Purification and Characterization of Membrane-bound Ferrochelatase from *Rhodopseudomonas sphaeroides* *

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Ferrochelatase (protohaem ferro-lyase EC 4.99.1.1) has been purified to apparent homogeneity from the facultative photosynthetic bacterium *Rhodopseudomonas sphaeroides*. The enzyme has been purified 1,640-fold with 43% recovery from isolated membrane fragments. The enzyme has a molecular weight of approximately 115,000 as estimated by both sodium dodecyl sulfate-polyacrylamide gel electrophoresis and gel filtration chromatography through Sephadex G-150 in the presence of 0.5% sodium deoxycholate. The purification procedure involves solubilization of ferrochelatase with sodium deoxycholate off of salt-washed membranes, followed by ammonium sulfate fraction, ion exchange chromatography on DEAE-Sephacel, followed by chromatography on Amicon dye matrix blue B, and finally Sephadex G-150. The enzyme has an extinction coefficient of 90,000 at 95 µm and an absorption spectrum reveals no chromoporic cofactors. Purified ferrochelatase is inhibited by iodoacetamide, N-ethylmaleimide, Hg, Pb, Cu, and hemin. The apparent $K_m$ values for mesoporphyrin IX, 20 µm; deuteroporphyrin IX, 95 µm; and iron, 20 µm.

The terminal step in the heme biosynthetic pathway is the insertion of ferrous iron into protoporphyrin IX to form protopheme. This unusual activity of transition metal insertion into a porphyrin macrocycle is catalyzed by the enzyme ferrochelatase (protohaem ferro-lyase, EC 4.99.1.1). The enzyme has been detected in a wide variety of tissues and organisms; and, in all cases, the activity has been found associated with the membrane fraction. In eukaryotes, ferrochelatase is bound to the inner face of the inner mitochondrial membrane (1); and, in plants, activity is also found in chloroplasts (2); while, in bacteria, it is bound to the cytoplasmic membrane (3, 4). Ferrochelatase will catalyze the insertion of the divalent cations of cobalt, zinc, and iron into the IX isomer, dicarboxylate porphyrins, but will not insert magnesium or ferric iron, nor will it utilize protein-bound porphyrins or porphyrinogens (2–6).

Ferrochelatase has been solubilized with detergents from membranes of both eukaryotic and prokaryotic organisms, and the kinetic characteristics of these preparations have been examined. The literature contains an assortment of confusing or conflicting reports attributable at least in part to the impurity of the enzyme samples or variations in assay procedures. The enzyme has been reported to be either stimulated (7, 8) or inhibited (9) by copper, to be stimulated to different extents depending on either specific head groups (10) or the degree of unsaturation of the fatty acyl chains (11) of phosphatidylcholine, and to be stimulated by calcium in the absence of unsaturated phosphatidylcholine (11). One area of general agreement is that the enzyme is sensitive to sulfhydryl reagents such as iodoacetamide and N-ethylmaleimide and that the end product, heme, inhibits activity (1, 7, 12).

There have been a few published attempts of the purification of ferrochelatase and only one of these (13) has been successful. Previously, the most highly purified bacterial preparation of ferrochelatase has been obtained from the bacterium *Spirillum itersonii* (7). The 1000-fold purified enzyme had an apparent molecular weight in detergent solution of about 50,000, and it was inhibited by N-ethylmaleimide and iodoacetamide. There was no evidence for a phospholipid requirement, but copper was found to strongly stimulate the purified enzyme (7).

In the present study, we have elected to examine ferrochelatase in the facultative-photosynthetic bacterium *Rhodopseudomonas sphaeroides*. This organism, which has high ferrochelatase activity (14), is of particular interest since it is capable of inserting either iron or magnesium into protoporphyrin to form either heme or bacteriochlorophyll. In switching from nonphotosynthetic aerobic growth to photosynthetic anaerobic growth conditions, there is about a 100-fold increase in protoporphyrin produced, but most of this additional porphyrin goes into bacteriochlorophyll rather than heme (15). Since protoporphyrin is the last common intermediate for biosynthesis of heme and bacteriochlorophyll, ferrochelatase then is placed at the branch point of the two pathways. It was felt that a better understanding of its catalytic functioning may yield some insight into possible regulatory mechanisms at this branch point. Here we report the purification and some properties of ferrochelatase from *R. sphaeroides*.

**MATERIALS AND METHODS**

Cultures of *R. sphaeroides* L (wild type) and L-57 (nonphotosynthetic carotenoidless mutant) were obtained from Dr. J. Lascelles of the University of California at Los Angeles, and strains 2.4.1, 2.4.7 (wild types), and Ga (green carotenoid mutant) were from Dr. J. Takemoto of Utah State University. All cultures were maintained on yeast extract-malate-glutamate deeps as previously described (16) except that for L-57 which was maintained on a slant. For nonpigmenting aerobic growth, cultures were grown in a 2.8-liter low form flask containing 500 ml of malate-glutamate medium (16) shaken at 120 rpm. For photosynthetic growth, cultures in the same medium in Roux bottles that were filled, stoppered, and placed in an aquarium with four 60-watt incandescent lamps as a light source. All cultures were grown at 30 °C. Cells were harvested after 24 h of growth and disrupted by sonication at 60 watts after being washed once in 10 mM Tris-acetate, pH 8.1.

For the purification of ferrochelatase, cultures were grown at 30 °C in 1-liter flasks containing 500 ml of yeast extract-malate-glutamate media at 140 rpm. A 5-ml 16-h culture grown in yeast extract-malate-
glutamate broth was used as the starter culture for each 500 ml. A total of 10 liters of culture was harvested by centrifugation at 8000 × g for 10 min after 24 h of growth. The cells were washed once in 10 mM Tris/acetate, pH 8.1, and then suspended to a final volume of 250 ml in the same buffer. All harvesting operations were carried out at 4 °C. Cells were lysed by sonication with a Heat Systems Cell Disrupter (Bloomington, IN) and the cell suspension was kept in an ice bath for 30 s between each sonication. The material was centrifuged at 8000 × g for 10 min at 4 °C to remove whole cells and large cell fragments.

Assays and Determinations—Protein concentrations were determined by the method of Lowry et al. (17) using bovine serum albumin as a standard. Ferrochelatase was assayed by the modification of the procedure described previously (7). The final reaction volume was 1 ml and consisted of 50 mM Tris/acetate, pH 8.1, 5 mM dithiothreitol, 0.2 mM ferrous ammonium citrate, 0.2% Triton X-100, 0.1 mM porphyrin, and enzyme preparation. For most assays, deuteroporphyrin was used. The reaction was at 37 °C in the dark for 30 min and was terminated by addition and mixing of 0.5 ml of 50 mM iodoacetamide. The product was quantitated as the pyridine hemochromogen using the extinction coefficients of Falk (18). Porphyrin solutions were prepared as described previously (3).

The purified ferrochelatase was subjected to electrophoresis and was visualized by silver staining (20). Chemicals—Hemin, protoporphyrin IX, mesoporphyrin IX, and deuteroheme were obtained from Porphyrin Products, Logan, UT. Matrix Gel Blue B was from Amicon Corp. Sephadex G-25 and G-150, DAE-Septacel, N-ethylmaleimide, iodoacetamide, pyridoxal phosphate, flavin mononucleotide, NAD, NADP, diithiothreitol, sodium deoxycholate, PMSF, and Brij 30 were purchased from Sigma. All other chemicals and reagents were of the highest grade available.

Purification of Ferrochelatase—All operations described below were carried out at 4 °C and, unless otherwise stated, the buffer used in all steps was 10 mM Tris/acetate, pH 8.1, 0.5 mM dithiothreitol, and 10 μg/ml of phenylmethylsulfonfluryl fluoride (Buffer A). The crude cell extract (250 ml) obtained as described above was centrifuged at 100,000 × g for 90 min. The soluble cytoplasmic fraction was poured off, leaving a firmly packed purple-red pellet. This pellet was suspended in Buffer A to a volume of 225 ml with a glass-Teflon Potter-Elvehjem homogenizer. Twenty-five ml of 5.0 M sodium thiocyanate were added to give a final concentration of 0.5 M. The membrane suspension was sonicated for 30 s at a 60-watt setting of a Heat Systems Cell Disrupter before being centrifuged at 100,000 × g for 90 min. The soluble salt wash was removed, leaving a firmly packed pellet. These salt-washed membranes were suspended to 237 ml in Buffer A as described above. The membrane suspension was brought to 0.5% (v/v) sodium deoxycholate, or the addition of 12.5 ml of a 10% (v/v) PMSF solution. This suspension was then centrifuged at 30,000 × g as described above and then centrifuged at 100,000 × g for 90 min. The reddish supernatant was carefully removed, leaving behind a firmly packed pellet. The volume of solubilized enzyme was usually about 220 ml and this material was fractionated with ammonium sulfate by the addition of a saturated solution of ammonium sulfate adjusted to pH 8.0. The solubilized enzyme solution was brought to 20% saturation and stirred for 10 min, and the precipitated material was removed by centrifugation at 10,000 × g for 10 min. The supernatant was removed and brought to 90% saturation and once again stirred for 10 min before centrifugation. The red pellet thus obtained was dissolved in a small volume of Buffer A (about 30 to 40 ml), and enzyme preparation was immediately passed through a Sephadex G-25 column (3.5 × 30 cm) to remove residual ammonium sulfate. This procedure also separated some brownish yellow material of unknown nature that was retarded by the column.

The enzyme preparation was applied to a DEAE-Septacel column (2.5 × 20 cm) that had been equilibrated with 1 liter of 10 mM Tris/acetate, pH 8.1, 0.5 mM dithiothreitol, 10 μg/ml of PMSF, and 0.2% (v/v) of Buffer A. After sample addition, Buffer A was applied to this column and buffer was washed through the column before the addition of 500 ml of Buffer B containing 8.10 M sodium thiocyanate. This wash eluted a major portion of the red pigment, but eluted no ferrochelatase activity. Ferrochelatase was then eluted with 0.15 M sodium thiocyanate. The fractions containing ferrochelatase activity were pooled and brought to 10% (v/v) propylene glycol. This material could be stored at 4 °C for several days with propylene glycol, but freezing resulted in the loss of most of the activity. Usually the material from the DEAE-Septacel column was immediately loaded onto an Amicon Blue B column. This column material binds ferrochelatase tightly, but has a low capacity and binds the enzyme slowly, so it was found that maximal binding was obtained by repeated passage of the enzyme solution through the column. Specifically, the solution was passed through a Blue B column (2.5 × 10 cm). The eluate was collected and passed through the column twice more. This procedure resulted in about 80% retention of ferrochelatase. The column was then washed with 300 ml of Buffer B containing 0.5 M KCl before ferrochelatase was eluted with 1.0 M sodium thiocyanate. The enzyme eluted from the Blue B was immediately concentrated by pressure dialysis with an Amicon YM-30 filter. Because much of the Brij is also concentrated in this procedure, the solution becomes noticeably viscous, but ferrochelatase activity is not affected. The enzyme was then chromatographed on a Sephadex G-150 column (1.5 × 100 cm); that was equilibrated with Buffer A plus 0.5% (w/v) sodium deoxycholate. The fractions containing ferrochelatase activity were pooled, brought to 10% (v/v) propylene glycol, and concentrated with the Amicon YM-30 filter before being stored at 4 °C.

RESULTS

Purification of Ferrochelatase

During the initial phases of our work, we examined several strains and mutants of R. sphaeroides grown under various growth conditions to determine if ferrochelatase levels were variable. Wild type strains L. 2.4.1. and 2.4.7 and the green mutant Ga were grown photosynthetically and aerobically in the dark. The albin mutant L-57 was grown aerobically. Among all of these strains, ferrochelatase activity was found to vary less than 10% under all growth conditions. In order to purify the enzyme, the following procedures were used:

- The culture of the mutant Ga was used as an enzyme source. It was found that the solubilized enzyme from this carotenoid mutant fractionated differently on the DEAE-Septacel column, possibly due to the lack of carotenoids usually found in the sample at this stage. Other strains were not tested.
- The purification scheme described above and shown on Table I and in Fig. 1 has been optimized for 10-liter cultures. The volumes and concentrations were found to be critical for good recovery from the DEAE-Septacel and Dye Matrix Blue B columns. Prior to solubilization, the isolated membrane fraction is subjected to a salt wash with 0.5 M sodium thiocyanate. Elimination of this particular salt wash results in subsequent poor solubilization, and, after elution from the Blue B column, the recoveries shown are those obtained after these concentration steps.

### Table I

| Fraction | Protein (mg/ml) | Recovery (%) | Specific activity (deuteroheme/min/mg) |
|----------|----------------|--------------|--------------------------------------|
| Crude membranes | 21.0 | 100 | 0.98 |
| NaSCN-washed membranes | 18.0 | 92 | 1.27 |
| Sodium deoxycholate solubilized | 6.5 | 85 | 5.13 |
| Ammonium sulfate fraction | 7.0 | 73 | 9.25 |
| DEAE-Septacel | 0.5 | 64 | 46.20 |
| Blue Bi | 0.5 | 46 | 1386 |
| Sephadex G-150 | 0.4 | 43 | 1600 |

* Specific activity is expressed as nanomoles of deuteroheme formed per min/mg of protein.

The abbreviations used are: SDS, sodium dodecyl sulfate; PMSF, phenylmethylsulfonfluryl fluoride.
Ferrochelatase of \textit{R. sphaeroides}

**Properties of Ferrochelatase**

**Molecular Weight and Absorption Spectrum**—The estimated molecular weight for ferrochelatase from \textit{R. sphaeroides} was 115,000 $\pm$ 5,000 as determined by SDS-polyacrylamide gel electrophoresis (Fig. 3) and 110,000 by gel filtration chromatography on Sephadex G-150 in the presence of 0.5\% (w/v) sodium deoxycholate. The ultraviolet and visible absorption spectrum of the purified enzyme is shown in Fig. 4. There was no evidence of any chromophoric cofactors such as pyridoxal or flavin in any spectra obtained from several different enzyme preparations. The calculated millimolar extinction coefficient of 90 was based upon an estimated molecular weight of 115,000.

**Purity of the Enzyme and Proteolytic Nicking**—SDS-polyacrylamide gel electrophoresis of the purified enzyme is shown in Fig. 1. As discussed below, ferrochelatase, even when stored in the presence of PMSF, is subject to proteolytic digestion with the ultimate product being a peptide of $M_r = 28,000$. The inclusion of 0.1 mM EDTA in all buffers did not prevent this.

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**FIG. 1.** SDS-polyacrylamide gel electrophoresis of purified ferrochelatase of \textit{R. sphaeroides}. The procedures for the 10\% acrylamide gels shown here are described under "Materials and Methods." The samples are in the first lane, 10 $\mu$g of purified ferrochelatase after Sephadex G-150 column chromatography; in the second lane 25 $\mu$g of material after DEAE-Sephacel; and the third lane, molecular weight markers (phosphorylase \textit{b}, bovine serum albumin, ovalbumin, and soybean trypsin inhibitor). B column, there are four major bands in addition to ferrochelatase. While it was found that Triton X-100, Brij 35, octylglucoside, sodium cholate, or sodium deoxycholate at concentrations of 0.5 to 1.0\% would solubilize ferrochelatase, sodium deoxycholate was chosen due to its cost and the ability to do ammonium sulfate fractionation in its presence. Chaotropic salts, such as sodium thiocyanate and sodium perchlorate, which have been used previously to solubilize a bacterial ferrochelatase (3) did not solubilize the enzyme from \textit{R. sphaeroides}. Optimal solubilization of ferrochelatase occurs with salt-washed membranes suspended to yield a protein concentration of about 20 mg/ml and with 0.5\% (w/v) sodium deoxycholate. At protein concentrations much above this, the enzyme was poorly solubilized.

While membrane-bound ferrochelatase is stable to freezing for months and storage at 4 $^\circ$C for at least several weeks, the solubilized enzyme is labile. In the absence of 0.5 mM dithiothreitol, a major portion of the activity of the solubilized enzyme is lost overnight at 4 $^\circ$C. Addition of 10\% (v/v) propylene glycol in addition to the dithiothreitol stabilizes the enzyme so that all but the most purified preparations can be safely stored for 1 to 2 weeks at 4 $^\circ$C. As discussed below, PMSF is kept in all buffers to reduce proteolytic destruction of the enzyme.

The DEAE-Sephacel column was equilibrated with 10 volumes of buffer containing 0.2\% Brij 35 prior to sample addition. This proved to be important since columns containing new DEAE-Sephacel equilibrated with less buffer retained the enzyme, and it was not possible to elute more than about 10\% of the total applied activity with the salt wash. For elution, a step gradient of sodium thiocyanate was used (Fig. 2) since neither potassium chloride nor sodium chloride was effective in removing the enzyme even when used at substantially higher concentrations. The enzyme eluting from DEAE-Sephacel may be stored for up to a week in the presence of 10\% propylene glycol with minimal loss of activity, but prolonged storage will result in poor recovery from the Blue B column. Normally the pooled fractions are loaded directly onto the Blue B column and eluted immediately.

The Blue B column yields the greatest single purification in this procedure. The matrix has a low capacity for ferrochelatase and the binding process is slow. However, once the enzyme is bound, it is held tenaciously with no activity being eluted with 0.5 M KCl. Sodium thiocyanate at 1 M was used to elute the enzyme although 2 M KCl will cause the elution of the enzyme, but in a large volume.

**FIG. 2.** DEAE-Sephacel column chromatography of solubilized ferrochelatase. The experimental details are listed under "Materials and Methods." Enzyme activity (-----) is expressed as the relative amount of deuteroheme produced by 0.1 ml of each fraction assayed. Protein concentration is shown as the absorbance at 280 nm (--.--). The arrow indicates where 0.15 M NaSCN was added to elute the enzyme, and the solid bar indicates the fractions pooled. Approximately 75\% of the columns activity was present in the pooled fractions.
Ferrochelatase of R. sphaeroides

**TABLE IV**

| Substrate      | Apparent $K_a$ (μM) | $V_{max}$ (pmol/mg·min) | pH optima |
|----------------|---------------------|--------------------------|-----------|
| Deuteroporphyrin| 95                  | 3175                     | 8.4       |
| Mesoporphyrin   | 20                  | 1220                     | 8.5       |
| Protoporphyrin  | 18                  | 110                      | 7.6       |
| Ferrous citrate | 22                  |                           |           |

$V_{max}$ values given as nanomole·min$^{-1}$·mg of protein$^{-1}$.

**TABLE II**

**Apparent $K_a$ values and pH optima for purified ferrochelatase**

Assays were carried out as described under "Materials and Methods." The pH of each assay was measured at 37°C, the assay temperature, and Tris acetate was the buffer in all cases.

**TABLE III**

**Effect of metal ions on ferrochelatase activity**

Assays were conducted as described under "Materials and Methods." Deuteroporphyrin was the porphyrin substrate used, and metal salts were added to give a final concentration of 50 μM. These data were obtained with enzyme from the G-150 column, but similar data were found for all solubilized enzyme preparations.

| Metal ions | Control activity % |
|------------|--------------------|
| None       | 100                |
| KCl        | 98                 |
| NaCl       | 97                 |
| MgCl₂      | 104                |
| MnCl₂      | 97                 |
| NiCl₂      | 99                 |
| CuSO₄      | 69                 |
| PbNO₃      | 50                 |
| HgCl₂      | 15                 |

**TABLE IV**

**Effect of sulfhydryl reagents on ferrochelatase activity**

Enzyme assays were carried out as described under "Materials and Methods" with deuteroporphyrin as substrate. The purified enzyme preparation was incubated at 37°C with the reagent at a final concentration of 5 μM. After 15 min, excess dithiothreitol was added to react with any remaining reagent. The remainder of the assay reagents was then added and the assay carried out as usual.

| Reagent          | Control activity % |
|------------------|--------------------|
| None             | 100                |
| Iodoacetamide    | 20                 |
| N-Ethylmaleimide | 25                 |

In the present study, ferrochelatase has been purified from the bacterium *R. sphaeroides*. This represents the first documented purification of this enzyme from any bacterial source. Recently, Taketani and Tokunaga (13) reported the purification of ferrochelatase from rat liver mitochondria where they obtained a 628-fold purification with a 25% yield. They reported a subunit molecular weight of 42,000 with $M_r$ = 240,000 by gel filtration. Herein, we report the 1,640-fold purification with 43% recovery from the isolated membrane fraction. Prior to this report, the most highly purified microbial ferrochelatase was from the bacterium *S. itersonii* (7). It was somewhat surprising to find that ferrochelatase from these two organisms differs so much in its physical properties. The *S. itersonii*

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**D I S C U S S I O N**

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enzyme was reported to have a molecular weight of about 50,000 and to be strongly stimulated by copper, while the *R. sphaeroides* enzyme has a molecular weight of 115,000 and is inhibited by copper. Except for this, both enzymes show similar responses to metal ions with marked inhibition occurring with lead and mercury and no substantial effect with any of the other tested salts. Also of interest is that, although both organisms are Gram-negative prokaryotes, the requirements for solubilization of ferrochelatase are quite different, with the enzyme from *S. itersonii* being readily solubilized by chaotropic salts, while the *R. sphaeroides* enzyme requires detergent for solubilization. Neither enzyme exhibited a requirement for phospholipid, although 0.2% Triton X-100 is always present in our assays and this may circumvent any such requirements.

As has been found with ferrochelatase from all sources examined, the highest activity is found with deuteroporphyrin followed by mesoporphyrin, and the lowest specific activity is with protoporphyrin. This same order is found with apparent K_M values, with deuteroporphyrin having the highest and protoporphyrin the lowest. The porphyrin substrate has no effect upon the apparent K_M for iron. The purified enzyme is inhibited by heme, but 50% inhibition is seen only at heme concentrations of around 40 μM, a concentration considerably higher than one would expect to find in vivo and also higher than the concentration that inhibits δ-aminolevulinate synthase. The kinetic properties of the purified enzyme are the same as those found with membrane-bound ferrochelatase.

A surprising finding was the large size of ferrochelatase from *R. sphaeroides*. The *M_r* = 115,000 for a single subunit membrane-bound enzyme is somewhat unusual, and earlier reports have calculated molecular weights for ferrochelatase of 40,000 to 50,000 (7, 13). The large size of the *R. sphaeroides* enzyme may contribute to the susceptibility of the protein to proteolytic cleavage. Our earliest attempts at purification were uniformly unsuccessful due to the rapid loss of activity after column chromatography. Inclusion of the serine protease inhibitor PMSF in all buffer dramatically improved enzyme recovery and stability. Even so, all ferrochelatase preparations obtained contain varying amounts of lower molecular weight peptides. Since it has not been possible to separate these by gel filtration, ion exchange chromatography, or non-denaturing gel electrophoresis, we assume that they represent proteolytically nicked ferrochelatase molecules that are only separated by denaturing conditions such as found in SDS-gel electrophoresis. Evidence that supports this view is the observation that freshly isolated ferrochelatase yields a major band of *M_r* = 115,000 ± 5,000 with faint minor bands around *M_r* = 60,000. After 1 week at 4 °C, about one-half of the enzyme activity is lost and SDS gels show more intense bands at *M_r* = 60,000 and 28,000 in addition to the large molecular weight band and if the enzyme preparation remains at 4 °C for 1 month, less than 1% of the activity is left and SDS gels show a single major band at *M_r* = 28,000 with faint bands at *M_r* = 60,000 and no visible band at *M_r* = 115,000. Gel filtration on Sephadex G-150 in the presence of detergent will separate the *M_r* = 28,000, but not the *M_r* = 60,000, peptides from the *M_r* = 115,000 ferrochelatase. One preparation was obtained from gel filtration on Sephadex G-150 of a 3-week-old enzyme preparation that contained bands in the *M_r* = 50,000 to 60,000 range and which was still partially active. Assuming that previous ferrochelatase preparations from other organisms did not suffer proteolytic nicking and their molecular weights are around 50,000, then a major point of interest is why *R. sphaeroides* has an enzyme of molecular weight of over 100,000. Perhaps the presence of both heme and bacteriochlorophyll biosynthetic pathways in this organism requires some additional enzymatic machinery at the iron insertion step. If this is true, then it may be of interest to examine ferrochelatase of plant chloroplasts versus mitochondria.

Because of the unusual nature of the reaction catalyzed and because of the confusion of previous reports, a variety of compounds were examined that might serve as possible cofactors in the reaction. Since the visible light spectrum of purified ferrochelatase contains no distinct absorption bands, any chromophoric cofactors would be expected to be noncovalently attached and separated during the purification procedure. Of all the compounds tested, including a variety of cations, pyridoxal phosphate, FMN, and NADH, none were found to be stimulatory, and considering the high recovery of enzyme, it would seem that none of these are required. Two divalent cations, mercury and lead, were found to be strongly inhibitory, possibly due to their ability to interact with free sulphydryl groups. Previously, it has been reported that ferrochelatase activity is inhibited by sulphydryl reactive compounds, and with purified *R. sphaeroides* ferrochelatase, both iodoacetamide and N-ethylmaleimide inhibit enzyme activity. With the availability of purified ferrochelatase, it is hoped that the nature of this inhibition can be more clearly delineated.

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