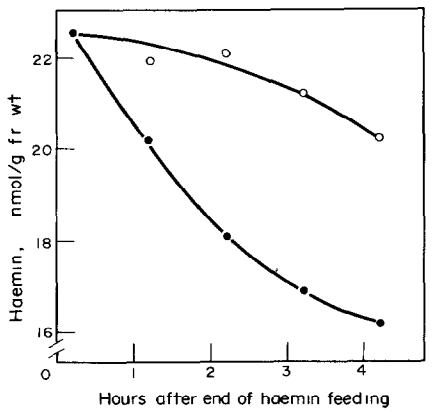

Fig. 1. The effect of light on haemin degradation in etiolated barley leaves. 7-day-old etiolated leaves were trimmed to 7 cm and the cut ends placed in 1 mM haemin for 16 hr. After transferring to buffer half of the samples were transferred to the light. At intervals dark and light treated leaves were assayed for haemin. The results are the mean of four determinations. O——O, light treatment; ●——●, dark controls.

Protohaem turnover

In the previous experiments it was shown that haemin inhibition of chlorophyll synthesis was overcome by a dark-incubation period in distilled water before light treatment. This suggests that the haemin in the tissue was rapidly degraded. By feeding haemin to leaves and measuring the rate of breakdown it is therefore possible to get an estimate of protohaem turnover.

One-thousand 7-day-old dark-grown primary leaves were trimmed to 7 cm, and placed in thimble flasks containing 1 mM haemin for 16 hr, and half the samples placed in the light or kept in the dark. At that time and at succeeding hourly intervals samples were removed and assayed for haemin. The buffer remaining in the flasks was also analysed to assess the amount of haemin leached from the cut leaves. Starting with identical haemin concentrations of 22.4 nmol/g fr. wt after 16 hr feeding, the amounts fell to 20.05 nmol after 4 hr in the light, a decrease of 10.5 % (Fig. 1). In leaves retained in the dark, the haemin declined to 16.25 nmol/g fr. wt after 4 hr a decrease of 27.5 %.

From the results of this experiment it is possible to calculate rates of haemin destruction in dark-grown and light-treated barley leaves. In a steady state the estimation of the haemin destruction rate would allow the calculation of the protohaem synthesis rate. Since 8-day-old dark-grown leaves were used when the decline in endogenous protohaem is minimal [5] the assumption was made that a steady state exists and therefore:

$$\frac{dP}{dt} = -AP + B = 0 \qquad \text{(equation 1)}$$

where P is the protohaem concentration (nmol), A is the rate of destruction and B is the rate of synthesis.

From the data in Fig. 1, A was calculated using equation 1. A (dark rate) was found to be 0.085 nmol/nmol/hr and A (light rate) 0.045 nmol/nmol/hr; that is the destruction rate constant is 0.085 in the dark and 0.045 in the light. It was asumed that no endogenous synthesis of protohaem took place during the experiment since haemin inhibits ferrochelatase activity [6]. Even if protohaem synthesis did occur the amount formed would be an order of magnitude less than the haemin taken up by the leaves (22.4 nmol/g fr. wt) and would not materially affect the calculations.

The Haemin half-life in dark-grown leaves

Accepting the assumption that only insignificant quantities of protohaem were synthesised during the experiment then:

$$\frac{dP}{dt} = -AP \qquad \text{(equation 2)}$$

and

$$\frac{dP}{dt} = -A\,dt \qquad \text{(equation 3)}$$

so intergrating:

$$\int_{P_0}^{P_{0.5}} \frac{dP}{P} = \int_{t_0}^{t_{0.5}} -A\,dt \qquad \text{(equation 4)}$$

taking logs:

$$\ln P_{0.5} - \ln P_0 + -A(t_{0.5} - 0) \qquad \text{(equation 5)}$$

however $A = 0.085$ nmol/hr,

$$\therefore \ln \frac{P_0}{P_{0\,5}} = At_{0\,5} = \frac{t_{0\,5}}{\frac{1}{0.085}} = \frac{t_{0\,5}}{11.765}$$

$$\therefore t_{0\,5} = \underline{8.15 \text{ hours}}$$

that is the half life of protohaem in the dark is 8.15 hr.

The effect of laevulinic acid on protohaem levels

Laevulinic acid (LA), a competitive inhibitor of ALA-dehydratase [7], causes the accumulation of ALA in leaves [8, 9] and inhibits the production of chlorophyll [10]. For the purpose of turnover rate calculations the effect of LA on the amount of endogenous protohaem was examined. Seedlings were grown in the dark for 7 days and half the material given a light treatment. At this time, 24, 48 and 72 hr later both light and dark-grown 6 cm leaf tops were incubated in 0.1 M LA, pH 7.5, for 7 hr and protohaem measured.

The effect of LA was to diminish the protohaem content of both dark and light-treated leaves (Table 7). The decrease in absolute terms (pmol) and in relative terms (% of control) was greatest in the illuminated leaves. In 7-day-old tops the LA-induced decrease was 30 pmol/leaf (11.2%) in the dark and 104 pmol/leaf (34.0%) in the light. After 24 hr light, 7 hr incubation in LA diminished protohaem by 181 pmol/leaf (39.2%) compared to only 46 pmol (20.6%) in 8-day-old dark-grown leaves. The decreases in both the light and dark were lower the older the leaf.

It is possible to test the accuracy of the protohaem half life caculation based on haemin destruction by considering the data (Table 7) obtained for LA inhibition of protohaem levels. However it is necessary to make two assumptions. Firstly, that LA inhibition of ALA-dehydratase diminishes equally the rates of synthesis of chlorophyll and protohaem. This assumption is not unreasonable if the two porphyrins are derived from a common pool of ALA. Secondly that a point can be established in the linear phase of chlorophyll synthesis where chlorophyll destruction is nil. Such a point would be approached shortly after the end of the period of photodestruction of newly formed chlorophyll (say after 2 hr illumination [5]) and before the onset of the plateau phase in chlorophyll concentration. Such a period would occur between 3 and 24 hr after the start of illumination.

Table 7. The effect of laevulinic acid (LA) on protohaem levels in leaves of barley

Leaf age (days)	Light (hr)	Protohaem (pmol/leaf) Cut controls	LA treated	Difference	LA inhibition (%)
7		269	239	−30	11.2
8		225	179	−46	20.6
9		179	170	−9	5.1
10		208	198	−10	4.8
7	7	306	202	−104	34.0
8	31	462	281	−181	39.2
9	55	595	496	−99	16.6
10	77	589	510	−79	13.4

Seedlings were grown in the dark for 7 days and half transferred to the light. At this time and 24, 48 and 72 hr later, the primary leaves of both light and dark-grown plants were cut 6 cm below the tip. LA (0.1 M, pH 7.5) was fed to the 'tops' for 7 hr in the light or dark. The tops were then ground in liquid N_2 and protohaem determined.

In order to test the calculated half-life of 8.15 hr for protohaem, the chlorophyll produced in the succeeding 7 hr after 12 hr pre-illumination was determined in cut leaves (5.22 nmol/leaf in 7-day-old dark-grown material). In similar samples treated with 0.1 M LA the chlorophyll content was 2.59 nmol, a reduction of 50.4%. As LA halved chlorophyll synthesis it was assumed that protohaem formation would also be inhibited by a similar amount. It was further assumed that the rate of protohaem synthesis was similar in light and dark-treated leaves.

Protohaem concentrations in 7.5-day-old material (Table 7) would be 250 pmol/leaf as derived by interpolation. In LA treated leaves after 7 hr incubation, the protohaem had declined to 210 pmol/leaf, again derived by interpolation. If 8.15 hr is the half-life of protohaem in the dark then 7 hr (the length of LA feeding) would cover 0.42 of a life and with a pool size of 250 pmol about 105 pmol would have been turned over.

Where $P_{t_0} - A + B = P_t$, (equation 6)

then $B_{LA} = 65$

That is where $t_{\frac{1}{2}} = 8.15$ hr, the calculated value for B is 65 pmol. B_{LA}, as calculated by a 50% inhibition (based on chlorophyll inhibition) of B control is 52.5 pmol (50% of 105). If the above assumptions are valid the half-life of protohaem would be nearer 9 hr than 8.15 hr. It may be, however, that LA directly or indirectly modifies protohaem turnover. In spite of this, the similarity between the two half-life computations of 8.15 and 9 hr indicates that protohaem turnover is relatively fast and that the assumption that rates of protohaem synthesis in the dark and light are similar, is correct.

The results described in Fig. 1 show that when leaves are illuminated the protohaem destruction rate is diminished. Henrikson and Gassman [4] found that protohaem concentrations rose significantly in etiolated bean leaves in the dark 24–48 hr after a 10 min light pulse. They attributed this increase to phytochrome. To help clarify this point 7-day-old dark-grown barley leaves were cut 7 cm below the tip and placed in 1 mM haemin, pH 7.6. Half of the samples were immediately exposed to a 60 sec light flash (10000 lx). After 16 hr incubation in haemin in the dark the flashed and dark-treated leaves were washed and placed in K–Pi buffer (50 mM, pH 7.6) for a further 6 hr in the dark. The haemin levels were measured in the buffer and in the leaves. The flashed leaves contained 50.40 nmol haemin/g fr. wt and the dark controls only 37.66 nmol. The difference was not due to leakage of haemin as the buffers in both treatments contained less than 1 nmol haemin/3 ml. The effect of a brief illumination was to suppress haemin destruction and implies that protohaem levels in barley are probably regulated by a phytochrome mechanism.

DISCUSSION

Exogenously supplied haemin affects magnesium–porphyrin metabolism in barley leaves in the dark and the light. In the dark, haemin inhibits protochlorophyll (P630) formation from exogenous ALA and inhibits

protochlorophyllide (P652) regeneration from endogenous ALA. In the light, haemin inhibits chlorophyll synthesis. After a period of dark-incubation in water for 2 hr or more, haemin no longer inhibits chlorophyll synthesis and in fact promotes it. This suggests that protohaem may have a regulatory function in chlorophyll synthesis in higher plants similar to that found in photosynthetic bacteria [11]. In a previous study [5] it was shown that light had two effects on haem metabolism in barley; it induced an increase in protohaem within 60 min of illumination and severely repressed the rate of degradation. Certainly here it would appear that the dark-incubation of leaves fed haemin, in distilled water caused a sufficient decrease in the internal haem concentrations to give amounts of chlorophyll similar to control values. In fact after haem feeding followed by an incubation period an enhanced amount of chlorophyll was often observed.

Protohaem turnover in the dark in barley leaves was relatively fast with a half-life of between 8 and 9 hr. The existence of an active degrading mechanism which controls the protohaem level was demonstrated in dark-grown leaves and confirms the suggestions of Castelfranco and Jones [2]. The rate constant for protohaem destruction in 8-day-old etiolated leaves was 85 pmol/nmol/hr. Light appears to be the principal regulator of protohaem turnover. In 8-day-old leaves illuminated for 4 hr the rate of destruction declined to 45 pmol/nmol/hr. There was no evidence that the protohaem synthesis rate changed on illumination. The enzyme catalysing the formation of protohaem from protoporphyrin IX, ferrochelatase, exhibits no increase in activity during the first 7 hr illumination in barley (Jones, O. T. G., personal communication). Ferrochelatase itself is inhibited by protohaem [6] and it may be that with sustained illumination and increasing haem concentration the rate of synthesis actually declines. It appears that the regulation of the size of the protohaem pool is largely through its breakdown. By regulating the breakdown of protohaem, plants could maintain relatively large pools of protohaem on illumination, without the need to divert additional amounts of protoporphyrin IX from the chlorophyll biosynthetic pathway. The maintenance of an elevated protohaem pool size would ensure adequate haem for haemoprotein synthesis although haemoprotein levels do not appear to increase in the first few hours of illumination [2].

Evidence for an inhibition by haem in dark-grown leaves of the conversion of ALA to protochlorophyll (P630) indicates that a step(s) beyond ALA is under regulatory control by haem. One obvious location for haem inhibition of the porphyrin pathway would be at the branch point of the iron-magnesium chelation of protoporphyrin IX. Haem inhibits ferrochelatase activity and may cause an accumulation of protoporphyrin IX and other intermediates. If a similar process occurs in plants then an accumulation of any of these precursors may result in feedback inhibition of either ALA synthesis or PBG formation. ALA dehydratase is inhibited by PBG and Gough [12] has produced evidence that Urogen and protoporphyrin IX inhibits ALA synthesis in barley mutants.

Certainly from the incorporation of labelled glycine, glutamate and glycollate into ALA in the presence of LA it appears that haem can regulate the production of the precursor for porphyrin synthesis (i.e. ALA) in higher plant systems.

EXPERIMENTAL

Plant material. Barley seeds (*Hordeum vulgare*, var. Proctor) were obtained from K. Wilson Ltd., Wellingborough, Northants, and stored at room temp. in bins. Seeds were soaked for 16 hr in H_2O, planted in trays of vermiculite, and germinated at 25° in the dark. Seedling illumination was from banks of Atlas flourescent super-white 65/80 W lamps with an illuminance, at seedling level, of 3500–4000 lx.

Porphyrin extraction and estimation. Chlorophylls and haem were extracted and estimated as previously described [5].

In-vivo *absorption spectra.* Methods for the *in-vivo* measurement of P652 regeneration and P630 synthesis from exogenous ALA have previously been described [13].

Haemin. Haemin, as crystalline bovine haemin (Sigma, U.K.), was dissolved initially in dil. NaOH and adjusted to pH 7.55 with minimal quantities of dil. HCl. Fresh haemin solns were used in all experiments.

Radiochemicals. L-Glutamate-[U-^{14}C] (275 mCi/mMol), glycine-[U-^{14}C] (114 mCi/mMol) and glycollate-[1-^{14}C] (7.2 mCi/mmol) were purchased from the Radiochemical Centre, Amersham.

Aminolaevulinic acid. After extraction, ALA was purified by high voltage paper electrophoresis. The electrolyte used to achieve full separation of ALA was HCO_2H–HOAc–H_2O (1:4:50), pH 1.95, at 85V/cm for 28 min (Mgly, 1.15). Paper chromatography in EtOH–HOAc–Py–H_2O (95:10.3:3) [14] showed that ALA purified by electrophoresis did not contain aminoacetone. ALA was measured spectrophotometrically [15].

Half life and rate constant calculations. These are based on definitions and equations presented by Reiner [16] and Atkins [17].

Acknowledgements—GAFH was in receipt of an SRC studentship award during the course of this work. AKS is grateful to the Royal Society, London, for financial support in the form of an equipment grant.

REFERENCES

1. Duggan, J. and Gassman, M. L. (1974) *Plant Physiol.* **53** 206.
2. Castelfranco, P. A. and Jones, O. T. G. (1975) *Plant Physiol.* **55**, 485.
3. Jones, O. T. G. (1976) *Phil. Trans. Proc. Roy. Soc. Lond. B.* **273**, 207.
4. Henrikson, L. and Gassman, M. L. (1976) *Plant Physiol.* **57**, 45 (report 238).
5. Hendry, G. A. F. and Stobart, A. K. (1977) *Phytochemistry* **16**, 1545.
6. Jones, M. S. and Jones, O. T.. G. (1970) *Biochem. Biophys. Res. Commun.* **41**, 1072.
7. Nandi, D. L. and Shemin, D. (1968) *J. Biol. Chem.* **243**, 1236.
8. Harel, E. and Klein, S. (1972) *Biochem. Biophys. Res. Commun.* **49**, 364
9. Beale, S. I. and Castelfranco, P. A. (1974) *Plant Physiol.* **53**, 291.
10. Hendry, G. A. F. (1976) Ph.D. Thesis, University of Bristol.
11. Lascelles, J. (1968) in *Porphyrins and Related Compounds* (Goodwin, T. W. ed.) p. 49. Academic Press, London
12. Gough, S. (1972) *Biochim. Biophys. Acta* **286**, 36.
13. Stobart, A. K., Shewry, P. R. and Thomas, D. (1972) *Phytochemistry* **11**, 571.
14. Elliot, W. H. (1960) *Biochem. J.* **74**, 90.
15. Urata, G. and Granick, S. (1963) *J. Biol. Chem.* **238**, 811.
16. Reiner, J. M. (1953) *Arch. Biochem. Biophys.* **461**, 53.
17. Atkins, G. L. (1969) in *Multicompartment Models in Biological Systems.* Methuen, London.