Biochem. J. (1968) **107**, 113
Printed in Great Britain

Ferrochelatase of Spinach Chloroplasts

By O. T. G. JONES
Department of Biochemistry, University of Bristol

(*Received* 15 *September* 1967)

Spinach chloroplasts catalyse the incorporation of Fe^{2+} into protoporphyrin, mesoporphyrin and deuteroporphyrin to form the corresponding haems. This ferrochelatase activity was detected by pyridine haemochrome formation with acetone-dried powders of chloroplasts, or from the formation of [^{59}Fe]haems by intact chloroplasts. Decreasing the mitochondrial contamination of the chloroplasts by density-gradient centrifugation did not cause any loss of activity: spinach ferrochelatase appears to be principally a chloroplast enzyme. The characteristics of the enzyme were examined by using [^{59}Fe]haem assay. The activity was pH-dependent: for both mesohaem and protohaem formation there were two pH maxima, a major peak at about pH 7·8 and a smaller peak at about pH 9·2. Lineweaver–Burk plots showed that the K_m for Fe^{2+} incorporation into protoporphyrin was 8 μM and that for Fe^{2+} incorporation into mesoporphyrin was 36 μM. At non-saturating Fe^{2+} concentrations the K_m for protoporphyrin was 0·2 μM and that for mesoporphyrin was 0·4 μM. Ferrochelatase was not solubilized by treatment of chloroplasts with ultrasound but was solubilized by stirring in 1% (w/v) Tween 20 at pH 10·4. Unlike the rat liver mitochondrial enzyme, chloroplast ferrochelatase was not stimulated by treatment with selected organic solvents. The spinach enzyme was inactive in aerobic conditions and it was shown by using an oxygen electrode that under such conditions the addition of Fe^{2+} to buffer solutions caused a rapid uptake of dissolved oxygen, believed to be due to the oxidation of Fe^{2+} to Fe^{3+}; Fe^{3+} is not a substrate for ferrochelatase.

The enzyme ferrochelatase (protohaem ferro-lyase, EC 4.99.1.1), which catalyses the incorporation of Fe^{2+} into porphyrins to form haems, appears to be localized in mitochondria in rat liver (Nishida & Labbe, 1959) and in pig liver (Porra & Jones, 1963*a*,*b*). Porra & Jones (1963*b*) also found ferrochelatase in particulate preparations from the photosynthetic bacterium *Chromatium* strain D and other micro-organisms and concluded that it was concerned in the formation of the haem prosthetic groups of such haemoproteins as the cytochromes, catalases and peroxidases.

Chloroplasts are particularly rich in tetrapyrrole pigments, chlorophylls as well as haem pigments, and, in *Euglena gracilis* at least, chloroplasts appear to contain many of the enzymes involved in the biosynthesis of protoporphyrin (Carell & Kahn, 1964); protoporphyrin is a probable intermediate in the formation not only of chlorophylls but also of haems. It therefore appeared possible that in green plant cells the synthesis of the haem pigments required for photosynthetic electron transport is carried out in the chloroplasts rather than in the mitochondria. This possibility was investigated with chloroplasts prepared from spinach and from maize. In the present paper some of the properties of a spinach chloroplast ferrochelatase are described. Evidence for the existence of such an enzyme has been briefly reported (Jones, 1967*a*).

MATERIALS AND METHODS

Protohaem IX, protoporphyrin IX and deuteroporphyrin IX were prepared as described by Falk (1964). Mesoporphyrin IX was obtained from Koch–Light Laboratories Ltd., Colnbrook, Bucks. Approximately 1 mM solutions of porphyrins were prepared by dissolving the tetrapyrrole in 2 N-NH_3, adjusting to pH 8·0 in the presence of sufficient Tween 80 to give a final concentration of 2% (v/v) of detergent and finally made 40 mM with respect to tris–HCl buffer, pH 8·0. The porphyrin concentrations of the solutions were determined spectroscopically in acid solution (Falk, 1964). $^{59}FeCl_3$ (specific activity about 10 c/g.) was obtained from The Radiochemical Centre, Amersham, Bucks.; 100 μc amounts were dissolved in 10 ml. of water as a stock solution.

Chloroplasts were prepared from commercially grown spinach. Two isolation methods were used. (1) Leaves (120 g.) were homogenized in a blender for 20 sec. in 350 mM-NaCl–100 mM-tris–HCl buffer, pH 7·0 (250 ml.), filtered through muslin and the chloroplasts centrifuged out in the 6 × 250 ml. rotor of the MSE 18 centrifuge, which was taken

to 9000rev./min. and then immediately slowed down. These crude chloroplasts were washed twice with 350mM-NaCl–100mM-tris–HCl buffer, pH7·0. (2) Purified chloroplasts, stripped of their outer membranes, were isolated from leaf homogenates, prepared in iso-osmotic sucrose–phosphate medium, by centrifugation through glycerol gradients, as described by James & Das (1957). These 'stripped' chloroplasts were free of mitochondria when examined by phase-contrast microscopy.

Acetone-dried powders of crude chloroplasts were prepared by homogenizing a chloroplast pellet in a large excess of acetone at −10° followed by filtration and washing of the precipitate with more cold acetone. The powder was finally dried and stored in a vacuum desiccator.

Ferrochelatase activity of chloroplast acetone-dried powders was determined by the pyridine haemochrome assay of Porra & Jones (1963*a*). The powder obtained from 180g. of leaves was evenly suspended in 200mM-tris–HCl buffer, pH8·0 (6·0ml.), and 0·5ml. of the suspension added to the main well of a Thunberg tube containing porphyrin substrates (25μM) in a final volume of 3ml. The tube was evacuated and flushed with N_2 five times, pre-incubated at 27° for 5min., and the reaction started by tipping $FeSO_4$ (0·2ml., 1mM) and GSH (0·2ml., 100mM) from the side arm. After 15min. incubation 1ml. of pyridine, 1ml. of 0·5N-NaOH and 1ml. of water were added and the haem formed was determined from the difference spectrum of the oxidized and reduced pyridine haemochromogens by using the $\Delta\epsilon_{mM}$ value given by Porra & Jones (1963*a*).

Ferrochelatase activity of intact and 'stripped' chloroplasts was determined from the rate of formation of [^{59}Fe]haems. The incubation mixture in 15ml. centrifuge tubes contained chloroplasts (0·5–1·0mg. of chlorophyll), 0·1ml. of 1mM-porphyrin solution, 2·0ml. of 200mM-tris–HCl buffer, pH8·0, 1ml. of 1M-mercaptoethanol and distilled water to 4·0ml. The reaction was started by the addition of 50μl. of $^{59}FeCl_3$ solution (0·5μC) and the tubes were incubated in a water bath at 27°. After 10min. incubation cyclohexanone (4ml.) and 4N-HCl (0·1ml.) were added, and the tubes were shaken, kept on ice for about 30min. and then centrifuged until the upper cyclohexanone layer was clear. A sample of the clear supernatant layer (0·1ml.) was plated on a planchet to determine the amount of cyclohexanone-soluble ^{59}Fe present by using a gas-flow end-window Geiger counter.

Previous workers who have used this assay (Krantz, Gallien-Lartigue & Goldwasser, 1963) have shown by paper chromatography that the lipid-soluble ^{59}Fe was present only in the form of haem. The substitution of cyclohexanone for the butanone extraction used by Krantz *et al.* (1963) was suggested by Dr E. Goldwasser. It was shown by reverse-phase chromatography of the cyclohexanone extract that nearly all the radioactivity was confined to the haem region after chloroplasts had been incubated with protoporphyrin, mesoporphyrin or deuteroporphyrin. Confirmation of these findings was obtained in experiments (Jones, 1967*a*) where [^{59}Fe]protohaem was crystallized and counted after the addition of carrier protohaem at the end of the incubation. Carrier protohaem was added in the form of blood (20ml.) and protohaem crystallized by the method of Nishida & Labbe (1957).

Measurements of oxygen evolution (Hill reaction) by chloroplasts and of concentrations of dissolved oxygen in incubation mixtures stirred with a magnetic stirrer were made with a Clark-type oxygen electrode fitted in a water-jacketed glass chamber.

RESULTS

Ferrochelatase activity of mitochondria and bacterial particles may readily be assayed by the pyridine haemochromogen assay of Porra & Jones (1963*a*), though this method may slightly underestimate the true activity of the enzyme (Porra, Vitols, Labbe & Newton, 1967). The very strong absorption of chloroplast pigments in the region used for assay (520–560mμ) interfered with the spectroscopic determination and it was found necessary to remove most of the chlorophylls and carotenoids from chloroplasts by preparing acetone-dried powders before this type of assay could be attempted. The concentration of haem pigments of the chloroplast acetone-dried powder alone was sufficiently high to interfere seriously with the assay, since their pyridine haemochromes, presumably arising from the prosthetic groups of cytochromes *b* and *f*, absorbed near the maxima of the pyridine haemochromes of protohaem and mesohaem. Only deuterohaem formation could be assayed satisfactorily since it was relatively rapid and also deuterohaem has maxima at slightly lower wavelengths than the endogenous haems. There was evidence, however, of significant haem formation, which was greatest with deuteroporphyrin as substrate.

The activity of the acetone-dried powders was variable; the best preparations gave a rate of deuterohaem synthesis corresponding to about 0·01mμmole/min./mg. of chlorophyll originally present in chloroplasts, but frequently no activity at all was present in the acetone-dried powders. This could be due to the removal of some lipids necessary for maximum activity, as had been found by Mazanowska, Neuberger & Tait (1966) in their experiments on metalloporphyrin formation by chromatophores of *Rhodopseudomonas spheroides*. To avoid these problems, ferrochelatase was assayed by measurement of [^{59}Fe]haem formation (see the Materials and Methods section), where intact chloroplasts could be used at high concentration.

It was found that after incubation with $^{59}FeCl_3$ and extraction with cyclohexanone the radioactivity in the cyclohexanone layer was proportional both to the time of incubation and to the amount of chloroplast material (estimated from chlorophyll content) added (Figs. 1*a* and 1*b*). In early experiments incubations were carried out under nitrogen, but it was later found that in high concentrations of mercaptoethanol haem synthesis took place in air in unshaken tubes. Determinations of dissolved oxygen by the oxygen electrode showed that about 250mM-mercaptoethanol was required to remove

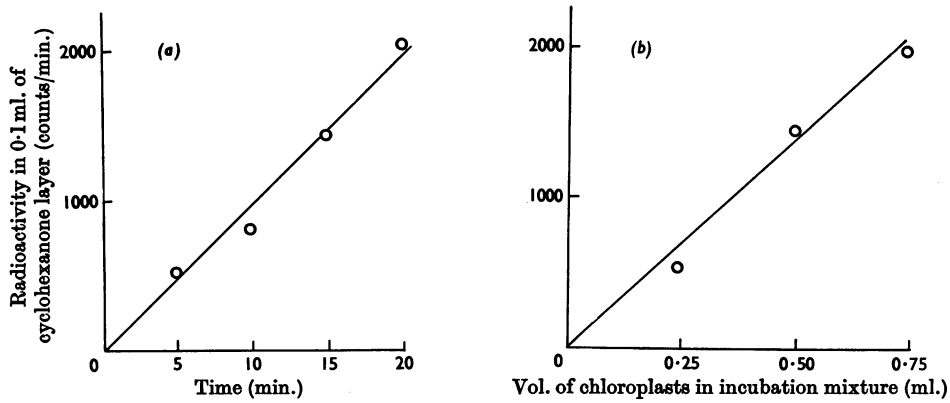

Fig. 1. Effect of varying time of incubation and chloroplast concentration on haem synthesis by spinach chloroplasts. (*a*) Tubes containing 250 μl. of chloroplast suspension (0·26 mg. of chlorophyll), 250 mM-mercaptoethanol, 25 μM-mesoporphyrin, 100 mM-tris–HCl buffer, pH 8·0, and 0·5 μc of $^{59}FeCl_3$ (10 c/g.) were incubated at 27° for the times indicated. Then 4 ml. of cyclohexanone and 0·1 ml. of 4 N-HCl were added to end the reaction, the tubes were centrifuged and the radioactivity in 0·1 ml. of each supernatant cyclohexanone layer was determined. (*b*) The volumes of chloroplasts added to the incubation mixture were varied, as indicated, and the tubes incubated for 10 min. Other procedures were as described for (*a*).

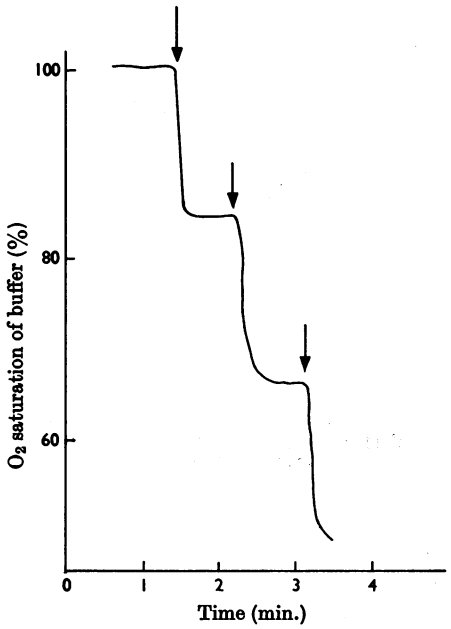

Fig. 2. Effect of additions of Fe^{2+} on the dissolved oxygen content of dilute buffer solution. To 4 ml. of 100 mM-tris–HCl buffer, pH 8·0, contained in a stirred water-jacketed chamber at 27°, 50 μl. quantities of 10 mM-$FeSO_4$ were added. The oxygen content was recorded with an oxygen electrode. The times of addition of $FeSO_4$ are indicated with arrows.

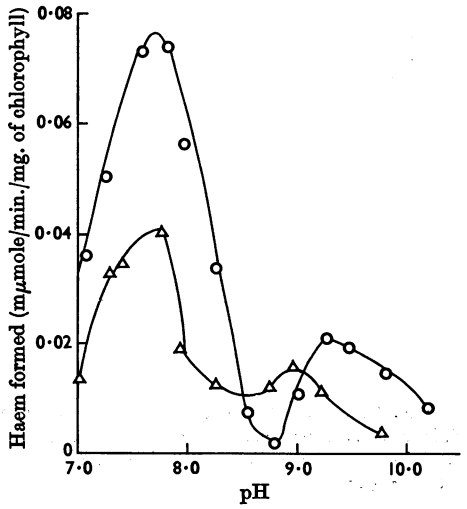

Fig. 3. Effect of pH on formation of protohaem and mesohaem by spinach chloroplasts. The buffer was 100 mM-tris–HCl and conditions for assay were as described in the Materials and Methods section. ○, Mesoporphyrin as substrate; △, protoporphyrin as substrate.

all the dissolved oxygen and that at this concentration ferrochelatase activity was highest. The importance of removing oxygen from the incubation mixture is shown in Fig. 2, where a rapid uptake of oxygen on the addition of Fe^{2+} to the incubation mixture can be observed, presumably resulting from the oxidation of Fe^{2+} to Fe^{3+}.

In Fig. 3 the effect of pH on the formation of protohaem and mesohaem in tris–hydrochloric acid buffer is shown. For both materials twin peaks were obtained with highest activity between pH 7·5 and

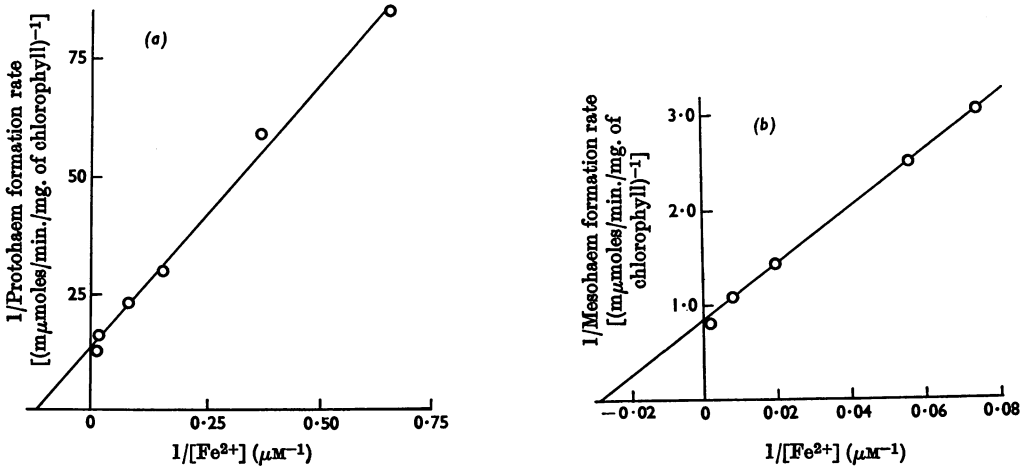

Fig. 4. Lineweaver–Burk plots of the effect of Fe^{2+} concentration on haem synthesis by spinach chloroplasts. (*a*) Protoporphyrin (25 μM) as substrate; (*b*) mesoporphyrin (25 μM) as substrate. Conditions for assay were as described in the Materials and Methods section, except that additions of $FeSO_4$ were made to give the indicated values.

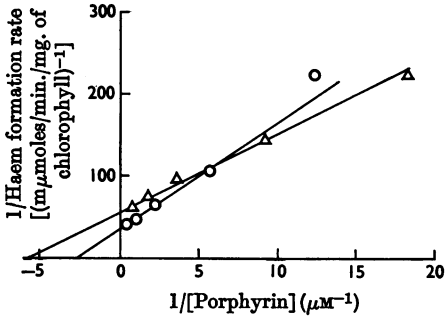

Fig. 5. Lineweaver–Burk plots of the effect of varying porphyrin concentration on haem synthesis by spinach chloroplasts. Conditions for assay were as described in the Materials and Methods section, except that porphyrin concentrations were varied as required. ○, Mesoporphyrin as substrate; △, protoporphyrin as substrate.

8·0 with a second, lower, peak between pH 9·0 and 9·5. In these assays, as in the determination of porphyrin K_m, the concentration of Fe^{2+} was kept low to minimize problems arising from the precipitation of Fe^{2+} at high pH.

Assays in glycine–sodium hydroxide buffer also resulted in a pH–activity curve with a second peak in the pH 9·0 region, as did assays carried out with purified 'stripped' chloroplasts.

When the concentration of Fe^{2+} in the incubation mixture was increased, ferrochelatase activity increased and in Figs. 4(*a*) and 4(*b*) are shown plots of $1/[Fe^{2+}]$ against 1/(rate of haem formation) indicating that the K_m for Fe^{2+} is lower for protohaem formation (8 μM) than for mesohaem formation (36 μM). Similarly it was found that ferrochelatase activity increased with increasing porphyrin concentration. From a plot of 1/[porphyrin] against 1/(rate of haem formation) (Fig. 5) the K_m values for protoporphyrin (0·2 μM) and for mesoporphyrin (0·4 μM) were determined.

Table 1. *Rates of protohaem synthesis by spinach leaf fractions: effect of chloroplast purification*

Spinach leaves were homogenized in 0·4 M-sucrose in 66 mM-potassium phosphate buffer, pH 7·2. Ferrochelatase was assayed in the filtrate obtained by filtering the homogenate through muslin, in a chloroplast fraction and a purified chloroplast fraction, stripped of outer membranes by centrifugation through a glycerol gradient (James & Das, 1957). Rates of formation of [^{59}Fe]protohaem were measured as described in the Materials and Methods section.

Spinach leaf fraction	Rate of protohaem formation (mμmole/min./mg. of chlorophyll)
(1) Crude homogenate, after filtration	0·0006
(2) Chloroplast fraction	0·018
(3) Purified 'stripped' chloroplasts	0·026

It was found that a crude chloroplast preparation, prepared by simply grinding the spinach leaves and filtering off debris, was very low in ferrochelatase activity when compared with purified 'stripped' chloroplasts or the normal washed chloroplasts (Table 1). The increase in activity on purification

Table 2. *Attempted solubilization of ferrochelatase of spinach chloroplasts by ultrasonic treatment and Tween 20 extraction*

For ultrasonic treatment, chloroplast suspensions (1·1 mg. of chlorophyll/ml.) were exposed to four 20 sec. treatments with a 60 w MSE ultrasonic disintegrator, with intervals for cooling. The suspensions were then centrifuged at 100000*g* for 30 min.; the pellet was made up in an equivalent volume of 350 mM-NaCl–100 mM-tris–HCl buffer, pH 7·0, for assay. Conditions for extraction of chloroplasts with 1% (w/v) Tween 20 in 100 mM-tris–HCl buffer, pH 10·4, were as described by Porra *et al.* (1967) for mitochondria. After gentle stirring for 3 hr. the suspension was centrifuged at 100000*g* for 30 min. The pellet was made up in an equivalent amount of 100 mM-tris–HCl buffer, pH 10·4, containing Tween 20 (1%). Rates are compared on a volume basis.

Chloroplast fraction	Protohaem formed (mμmole/min./0·5 ml. of extract)	Deuterohaem formed (mμmole/min./0·5 ml. of extract)
(1) Normal chloroplasts	0·020	0·035
(2) Supernatant from ultrasonically treated suspension	0·0008	0·0008
(3) Pellet from ultrasonically treated suspension	0·017	0·030
(4) Suspension in Tween 20–tris	0·002	
(5) Supernatant from Tween 20–tris suspension	0·0012	
(6) Pellet from Tween 20–tris suspension	0·0008	

may be due to the existence of natural binding factors for Fe^{2+} or other inhibitors present in crude preparations or indeed may be due to the selective use of endogenous iron compounds in preference to the ^{59}Fe supplied in the incubation. When the purified 'stripped' chloroplasts were exposed to a 60 w ultrasonic disintegrator for 80 sec. there was very little release of ferrochelatase activity into the soluble fraction (Table 2). A soluble ferrochelatase, apparently specific for deuterohaem formation, was, however, released during ultrasonic treatment of *Rhodopseudomonas spheroides* chromatophores (Johnson & Jones, 1964; Jones, 1967*b*). It was possible partly to solubilize ferrochelatase activity from chloroplasts by extraction in alkaline Tween 20 (Table 2), conditions that have been successfully used in the solubilization of mitochondrial ferrochelatase (Labbe & Hubbard, 1961; Porra *et al.* 1967). However, the experiments are not clear, partly because of the marked inhibition caused by Tween. No attempt was made to see if activity would be increased by dialysis of the soluble extract. Ferrochelatase was similarly extracted from acetone-dried powders of chloroplasts by 1% Tween 20 at pH 10.

Organic solvents such as ether, ethanol, acetone and ethyl acetate stimulate the formation of metalloporphyrins by chromatophores of *R. spheroides* and by liver mitochondria (Mazanowska *et al.* 1966; Neuberger & Tait, 1964), but at the concentrations used by Mazanowska *et al.* (1966) the solvents used by these workers all proved to be inhibitory to chloroplast ferrochelatase.

The rates of oxygen evolution by the spinach chloroplasts, under the conditions of pH and temperature used, were about 4·5 μg.atoms of oxygen/min./mg. of chlorophyll, at pH 8·1, in the presence of 1·2 mM-ammonium chloride, a rate of electron flow some 50000-fold greater than the rate of H^+ removal from porphyrins by ferrochelatase. There was no evidence that the reducing conditions favouring ferrochelatase could be obtained from reduced intermediates of photosynthesis. Haem synthesis was not stimulated by illumination even in the presence of benzyl viologen or methyl viologen either in the presence or absence of ferricyanide or mercaptoethanol.

DISCUSSION

The use of the ^{59}Fe assay with intact spinach chloroplasts confirms the finding obtained by spectrophotometric assays with acetone-dried powders of chloroplasts that isolated chloroplasts contain the enzyme for the final stage of haem synthesis and has made possible an examination of the characteristics of ferrochelatase. Carell & Kahn (1964) found that purified *Euglena* and spinach chloroplasts contained δ-aminolaevulate dehydratase and that when supplied with δ-aminolaevulate the *Euglena* chloroplast preparation synthesized a range of porphyrins up to and including protoporphyrin. The enzyme δ-aminolaevulate synthetase has not been found in green plants, although Nandi & Waygood (1965) have shown that succinyl-CoA synthetase, which may be involved in the synthesis of the substrate for δ-aminolaevulate synthetase, can be extracted from acetone-dried powders of wheat leaves, where it appears to be localized in chloroplasts. It seems reasonable to assume that haem pigments are synthesized through the same intermediates in plants and in animal cells, but that the distribution of soluble and particulate enzymes may be different. In animal cells δ-aminolaevulate is synthesized in

mitochondria and is then converted into coproporphyrinogen by extramitochondrial enzymes; coproporphyrinogen is then converted into protoporphyrin by another series of mitochondrial enzymes (Sano & Granick, 1961); chloroplasts are apparently self-sufficient in protoporphyrin biosynthesis. The maximum rate of haem synthesis found in spinach chloroplasts (about 1·0mμmole/min./mg. of chlorophyll when the Fe^{2+} concentration was 500μM) corresponds reasonably well with the rates of the δ-aminolaevulate dehydratase reaction reported for purified spinach chloroplasts of about 0·2mμmole of porphobilinogen/min./mg. of chlorophyll (Carell & Kahn, 1964).

Attempts to detect Mg^{2+} incorporation by the chloroplast preparations were unsuccessful, nor did Mg^{2+} compete with Fe^{2+} in ferrochelatase determinations, and it appears unlikely that Fe^{2+} incorporation results from the lack of metal ion specificity of an enzyme concerned in the synthesis of magnesium prophyrins. This view is supported by the general similarity in properties of spinach ferrochelatase and the rat liver mitochondrial ferrochelatase. Both incorporate Fe^{2+} into protoporphyrin, mesoporphyrin and deuteroporphyrin, with protoporphyrin giving the lowest rates. Both mitochondrial and chloroplast ferrochelatase give pH–activity curves with two peaks with protoporphyrin as substrate, though Porra & Jones (1963*a*) found a shoulder rather than a second peak when mesoporphyrin was substrate for the mitochondrial enzyme. This second maximum may be due to the presence of two enzymes of overlapping specificity or to the presence of an ionizing group of a high pK, perhaps the hydroxyl group of tyrosine, near the active site of the enzyme, or, as suggested by Phillips (1967), it may reflect the existence of two different reaction pathways in the same enzyme. The lower pH optimum could refer to a mechanism of metal ion incorporation based on a displacement reaction where porphyrin and metal ion form a complex from which protons subsequently dissociate, and the high pH optimum a dissociation reaction where proton dissociation first occurs from the central nitrogen and the resulting porphyrin anion then reacts with the metal cation.

The K_m for Fe^{2+} incorporation into protoporphyrin is similar to those that have been published for ferrochelatase from other sources. Thus for pig liver mitochondrial enzyme it would appear from the results of Labbe & Hubbard (1961) to be about 12μM or from Yoneyama, Tamai, Yasuda & Yoshikawa (1965) to be 60μM, whereas Neuberger & Tait (1964) found K_m 40μM for the incorporation of Zn^{2+} into protoporphyrin by extracts of *R. spheroides*. The relatively high affinity for Fe^{2+} would lower the concentration of this toxic ion that has to be present within the chloroplast in order for haem synthesis to occur. The K_m values for the porphyrin substrates of spinach ferrochelatase are very considerably lower than the value of 100μM reported for protoporphyrin for pig liver ferrochelatase by Yoneyama *et al.* (1965) and for zinc protoporphyrin chelatase of *R. spheroides* (200μM) found by Neuberger & Tait (1964). It is noteworthy that neither of these groups of workers used porphyrin substrates solubilized with detergents as was done in the present work; Porra & Jones (1963*a*) found that at physiological pH porphyrins are very poorly soluble and readily centrifuged out of solution. It may be that the low K_m values found for spinach ferrochelatase would also be found for the liver and bacterial systems if the substrates were provided in true solution. However, since technical problems arising from iron precipitation prevent the determination of porphyrin K_m satisfactorily at saturating Fe^{+} concentration, the value obtained may only be approximate.

The chloroplast enzyme appeared to differ from liver and *R. spheroides* ferrochelatase in that it was not readily released by ultrasonic treatment and was not stimulated by organic solvents (Table 2). Indications are that it is possible to extract a soluble form in Tween 20 at pH 10, from chloroplasts as well as from mitochondria and chromatophores. The experiments described in this paper also show that, under aerobic conditions in the absence of a reducing material such as mercaptoethanol, no haem synthesis took place. As shown in Fig. 2 one cause of this sensitivity to oxygen may be simply the rapid oxidation of Fe^{2+} to Fe^{3+}, which is not a substrate for the enzyme. Tokunaga & Sano (1966) have shown that non-enzymic protohaem formation could take place in the presence of sodium dithionite. Though the mechanism may be similar to the enzymic process, requiring the formation of an activated form of Fe^{2+}, the enzymic reaction did not have an absolute requirement for dithionite, and was stimulated, not inhibited, by the addition of detergent.

The presence of a ferrochelatase enzyme system in chloroplasts is another example of the biochemical independence of the plastid, of great importance in view of the genetic independence of the chloroplast (cf. Kirk & Tilney-Bassett, 1967).

This project was supported by a Research Grant from the Science Research Council.

REFERENCES

Carell, E. F. & Kahn, J. S. (1964). *Arch. Biochem. Biophys.* **108**, 1.

Falk, J. E. (1964). *Porphyrins and Metalloporphyrins*, p. 174. Amsterdam, London and New York: Elsevier Publishing Co.

James, W. O. & Das, V. S. R. (1957). *New Phytol.* **56**, 325.

Johnson, A. & Jones, O. T. G. (1964). *Biochim. biophys. Acta*, **93**, 171.
Jones, O. T. G. (1967*a*). *Biochem. biophys. Res. Commun.* **28**, 671.
Jones, O. T. G. (1967*b*). *Phytochemistry*, **6**, 1355.
Kirk, J. T. O. & Tilney-Bassett, R. A. E. (1967). *The Plastids*, p. 576. London and San Francisco: W. H. Freeman and Co.
Krantz, S. B., Gallien-Lartigue, O. G. & Goldwasser, E. (1963). *J. biol. Chem.* **238**, 4085.
Labbe, R. F. & Hubbard, N. (1961). *Biochim. biophys. Acta*, **52**, 130.
Mazanowska, A. M., Neuberger, A. & Tait, G. H. (1966). *Biochem. J.* **98**, 117.
Nandi, D. L. & Waygood, E. R. (1965). *Canad. J. Biochem.* **43**, 1605.
Neuberger, A. & Tait, G. H. (1964). *Biochem. J.* **90**, 607.
Nishida, G. & Labbe, R. F. (1957). *Biochim. biophys. Acta*, **26**, 437.
Nishida, G. & Labbe, R. F. (1959). *Biochim. biophys. Acta*, **31**, 519.
Phillips, J. N. (1967). *Enzymologia*, **32**, 13.
Porra, R. J. & Jones, O. T. G. (1963*a*). *Biochem. J.* **87**, 181.
Porra, R. J. & Jones, O. T. G. (1963*b*). *Biochem. J.* **87**, 186.
Porra, R. J., Vitols, K. S., Labbe, R. F. & Newton, N. A. (1967). *Biochem. J.* **104**, 321.
Sano, S. & Granick, S. (1961). *J. biol. Chem.* **236**, 1173.
Tokunaga, R. & Sano, S. (1966). *Biochem. biophys. Res. Commun.* **25**, 489.
Yoneyama, Y., Tamai, A., Yasuda, T. & Yoshikawa, H. (1965). *Biochim. biophys. Acta*, **105**, 100.