Genetics. In the article "Use of the DNA polymerase chain reaction for homology probing: isolation of partial cDNA or genomic clones encoding the iron–sulfur protein of succinate dehydrogenase from several species" by Stephen J. Gould, Suresh Subramani, and Immo E. Scheffler, which appeared in number 6, March 1989, of Proc. Natl. Acad. Sci. USA (86, 1934–1938) the partial amino acid sequence reported for the iron–sulfur protein of succinate dehydrogenase from Drosophila melanogaster is in error. The entire gene has recently been cloned (H. Au and I.E.S., unpublished work), using the same partial cDNA clone in the screening of a D. melanogaster genomic library. There are a significant number of sequence changes (approximately 72% sequence identity with the mammalian coding sequence), which make us believe that the original clone arose from a mix-up or a contamination. The paper described a relatively unusual, at the time, application of the polymerase chain reaction, and it was illustrated by cloning partial genomic or cDNA sequences from several species. The human and Saccharomyces cerevisiae sequences have since been confirmed independently by us and by others (1, 2).


Biochemistry. In the article "Marek disease virus encodes a basic-leucine zipper gene resembling the fos/jun oncogenes that is highly expressed in lymphoblastoid tumors" by Dan Jones, Lucy Lee, Juinn-Lin Liu, Hsing-Jien Kung, and Joanne K. Tillotson, which appeared in number 9, May 1, 1992, of Proc. Natl. Acad. Sci. USA (89, 4042–4046), the authors request that the following correction be noted. In the sequence presented in Fig. 2 on p. 4044, a G residue should be inserted after base +1053. This sequence correction does not affect the protein motifs (basic-leucine zipper or proline repeats) reported in the earlier paper. The correct DNA sequence has been deposited in GenBank.

Cell Biology. In the article "Interaction with basement membrane serves to rapidly distinguish growth and differentiation pattern of normal and malignant human breast epithelial cells" by Ole William Petersen, Lone Rønnov-Jessen, Anthony R. Howlett, and Mina Bissell, which appeared in number 19, October 1, 1992, of Proc. Natl. Acad. Sci. USA (89, 9064–9068), the authors request that the following correction be noted. On p. 9065, left column, line 1, µg/ml should be replaced with mg/ml.

Cell Biology. In the article "Regulation of cell cycle progression and nuclear affinity of the retinoblastoma protein by protein phosphatases" by Arthur S. Alberts, Andrew M. Thorburn, Shirish Shenolikar, Marc C. Mummy, and James R. Feramisco, which appeared in number 2, January 15, 1993, of Proc. Natl. Acad. Sci. USA (90, 388–392), the authors request that the following correction be noted. In the legend to Fig. 5, the locant designations were incorrect. The correct legend should read as follows:

FIG. 5. Effects of nuclear injection of protein phosphatases on progression through S phase. BrdUrd uptake was monitored in cells injected directly into nuclei as described in Fig. 4 and Materials and Methods. (A–C) REF-52 fibroblasts injected in the nucleus with SlgiG (3 mg/ml). (A) FITC donkey anti-SlgG antibody. (B) Texas Red/ BrdUrd. (C) Double exposure of both fields. In double exposures, cells positive for both injection (FITC) and BrdUrd incorporation (Texas Red) appear yellow. Double exposures of phosphatase-injected nuclei are shown in D, E, and F (PP1, PP2AC, and PP2A, respectively). Nuclei appearing green have been injected but have not made DNA.

Neurobiology. In the article "Physiological and anatomical evidence for a magnocellular defect in developmental dyslexia" by Margaret S. Livingstone, Glenn D. Rosen, Frank W. Drislane, and Albert M. Galaburda, which appeared in number 18, September 15, 1991, of Proc. Natl. Acad. Sci. USA (88, 7943–7947), the authors request that the following correction be noted. On p. 7944, the first sentence of the first paragraph should read as follows: "We examined the LGN in autopsy specimens from five dyslexic subjects (four males and one female; mean age, 34.2 ± 13.7 years) and five nondyslexic subjects (four males and one female; mean age, 40 ± 11.2 years)."

Biochemistry. In the article "Purification and characterization of cytosolic aconitase from beef liver and its relationship to the iron-responsive element binding protein" by Mary Claire Kennedy, Liane Mende-Mueller, George A. Blondin, and Helmut Beinert, which appeared in number 24, December 15, 1992, of Proc. Natl. Acad. Sci. USA (89, 11730–11734), the authors wish that the following correction be noted. In Table 4, entry 6 (amino acid sequence of domain 4), Arg should be below the first Lys (position 732) and not below the second Lys (position 736).
Purification and characterization of cytosolic aconitase from beef liver and its relationship to the iron-responsive element binding protein

(iron-sulfur protein/iron regulation)

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Contributed by Helmut Beinert, September 18, 1992

ABSTRACT In recent reports attention has been drawn to the extensive amino acid homology between pig heart, yeast, and Escherichia coli aconitases (EC 4.2.1.3) and the iron-responsive element binding protein (IRE-BP) of mammalian cells (Rouault, T. A., Stout, C. D., Kaptein, S., Harford, J. B. & Klausner, R. D. (1991) Cell 64, 881–883; Hentze, M. W. & Argos, P. (1991) Nucleic Acids Res. 19, 1739–1740; Prodro- mon, C., Artyminuk, P. J. & Guest, J. R. (1992) Eur. J. Biochem. 204, 599–609). Iron-responsive elements (IREs) are stem-loop structures located in the untranslated regions of mRNAs. IRE-BP is required in the posttranscriptional regulation of ferritin mRNA translation and stabilization of transferrin receptor mRNA. In spite of substantial homology between the amino acid sequences of mammalian mitochondrial aconitase and IRE-BP, the mitochondrial protein does not bind IREs. However, there is a second aconitase, found only in the cytosol of mammalian tissues, that might serve as an IRE-BP. To test this possibility, we have prepared sufficient quantities of the heretofore poorly characterized beef liver cytosolic aconitase. This enzyme is isolated largely in its active [4Fe-4S] form and has a turnover number similar to that of mitochondrial aconitase. The EPR spectra of the two enzymes are markedly different. The amino acid composition, molecular weight, isoelectric point, and the sequences of six random peptides clearly show that these physicochemical and structural characteristics are identical to those of IRE-BP, and that c-aconitase is distinctly different from m-aconitase. In addition, both cytosolic aconitase and IRE-BP can have aconitase activity or function as IRE-BPs, as shown in the following paper and elsewhere [Zheng, L. Kennedy, M. C., Blondin, G. A., Beinert, H. & Zalkin, H. (1992) Arch. Biochem. Biophys., in press]. This leads us to the conclusion that cytosolic aconitase is IRE-BP.

In the past 10 years evidence has been obtained that intracellular iron levels are controlled by a posttranscriptional mechanism which correlates translation of mRNA for the H subunit of ferritin and stabilization of transferrin receptor mRNA. This is accomplished by the interaction of a cytosolic protein with iron-responsive elements (IREs), which are stem-loop structures located in the untranslated regions of the respective mRNAs (1–3). Small quantities (nanograms to micrograms) of a cytosolic protein of ~100 kDa that binds to IREs (IRE binding protein, IRE-BP) have been isolated (4–6). This research took an unexpected turn when the cDNA sequence for the protein from human liver was determined and the protein sequence deduced was found to have a striking homology to the amino acid sequence of pig heart mitochondrial aconitase (m-aconitase) (7). All active-site residues identified in the aconitase crystal structure are conserved (8). The three cysteine residues that are ligands to the iron atoms of the Fe-S cluster of aconitase are the only cysteine residues conserved in all the proteins that can now be compared, which include yeast and Escherichia coli aconitases and isopropylmalate isomerases (9, 10), enzymes with a similar function. In spite of this homology, mammalian m-aconitase does not function as an IRE-BP. However, in mammals there exists a separate aconitase in the cytosol, which has received relatively little attention thus far (11–13). In the context of these observations we were prompted to prepare this enzyme in quantity sufficient for characterization with respect to its relationship to m-aconitase and the IRE-BP reported by others. We report here details of its purification and several properties as well as the sequences of six peptides obtained by cyanogen bromide cleavage. These peptides are located in all four domains of the enzyme, when modeled according to m-aconitase (7). Our results here and in a previous publication (14) and those reported by Kaptein et al. (15), by Haile et al. (16), and in the following paper (17) leave little doubt that IRE-BP and cytosolic aconitase (c-aconitase) are identical.

MATERIALS AND METHODS

m-Aconitase was prepared and enzyme activation, assay, and analysis for S²⁺, S⁶⁺, and Fe were carried out as described (18–20). Protein was determined by a biuret method, standardized for m- or c-aconitase, respectively, by amino acid analysis.

Purification of c-Aconitase. m-Aconitase is the least desirable contaminant of c-aconitase. Hence, we chose as the initial part of the purification procedure the separation of cytosol and mitochondria by a method previously used for the large-scale preparation of mitochondria from slaughterhouse tissue (21); the following modifications were incorporated: (i) 2 mM Hepes (pH 7.2) containing 2 mM citrate was used as buffer, (ii) the tissue grinding step was omitted, (iii) blending time was only 30 sec, and (iv) the homogenate was centrifuged at 1300 × g for 60 min. All manipulations were done at 0–4°C and the initial ratio of liver to buffer was 1:3.5 (wt/vol). The supernatant obtained after the sedimentation of the mitochondria was made 20% (vol/vol) in CHCl₃ and was stirred vigorously for 30 sec to remove lipids. The emulsion was cleared by centrifugation (1300 × g, 60 min) and the two layers were separated. The aqueous phase was brought to 35% saturation with solid ammonium sulfate and allowed to stand 2 hr. After centrifugation (27,000 × g, 25 min) the supernatant was brought to 75% saturation with ammonium

Abbreviations: IRE, iron-responsive element; IRE-BP, IRE binding protein; c- and m-aconitase, cytosolic and mitochondrial aconitase, respectively; PVDF, poly(vinylidene difluoride).

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sulfate and allowed to stand overnight. The light brown precipitate obtained on centrifugation was dissolved in 2 mM potassium citrate, pH 6.5 (KC65). The dark reddish brown solution was desalted on a Bio-Gel P-6DG column (Bio-Rad) with elution by 2 mM KC65. The combined protein fractions were adsorbed onto a column (5 x 20 cm) of CM-52 (Whatman microgranular) resin equilibrated with 2 mM KC65. The resin was washed with 2 mM KC65 until all the dark brown material was eluted; this was followed by 10 mM KC65. The fractions containing enzyme activity were combined, diluted 1:1 with distilled water, and concentrated to 20 ml by using a P30 membrane (Amicon). The solution was diluted 1:5 with water (final concentration, ~1 mM citrate), clarified by centrifugation, and applied to a column (2.5 x 30 cm) of CM-52 resin in 2 mM KC65. The protein was eluted with a linear gradient of 10 mM KC65 in 2 mM KC65. After concentration, the active fractions were further purified by gel permeation chromatography on a column (2.5 x 90 cm) of Sephadex G-100 superfine (Pharmacia) equilibrated with 10 mM Hepes-KOH/2 mM citrate/50 mM NaCl, pH 7.5. The active fractions were combined and concentrated as above and stored under liquid N2.

**Electrophoresis.** SDS/PAGE was performed according to Laemmli (22) and isoelectric focusing according to the manufacturer's instructions with the Bio-Rad model 111 Mini-IEF cell. The ampholytes used were Bio-Lyte 3/10.

**Amino Acid Composition.** c-Aconitase was subjected to SDS/7.5% PAGE. M-Aconitase and the low molecular weight standards from Bio-Rad were used as standards. Immediately after electrophoresis the slab gel was equilibrated in electroblotting-transfer buffer (10% methanol/25 mM N-ethylmorpholine formate, pH 8.3) for 5 min and the proteins were electroblotted on to poly(vinylidene difluoride) (PVDF) membranes (Immobilon-P; Millipore) (23). The membranes were stained with amido black (0.1% in acetic acid/methanol/water, 7:45:48, vol/vol). The gel was stained with Coomassie brilliant blue to detect remaining protein. Membranes were air-dried and the c-aconitase bands were excised. Similar-sized pieces of the same membrane were excised in an area with no detectable protein. Data from these pieces were used as background values. Hydrolysis was carried out in 5.7 M HCl/0.02% 2-mercaptoethanol at 110°C for 20 hr under N2. Samples were dried under vacuum and analyzed with the Beckman model 6300 analyzer using a post-column ninhydrin detection system.

**Cyanogen Bromide Digest and Peptide Sequencing.** Purity of the enzyme was determined by SDS/PAGE as described above. c-Aconitase from two different batches, 440 and 500 µg, respectively, was digested for 48 hr at room temperature in the dark with a 200-fold excess of cyanogen bromide (Sigma) over methionine in 80% formic acid under N2 with constant stirring. The digest was diluted 1:1 with water and dried under vacuum with a Savant Speed-Vac system, dissolved in water, and redried to evaporate all acid. The sample was dissolved in SDS/PAGE sample buffer (22); 11- and 22-µg samples and the low molecular weight standards were run in a 5–25% gradient gel (Bio-Rad Mini-Protein apparatus). Gels were electroblotted as above. Membranes were stained with amido black and peptide bands were excised for sequence determination on an Applied Biosystems model 477A pulsed liquid-phase sequencer or a Beckman/Porton model LF 3000 sequencer.

**Protein Sequencing.** The intact protein, separated in an SDS/7.5% polyacrylamide gel as described above, was electroblotted. The band corresponding to c-aconitase was excised and subjected to sequencing in the Applied Biosystems model 477A. After sequencing for 10 steps the PVDF membrane was removed from the sequencer and hydrolyzed as described above for amino acid analysis. Although >100 pmol of the intact protein had been applied to the sequencer, sequencing did not proceed as expected. By amino acid analysis after sequencing, 66 pmol of the protein could be recovered from the PVDF strips and the composition did not differ significantly from that obtained without sequencing. This suggests that the N-terminal amino acid may be modified and not available for Edman degradation.

**RESULTS AND DISCUSSION**

Our attempts to produce c-aconitase in quantities sufficient for various spectroscopic analyses were unsuccessful when we used variations of published procedures (11–13). A summary of a typical run of the purification method developed is given in Table 1. Mitochondria were first removed from liver homogenates to eliminate contamination by m-aconitase. The degree of purification therefore is higher than that given in Table 1, as it would be impossible to differentiate c-aconitase activity from that of m-aconitase.

**SDS/PAGE** (Fig. 1) of the purified protein gave anomalous

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**Fig. 1.** SDS/PAGE of purified bovine c-aconitase. Enzyme (2.2 µg of protein) was denatured with 2% SDS at 95°C for 10 min and resolved by electrophoresis in an SDS/7.5% polyacrylamide gel. During the denaturation step the samples contained EDTA and 2-mercaptoethanol as indicated. Proteins were stained with Coomassie blue. The conditions for denaturation were varied in lanes 1–3. Lanes: 1, 5 mM EDTA and 0.45 M 2-mercaptoethanol; 2, 1.3 M 2-mercaptoethanol; 3, 5 mM EDTA; 4, molecular mass standards (from top to bottom, myosin heavy chain, β-galactosidase, phosphorylase b, bovine serum albumin, ovalbumin, and carbonic anhydrase). Molecular masses are given in kilodaltons.
results. A major single band was obtained only when the enzyme was (i) treated with EDTA in the absence of thiol or (ii) treated with very high thiol concentrations—i.e., \( \approx 1.4 \) M 2-mercaptoethanol. At intermediate thiol concentrations a doublet appeared. The same phenomenon is seen with IRE-BP isolated from human placenta (figure 5 of ref. 6). Similar results have been observed previously with \( \beta \)-lactamase, where reduction of a disulfide bond has been implicated in this behavior (25).

A comparison of the results of isoelectric focusing of the three forms of both c-aconitase and m-aconitase is shown in Fig. 2. Under the conditions used, the pI values of the [3Fe-4S] and [4Fe-4S] forms of c-aconitase are close to 8.0, while those of the same forms of m-aconitase are between 7.5 and 7.8. The apoprotein, formed by oxidative removal of the cluster with ferricyanide (19), also shows different behavior. The pI of apo c-aconitase decreases to \( \approx 7.7 \), whereas that of apo m-aconitase increases to 8.0. The result for holo c-aconitase, pI \( \approx 8.0 \), is different from the pI of 5.4 (12) or 6.7 (13) reported previously; a pI of 6.21 was calculated from the amino acid composition based upon the cDNA sequence for IRE-BP, without added Fe or S (6).

Although both c-aconitase and m-aconitase require an intact [4Fe-4S] cluster for activity, a significant difference between the stability of the clusters was observed. m-Aconitase is isolated predominantly in the [3Fe-4S] form (Fe/S ratio of 0.73 \pm 0.02) and must be activated by the addition of ferrous iron. However, c-aconitase, as isolated is \( \approx 80\% \) active with an Fe/S ratio of 1.10 \pm 0.06 (six different preparations). Enzymatic activities with all three substrates as measured in units/nmol of cluster are very similar to those of the mitochondrial enzyme (Table 2). Apo c-aconitase contains S\( ^0 \) as does apo m-aconitase (19). The cytosolic enzyme also behaves similarly to the mitochondrial enzyme in regard to the tight binding of substrate. Active enzyme, when incubated with radiolabeled substrate and then rapidly desalted by centrifugation on a gel column, will retain substrate (26). The [3Fe-4S] form of c-aconitase on a second passage through the gel column still retains 0.6 mol of substrate per mol of Fe-S cluster. This is significantly different for the [3Fe-4S] form of m-aconitase, which, after a second passage through gel, retains negligible substrate.

The EPR spectrum of c-aconitase in the [3Fe-4S]\( ^+ \) form (Fig. 3) is distinctly different from that of m-aconitase, so that cross contamination can be readily detected. The c-aconitase spectrum was simulated with g values of 2.015, 2.015, and 2.033. In the [4Fe-4S]\( ^+ \) state at pH 8.5, rhombic spectra typical for this cluster state were observed with g values at 2.07, 1.95, and 1.86 without substrate and 2.06, 1.85, and 1.77 with substrate. The corresponding values for m-aconitase are 2.06, 1.93, and 1.86 and 2.04, 1.85, and 1.78, respectively. In either case there is a similar increase in rhombicity in the presence of substrate.

Table 3 the average of four amino acid analyses of purified c-aconitase is compared with the theoretical results as deduced from the cDNA sequence for human IRE-BP (6). The mole fraction of at least five different residues clearly distinguishes between IRE-BP and m-aconitase. For all of the amino acids presented in the table the percentage of residues with the assigned charge xmol% for c-aconitase, m-aconitase, and IRE-BP and their corresponding standard deviations are given for cDNA and c-DNA.

Table 2. Comparison of enzymatic activities of c- and m-aconitase

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Activity, units/nmol of Fe-S cluster</th>
<th>c-Aconitase</th>
<th>m-Aconitase</th>
</tr>
</thead>
<tbody>
<tr>
<td>citrate ( \rightarrow ) cis-aconitate</td>
<td>1.6</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>citrate ( \rightarrow ) isocitrate</td>
<td>1.2</td>
<td>1.1</td>
<td></td>
</tr>
<tr>
<td>cis-aconitate ( \rightarrow ) isocitrate</td>
<td>3.4</td>
<td>3.7</td>
<td></td>
</tr>
<tr>
<td>isocitrate ( \rightarrow ) cis-aconitate</td>
<td>3.3</td>
<td>4.3</td>
<td></td>
</tr>
</tbody>
</table>

Activities were measured under \( V_{\text{max}} \) conditions (26).

Table 3. Comparison of amino acid composition of c- and m-aconitase and IRE-BP

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>IRE-BP(^ a )</th>
<th>c-Aconitase(^ b )</th>
<th>m-Aconitase(^ c )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asx</td>
<td>11.0</td>
<td>10.90</td>
<td>10.5</td>
</tr>
<tr>
<td>Thr</td>
<td>4.6</td>
<td>5.04</td>
<td>4.7</td>
</tr>
<tr>
<td>Ser</td>
<td>5.3</td>
<td>5.15</td>
<td>4.9</td>
</tr>
<tr>
<td>Glx</td>
<td>10.1</td>
<td>9.62</td>
<td>10.7</td>
</tr>
<tr>
<td>Pro</td>
<td>7.0</td>
<td>6.41</td>
<td>7.8</td>
</tr>
<tr>
<td>Gly</td>
<td>8.9</td>
<td>8.15</td>
<td>8.6</td>
</tr>
<tr>
<td>Ala</td>
<td>7.7</td>
<td>7.56</td>
<td>7.7</td>
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<tr>
<td>Val</td>
<td>8.8</td>
<td>8.58</td>
<td>9.2</td>
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<tr>
<td>Met</td>
<td>1.9</td>
<td>2.40</td>
<td>2.0</td>
</tr>
<tr>
<td>Ile</td>
<td>5.8</td>
<td>6.18</td>
<td>5.8</td>
</tr>
<tr>
<td>Leu</td>
<td>9.6</td>
<td>9.16</td>
<td>10.4</td>
</tr>
<tr>
<td>Tyr</td>
<td>3.2</td>
<td>3.56</td>
<td>3.4</td>
</tr>
<tr>
<td>Phe</td>
<td>4.9</td>
<td>5.04</td>
<td>5.3</td>
</tr>
<tr>
<td>His</td>
<td>1.9</td>
<td>1.93</td>
<td>1.4</td>
</tr>
<tr>
<td>Lys</td>
<td>5.0</td>
<td>5.60</td>
<td>4.3</td>
</tr>
<tr>
<td>Arg</td>
<td>4.0</td>
<td>4.68</td>
<td>3.7</td>
</tr>
</tbody>
</table>

\( a \) Average of four determinations on three preparations.

\( b \) Ref. 6.

\( c \) Faint lower band in lanes 1 and 2 of Fig. 1.

\( d \) Ref. 27. Arrows in this column indicate residues showing maximum differences.
these c-aconitase is, within error, the same as IRE-BP. Also included in Table 3 are the results of the amino acid analysis of minor, slower-moving band (Fig. 1, lanes 1 and 2). This band (∼4% of the protein) coincides with the single band obtained by treating c-aconitase with EDTA in the absence of thiol. The composition of this material (column 3 of Table 3) indicates that this protein is also c-aconitase and not m-aconitase. Performic acid oxidation of c-aconitase shows that 11 cysteine residues are present. This is different from the 9 cysteines reported for human IRE-BP (6) and similar to the 12 found in rat liver IRE-BP (28). Analysis of m-aconitase and a standard similarly treated indicates that the method used usually gives results on the low side, minus 10–15%, and thus it may be that there are actually 12 cysteines in beef liver c-aconitase.

SDS/PAGE of the purified enzyme (Fig. 1) has established its apparent molecular mass to be close to 97 kDa, which is very similar to that recorded for the human IRE-BP. The latter has also been shown to have aconitase activity when isolated from cells grown replete with iron (16). To further establish the identity of the two proteins, sequencing of six peptides obtained from cyanogen bromide cleavage of the protein was carried out (Table 4). (Sequencing of the intact cDNA of the human IRE-BP (6) and both of these are conservative changes. This sequence identity plus the aforementioned aconitase activity of IRE-BP, along with the observations that under appropriate conditions c-aconitase can bind to the IREs of ferritin mRNA (17) and the mRNA of m-aconitase (14), leaves little doubt as to the identity of these two proteins.

Conclusions. We conclude from the work reported in this and the accompanying paper (17) that IRE-BP and c-aconitase are identical proteins. We know, however, that enzymatic activity and IRE-binding ability are mutually exclusive (16). The iron-free apoprotein as prepared in vitro can bind IRE only in the presence of high concentrations of thiol, whereas IRE-BP produced in iron-depleted cells binds IRE without thiol addition. Since the apo form of aconitase is known to contain S2 and disulfide bonds, it appears likely that a significant difference between the forms produced in vitro and the form produced in the cell is the disposition of the sulfur components and ligands when the Fe-S cluster is chemically removed. It is the intact Fe-S cluster that is required for aconitase activity but prevents RNA binding.

We are indebted to Drs. R. D. Klausner and T. A. Rouault for providing information before publication and for discussions, to Dr. W. A. Antholine for collaboration in the EPR experiments, to Dr. J. B. Howard for valuable comments on the manuscript, and to Dr. Howard Zalkin for drawing our attention to ref. 25 concerning SDS/PAGE. We acknowledge the use of instrumentation at the National Biomedical ESR Center (National Institutes of Health, Research Resources Grant RR01006), and of equipment at the Institute for Enzyme Research, University of Wisconsin, Madison. The amino acid and sequence analyses were carried out in the Protein and Nucleic Acid Shared Facility of the Medical College of Wisconsin. We acknowledge the assistance of Brady Stoner in these analyses. This work was supported by a research grant from the National Institutes of Health (GM34812) to H.B.

We are indebted to Drs. R. D. Klausner and T. A. Rouault for providing information before publication and for discussions, to Dr. W. A. Antholine for collaboration in the EPR experiments, to Dr. J. B. Howard for valuable comments on the manuscript, and to Dr. Howard Zalkin for drawing our attention to ref. 25 concerning SDS/PAGE. We acknowledge the use of instrumentation at the National Biomedical ESR Center (National Institutes of Health, Research Resources Grant RR01006), and of equipment at the Institute for Enzyme Research, University of Wisconsin, Madison. The amino acid and sequence analyses were carried out in the Protein and Nucleic Acid Shared Facility of the Medical College of Wisconsin. We acknowledge the assistance of Brady Stoner in these analyses. This work was supported by a research grant from the National Institutes of Health (GM34812) to H.B.