Complex Formation between Glutamyl-tRNA Reductase and Glutamate-1-semialdehyde 2,1-Aminomutase in Escherichia coli during the Initial Reactions of Porphyrin Biosynthesis*

Received for publication, January 13, 2005, and in revised form, March 4, 2005
Published, JBC Papers in Press, March 9, 2005, DOI 10.1074/jbc.M500440200

Corinna Lüer‡, Stefan Schauer§, Kalle Möbius‡, Jörg Schulze¶, Wolf-Dieter Schubert¶,
Dirk W. Heinz‡, Dieter Jahn‡, and Jürgen Moser‡‡

From the ‡Institute of Microbiology, Technical University Braunschweig, Spielmannstrasse 7, D-38106 Braunschweig, Germany, §Institute for Molecular Biology and Biophysics, Swiss Federal Institute of Technology, Schafmattstrasse 20, CH-8093 Zürich, Switzerland, and ¶Division of Structural Biology, German Research Center for Biotechnology, Mascheroder Weg 1, D-38124 Braunschweig, Germany

In Escherichia coli the first common precursor of all tetrapyrroles, 5-aminolevulinic acid, is synthesized from glutamyl-tRNA (Glu-tRNA\(^{\text{Glu}}\)) in a two-step reaction catalyzed by glutamyl-tRNA reductase (GluTR) and glutamate-1-semialdehyde 2,1-aminomutase (GSA-AM). To protect the highly reactive reaction intermediate glutamate-1-semialdehyde (GSA), a tight complex between these two enzymes was proposed based on their solved crystal structures. The existence of this hypothetical complex was verified by two independent biochemical techniques. Co-immunoprecipitation experiments using antibodies directed against E. coli GluTR and GSA-AM demonstrated the physical interaction of both enzymes in E. coli cell-free extracts and between the recombinant purified enzymes. Additionally, the formation of a GluTR-GSA-AM complex was identified by gel permeation chromatography. Complex formation was found independent of Glu-tRNA\(^{\text{Glu}}\) and cofactors. The analysis of a GluTR mutant truncated in the 80-amino acid C-terminal dimerization domain (GluTR-A338Stop) revealed the importance of GluTR dimerization for complex formation. The in silico model of the E. coli GluTR-GSA-AM complex suggested direct metabolic channeling between both enzymes to protect the reactive aldehyde species GSA. In accordance with this proposal, side product formation catalyzed by GluTR was observed via high performance liquid chromatography analysis in the absence of the GluTR-GSA-AM complex.

In plants, green algae, archaea, and most bacteria the common precursor molecule of all tetrapyrroles, 5-aminolevulinic acid (ALA), is synthesized from tRNA-bound glutamate (Glu-tRNA\(^{\text{Glu}}\)) in a two-step reaction catalyzed by glutamyl-tRNA reductase (GluTR) and glutamate-1-semialdehyde 2,1-aminomutase (GSA-AM). To protect the highly reactive reaction intermediate glutamate-1-semialdehyde (GSA), a tight complex between these two enzymes was proposed based on their solved crystal structures. The existence of this hypothetical complex was verified by two independent biochemical techniques. Co-immunoprecipitation experiments using antibodies directed against E. coli GluTR and GSA-AM demonstrated the physical interaction of both enzymes in E. coli cell-free extracts and between the recombinant purified enzymes. Additionally, the formation of a GluTR-GSA-AM complex was identified by gel permeation chromatography. Complex formation was found independent of Glu-tRNA\(^{\text{Glu}}\) and cofactors. The analysis of a GluTR mutant truncated in the 80-amino acid C-terminal dimerization domain (GluTR-A338Stop) revealed the importance of GluTR dimerization for complex formation. The in silico model of the E. coli GluTR-GSA-AM complex suggested direct metabolic channeling between both enzymes to protect the reactive aldehyde species GSA. In accordance with this proposal, side product formation catalyzed by GluTR was observed via high performance liquid chromatography analysis in the absence of the GluTR-GSA-AM complex.

* This work was supported by grants from the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie (to D. W. H. and D. J.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

†To whom correspondence should be addressed. Tel.: 49-531-391-5808; Fax: 49-531-391-5854; E-mail: j.moser@tu-bs.de.

‡The abbreviations used are: ALA, 5-aminolevulinic acid; DTT, 1,4-dithio-bis(o-carboxyethyl) disulfide; GluRS, glutamyl-tRNA synthetase; GluTR, glutamyl-tRNA reductase; Glu-tRNA\(^{\text{Glu}}\); glutamyl-tRNA\(^{\text{Glu}}\); GSA-AM, glutamate-1-semialdehyde 2,1-aminomutase; GSA, glutamate-1-semialdehyde; HPLC, high performance liquid chromatography; PIPES, piperazine-N,N'-bis[2-ethanesulfonic acid]; PLP, pyridoxal 5'-phosphate.

The Journal of Biological Chemistry
Vol. 280, No. 19, Issue of May 13, pp. 18568–18572, 2005
Printed in U.S.A.
**GluTR protein.** The resulting combined model of the ternary complex (GluTR, tRNA, and GSA-AM) did not lead to steric clashes. Additional strong evidence for the model complex came from the observation that the putative active site entrance of each GSA-AM monomer is positioned opposite a partly opened depression of the catalytic domain of GluTR. This depression and the GluTR active site pocket are separated from each other only by the conserved arginine 50 (M. kandleri GluTR numbering). In our current hypothesis the GluTR product GSA leaves the enzyme via this “back door” of the GluTR active site pocket and subsequently enters the active site of GSA-AM. This way direct channeling of labile GSA to the active site of GSA-AM without exposure to the aqueous environment is possible. Here we provide the first experimental evidence for the GluTR-GSA-AM complex using two independent biochemical techniques. Additionally, we created the homologous *E. coli* model complex in an *in silico* experiment (Fig. 1) supporting our results.

**EXPERIMENTAL PROC EDURES**

*Overexpression and Purification of E. coli GluTR—Details for recombinant production, refolding, and purification of recombinant E. coli GluTR have been published elsewhere (7).*

*Construction of the Gene for the GluTR Mutant A338Stop by Site-directed Mutagenesis—A deletion mutant lacking the dimerization domain (GluTR-A338Stop) was generated using the plasmid pBKCwt (6) and the QuickChange™ kit (Stratagene, La Jolla, CA) according to the manufacturer’s instructions. The following oligonucleotide was employed to introduce a stop codon into the *E. coli* GluTR sequence: 5’-GCGTG-GCGGCGCCTGGCATAACGAAAGCCGCAAGC-3’ (stop-codon underlined).*

*Purification and Characterization of the E. coli GluTR Deletion Mutant A338Stop—Purification and refolding of the truncated protein was performed in analogy to the wild type enzyme (7). The yield of refolded protein was 4 mg from 0.6 g of inclusion bodies. In the final concentrated fraction a single protein band on a SDS-polyacrylamide gel was visible after Coomassie Blue staining. The calculated molecular mass of the mutant enzyme deduced from the gene sequence (40,112 Da) was experimentally confirmed using electrospray ionization mass spectrometry and by N-terminal protein sequencing as described above (data not shown). Analytical gel filtration chromatography, performed as described previously (12) resulted in a single, well resolved peak. The cofactor absorption spectrum indicated a peak at 330 nm and another peak at 430 nm as described previously (3). The CD spectrum of the *E. coli* GSA-AM was comparable with that of the Synchococcus enzyme (10).*

*Co-immunoprecipitation Experiments Using Cell-free E. coli Extracts—Polyclonal rabbit antibodies against recombinant *E. coli* GluTR and GSA-AM were generated by Eurogentec (Seraing, Belgium). For co-immunoprecipitation using cell free extracts, a total of 0.5 g of aerobically grown *E. coli* BL21 (DE3) cells were harvested in the early exponential phase. The bacterial cell pellet was resuspended in 5 ml of Tris-HCl buffer, pH 8.0, containing 150 mM NaCl, 10 mM DTT, and 0.5% (v/v) of the detergent Nonitet P-40 (lysis-buffer). Cells were disrupted by sonication, and cell debris was removed by centrifugation for 45 min at 4 °C. The supernatant was loaded onto a 25-ml DEAE-Sepharose Fast Flow column ( XK 16 column, Amersham Biosciences, Freiburg, Germany) equilibrated with buffer A. After washing the column with 2 column volumes of buffer A, proteins were eluted with a linear gradient of 5 column volumes ranging from 0 to 1 M NaCl in buffer B. Fractions containing GSA-AM were pooled and concentrated by ultrafiltration using a Vivaspin-15 centrifugal concentrator with a molecular weight cut-off of 10,000 (Vivascience, Hannover, Germany). A final volume of 2 ml with a protein concentration of 45 mg/ml was chromatographed on a Superdex 75 prep grade, high load 26/60 gel filtration column (Amersham Biosciences) equilibrated previously with 20 mM HEPES-NaOH, pH 7.9, 100 mM NaCl, 10 mM DTT at a flow rate of 2.0 ml/min. Fractions containing GSA-AM were pooled and concentrated to 30 mg/ml (Vivascience-15 concentrator, molecular weight cut-off of 20,000). The newly established purification procedure for the *E. coli* GSA-AM yielded ~50 mg of protein/liter of bacterial culture purified to apparent homogeneity as judged by SDS-PAGE. The integrity of the enzyme preparation was experimentally verified by electrospray ionization mass spectrometry and by N-terminal protein sequencing as described above (data not shown). Analytical gel filtration chromatography, performed as described previously (12) resulted in a single, well resolved peak. The cofactor absorption spectrum indicated a peak at 330 nm and another peak at 430 nm as described previously (3). The CD spectrum of the *E. coli* GSA-AM was comparable with that of the Synchococcus enzyme (10).*
separated proteins were transferred onto polyvinylidene difluoride membranes using a Trans-Blot apparatus (semi-dry transfer cell, Bio-Rad) according to the manufacturer's instructions. The mem-
brane was first incubated with anti-GSA-AM or anti-GluTR rabbit
antibodies (1:30,000 in phosphate-buffered saline (13) with 3% bovine serum albumin), washed three times with phosphate-buffered saline, and then incubated with alkaline phosphatase-conjugated sheep anti-rabbit antibodies (1:20,000 in phosphate-buffered saline with 3% bovine serum albumin) from Pierce (Bonn, Germany). The detection of immunoreactive bands was performed using the nitro blue tetra-
zolium/5-bromo-4-chloro-3-indolyl phosphate color developing method from Promega (Mannheim, Germany).

Co-immunoprecipitation Experiments Using Purified Recombinant E. coli GluTR and GSA-AM—For co-immunoprecipitation using purified enzymes 1 \( \mu \)g wild type GluTR or GluTR deletion mutant A33SStop, respectively, and 1 \( \mu \)g of GSA-AM were analyzed in 50 mM HEPES-NaOH, pH 8.0, 150 mM NaCl, 10 mM MgCl\(_2\), 5 mM DTT, 10% (v/v) glycerol, 0.1% (w/v) bovine serum albumin, and 0.05% (v/v) Tween 20 ( assay buffer) containing 500 \( \mu \)M glutamate, 4 mM ATP, 2 mM NADPH, 500 \( \mu \)M PLP, 1 \( \mu \)g E. coli glutamyl-tRNA synthetase (GluRS), and 20 \( \mu \)g E. coli tRNA preparation containing ~37% tRNA\(^{\text{Glu}}\) acceptor activity prepared as described elsewhere (14). 100 \( \mu \)l of this assay mixture were incubated for 10 min at 4 °C or alternatively for 2 min at 37 °C with 300 \( \mu \)l of 1 \( \mu \)l anti-GluTR (5 mg/ml) or anti-GSA-AM serum (8 mg/ml), respectively, was added to the assay mixture and incubation was continued for 30 min at 4 °C. Co-
precipitation and immunodetection was performed analogous to the experiments using cell-free extracts as described above.

Analysis of GluTR-GSA-AM Interaction by Gel Filtration according to Hummel and Dreyer—The Hummel and Dreyer method (15) is based on the detection of the equilibration of a gel filtration column equilibrated with a given concentration of a compound of interest (GSA-AM) by the presence of a weak interaction partner (GluTR). For this purpose a defined amount of the tested interaction partner (GluTR) is injected onto a gel filtration system equilibrated with GSA-AM, and elution profiles are recorded spectroscopically. The binding of GluTR to GSA-AM results in a local deficit of GSA-AM in the eluent, allowing the determination of the binding constant (15, 16). An analytical Superdex 200 PC 3.2/100 (Amer-
sham Biosciences) was used to investigate the binding of GluTR to GSA-
AM. The chromatography buffer contained 50 mM HEPES-NaOH, pH 8.0, 150 mM NaCl, 5 mM DTT, and 10 \( \mu \)g GSA-AM. After equilibration with 2 column volumes of chromatography buffer a 20-\( \mu \)l injection of various concentrations of GluTR (2–20 \( \mu \)g) dissolved in the chromatography buffer, including 10 \( \mu \)g GSA-AM, was performed. Chromatography was set at a flow rate of 100 \( \mu \)l/min. Protein concentration determination was followed by monitoring of absorbance at 280 nm. Analogous experiments were per-
fomed using GSA-AM at a concentration of 5 \( \mu \)g.

Enzyme Assays and HPLC Analysis of Reaction Products—The substrate \([14\text{C}]\text{Glu-tRNAGlu}\) was prepared in a bulk reaction using 100 \( \mu \)l of [14C]Glu-tRNAGlu and incubated at 37 °C for 8 min. HPLC analysis revealed the ratio of reaction products. Reactions with GluTR were performed in the presence of a weak interaction partner (GluTR). For this purpose a defined amount of a compound of interest (GSA-AM) was added to the assay mixture and incubation was continued for 30 min at 4 °C. The presence of GSA-AM in the eluent, allowing the determination of the corresponding genes was overexpressed. Both of the comple-
ments between GluTR from M. kandleri and E. coli, as well for GSA-AM from Synechococcus and E. coli, were carried out using the program ClustalW (18). The modeled E. coli structures were generated using the program BRAGI (19). The model complex was created by placing GSA-AM in the open space delimited by GluTR and docking them along their 2-fold symmetry axes as described previously (5). The image was generated by using PyMOL (20).

RESULTS AND DISCUSSION
E. coli GluTR and GSA-AM form a Complex in Cell-free Extracts—In silico experiments suggested a complex between GluTR and GSA-AM to protect the labile GSA from hazardous exposure to the aqueous environment. To analyze for the presence of the proposed complex, co-immunoprecipitation experi-
ments were conducted. For this purpose rabbit anti-GluTR and anti-GSA-AM antibodies were generated. The employed strategy involved the recognition of one protein with the specific antibody, immobilization of the antibody-antigen complex on Protein A-Sepharose, and its isolation via centrifugation and washing. In the case of a co-immunoprecipitated interaction partner, this protein was visualized in Western blot experi-
ments using a second antibody directed against it. Co-immunoprecipitation experiments with anti-GluTR and anti-
GSA-AM antibodies were performed first with cell-free extracts prepared from wild type E. coli BL21 (DE3) cultures harvested in the early exponential growth phase. These cells contained the natural amounts of both enzymes because none of the corresponding genes was overexpressed. Both of the comple-
ments co-immunoprecipitation experiments resulted in the precipitation of the postulated GluTR-GSA-AM complex. Sig-
nificant amounts of complexed protein were detected with the corresponding anti-GSA-AM antibody (Fig. 2A, lane 2) and anti-GluTR antibody, respectively (Fig. 2B, lane 2). In the supernatant of the co-precipitates, only residual amounts of the interacting protein partners were detected using Western blot analy-
sis (data not shown). In a control experiment E. coli strain EV61, which carries a disrupted gene for GluTR, was cultivated (21), and a cytosolic extract was prepared analogously to E. coli BL21 (DE3). No immunoprecipitation of GluTR or of GSA-AM by anti-GluTR antibodies was observed (data not shown). In agreement, neither of the pre-immune sera taken

FIG. 2. Western blot analysis of the co-immunoprecipitation of the GluTR-GSA-AM complex from cell-free E. coli extracts. Proteins of an E. coli BL21 (DE3) cell-free extract (40 mg/ml total protein) were immunoprecipitated (IP) using the following antibodies and sub-
sequently Protein A-Sepharose: A, lane 1, with rabbit pre-immune serum (negative control); lane 2, with rabbit anti-GluTR; lane 3, with rabbit anti-GSA-AM; B, lane 1, with rabbit pre-immune serum (nega-
tive control); lane 2, with rabbit anti-GSA-AM; lane 3, with rabbit anti-GluTR. Complex formation between E. coli GluTR and GSA-AM was visualized by the detection (DT) of the co-precipitated corresponding protein partner with anti-GSA-AM antibodies in A and anti-GluTR antibodies in B via Western blotting.
prior to the immunization of the rabbits reacted with E. coli GluTR or GSA-AM, respectively (Fig. 2, A and B, lane 1) Because of the known low cellular concentration of both enzymes, only highly concentrated extracts from 20 to 40 mg/ml protein resulted in clear co-immunoprecipitation results. Interestingly, cell-free extracts prepared from stationary phase-grown E. coli did not contain detectable amounts of the GluTR-GSA-AM complex (data not shown). Possibly because of lower heme requirements in the stationary phase, heme-induced GluTR proteolysis decreased cellular GluTR concentrations (22). Clearly, a stable GluTR-GSA-AM complex detectable via co-immunoprecipitation is present in E. coli cell-free extracts.

Complex Formation between Purified E. coli GluTR and GSA-AM is Glutamyl-tRNA- and Cofactor-independent—To further study the prerequisites for the observed interaction between E. coli GluTR and GSA-AM, co-immunoprecipitation experiments using recombinant purified enzymes at protein concentrations of 1 μM were performed. Incubation of the assay mixture prior to immunoprecipitation was carried out in the presence and absence of Glu-tRNA<sub>Glu</sub> and catalytically important cofactors such as NADPH and PLP at both 4 °C and 37 °C. At both preincubation temperatures the GluTR-GSA-AM complex was precipitated from the assay mixture independently of the addition of the mutant enzyme compared with the precipitated GluTR with anti-GluTR antibody is lower in the case of the GluTR-A338Stop.

GluTR Dimerization Enhances GluTR-GSA-AM Complex Formation—To study the role of the C-terminal domain in complex formation in vitro co-immunoprecipitation experiments were performed using the GluTR-A338Stop mutant lacking the dimerization domain. Gel filtration chromatography indicated a native relative molecular mass of 39,000 ± 3,000 Da for the truncated protein. Based on these results it was concluded that the GluTR-A338Stop-mutant (40,112 Da calculated molecular mass) is a globular monomeric two-do-
Fig. 5. Comparison of sequential and coupled GluTR- and GSA-AM-mediated ALA formation. The reaction products derived from $[^{14}C]$Glu-tRNA$_{tRNAGlu}$ isolated from different assay mixtures were separated via HPLC chromatography at a flow rate of 0.7 ml/min on the Waters Bondapack C$_{18}$ reverse phase column (3.9 × 150 mm, 125 Å pore size, 10-μm particle diameter). 350-μl fractions were collected, and the $[^{14}C]$ reaction products were quantified using scintillation counting. The C$_{18}$ reverse phase column was equilibrated using $[^{14}C]$glutamate (Glu), $[^{14}C]$ALA, and $[^{14}C]$GSA (data not shown). The reaction products were identified by scintillation counting. Because of the spontaneous hydrolysis of the substrate Glu-tRNA$_{tRNAGlu}$, an additional radioactive compound (2.6 min) besides the $[^{14}C]$ALA end product.

Additional compound at 2.6 min was no substrate for GSA-AM catalysis (Fig. 5B). In coupled in vitro assays, allowing complex formation prior to substrate addition, this additional compound was not detectable (Fig. 5C). On the basis of these observations one might speculate that one essential role of GluTR-GSA-AM complex formation is to prevent the side reaction of the reactive GSA aldehyde species, possibly with cellular compounds or the solvent. Another possible reaction has been described earlier during the chemical synthesis of GSA in which a cyclization of GSA to 2-hydroxy-3-aminoetathypyrany-1-one was described (23). To date no physical characterization of that compound has been reported. Because of the minimal amounts of intermediate formed in the assay mixture (~5 pmol) no further characterization of this side product was possible. The experiments clearly demonstrated that the semialdehyde species was protected from an inefficient side reaction by the presence of GSA-AM. However they do not rule out the possibility that a very rapid GSA-AM reaction in the coupled assay might also result in the protection of GSA. Nevertheless, these findings are in clear agreement with the postulated substrate channeling pathway as indicated by x-ray crystallography and by modeling experiments (5) in which the intermediate aldehyde is prevented from exposure to the aqueous environment.

The current investigation was one of the rare cases in which the structural biology of related enzymes from different organisms directly give the answer to a metabolic question. The present investigation demonstrates the existence of a GluTR-GSA-AM complex in E. coli, which might indicate that the original structure-based complex model can be regarded of general significance for the GlutTR-GSA-AM interaction in plants, archaea, and all bacteria synthesizing ALA from Glu-tRNA$_{tRNAGlu}$.

Acknowledgments—We thank Rita Getzlaff for N-terminal protein sequencing and Manfred Nimtz (both from the German Research Center for Biotechnology, Braunschweig) for mass spectrometry analysis. We are indebted to Nicole Franken-Kendel for the gel filtration column and Ronja Tasler for advice during the Hummel-Dreyer experiments.

REFERENCES
1. Schön, A., Krupp, G., Gough, S., Berry-Lowe, S., Kannangara, C. G., and Soll, D. (1986) Nature 322, 281–284
2. Jahn, D., Verkamp, E., and Soll, D. (1992) Trends Biochem. Sci. 17, 215–218
3. Ilag, L. L., and Jahn, D. (1992) Biochemistry 31, 7143–7151
4. Moser, J., Lorenz, S., Hubscherlein, C., Rompf, A., and Jahn, D. (1999) J. Biol. Chem. 274, 30679–30685
5. Moser, J., Schubert, W. D., Beier, V., Bringemeier, I., Jahn, D., and Heinz, D. W. (2001) EMBO J. 20, 6583–6590
6. Schauer, S., Chaturvedi, S., Randau, L., Moser, J., Kitabatake, M., Lorenz, S., Verkamp, E. M., Wall, W. D., Nakayashiki, T., Murai, M., Wall, X., Thamm, H. U., Heinz, D. W., Inokuchi, H., Soll, D., and Jahn, D. (2002) J. Biol. Chem. 277, 48657–48663
7. Schauer, S., Luer, C., and Moser, J. (2003) Protein Expression Purif. 31, 171–275
8. Schubert, W. D., Moser, J., Schauer, S., Heinz, D. W., and Jahn, D. (2002) Photosynth. Res. 74, 205–215
9. Hooper, J. K., Kahn, A., Ash, D. E., Gough, S., and Kannangara, C. G. (1988) Carlsberg Res. Commun. 53, 11–25
10. Hennig, M., Grimm, B., Contestabile, R., John, R. A., and Jansonius, J. N. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 4866–4871
11. Ilag, L. L., Jahn, D., Eggertsson, G., and Soll, D. (1991) J. Bacteriol. 173, 3408–3413
12. Franken-Kendel, H., Heinz, D. W., and Jahn, D. (1999) Biochimie 81, 1096–10975
13. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd Ed., pp. 18.47–18.55, B12, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
14. Borel, F., Hartlein, M., and Leberman, R. (1993) FEBS Lett. 324, 162–166
15. Hummel, J. P., and Dreyer, W. J. (1990) Biochim. Biophys. Acta 630, 530–532
16. Beekmann, S. (1999) Methods 19, 278–305
17. Lin, S. X., Brison, A., Liu, J., Roy, P. H., and Lapointe, J. (1992) Protein Expression Purif. 3, 71–74
18. Chenna, R., Sugawara, H., Kikue, T., Lopez, R., Gibson, T. J., Higgins, D. G., and Thompson, J. D. (2003) Nucleic Acids Res. 31, 3967–3970
19. Reichelt, J., Dieterich, G., Kvesic, M., Schomburg, D., and Heinz, D. W. (2005) Bioinformatics 21, 1291–1293
20. DeLano, W. L. The PyMOL Molecular Graphics System (2002) DeLano Scientific, San Carlos, CA
21. Verkamp, E., and Chelmin B. K. (1989) J. Bacteriol. 171, 4728–4735
22. Wang, L., Elliott, M., and Elliott, T. (1999) J. Bacteriol. 181, 1211–1219
23. Jordan, P. M. (1990) in Biosynthesis of Heme and Chlorophylls (Dailey, H. A., ed) McGraw-Hill, New York