The initial reaction of tetrapyrole formation in archaears is catalyzed by a NADPH-dependent glutamyl-tRNA reductase (GluTR). The hema gene encoding GluTR was cloned from the extremely thermophilic archaeon Methanopyrus kandleri and overexpressed in Escherichia coli. Purified recombinant GluTR is a tetrameric enzyme with a native $M_\text{r} = 190,000 \pm 10,000$. Using a newly established enzyme assay, a specific activity of 0.75 nmol h$^{-1}$ mg$^{-1}$ at 56 °C with E. coli glutamyl-tRNA as substrate was measured. A temperature optimum of 90 °C and a pH optimum of 8.1 were determined. Neither heme cofactor, nor flavin, nor metal ions were required for GluTR catalysis. Heavy metal compounds, Zn$^{2+}$, and heme inhibited the enzyme. GluTR inhibition by the newly synthesized inhibitor glutamyacin, whose structure is similar to the $3\'$ end of the glutamyl-tRNA substrate, revealed the importance of an intact chemical bond between glutamate and tRNA$^{\text{Glu}}$ for substrate recognition. The absolute requirement for NADPH in the reaction of GluTR was demonstrated using four NADPH analogues. Chemical modification and site-directed mutagenesis studies indicated that a single cysteiny1 residue and a single histidiny1 residue were important for catalysis. It was concluded that during GluTR catalysis the highly reactive sulfhydryl group of Cys-48 acts as a nucleophile attacking the $\alpha$-carbonyl group of tRNA-bound glutamate with the formation of an enzyme-localized thioester intermediate and the concomitant release of tRNA$^{\text{Glu}}$. In the presence of NADPH, direct hydride transfer to enzyme-bound glutamate, possibly facilitated by His-84, leads to glutamate-1-semialdehyde formation. In the absence of NADPH, a newly discovered esterase activity of GluTR hydrolyzes the highly reactive thioester of tRNA$^{\text{Glu}}$ to release glutamate.

ALA$^1$ is the general precursor molecule for the biosynthesis of tetrapyroles like chlorophylls, hemes, and coenzyme B$_{12}$ (1).

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank$^{17}$ / EBI Data Bank with accession number(s) AJ131561.

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‡ The abbreviations used are: ALA, 5-aminolevulenic acid; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GluRS, glutamyl-tRNA synthetase; GluTR, glutamyl-tRNA reductase; GSA-AT, glutamate-1-semialdehyde-2,1-aminomutase; HPLC, high performance liquid chromatography; IPTG, isopropyl-$\beta$-thiogalactopyranoside; TPCK, N-tosyl-l-phenylalaninchloromethyl ketone.

Plants, archaea, and the majority of bacteria form ALA in a two-step reaction from the C$_2$-skeleton of glutamate bound to tRNA$^{\text{Glu}}$ (glutamyl-tRNA) (2). The initial enzyme of the pathway GluTR, encoded by hema, reduces the activated $\alpha$-carboxyl group of glutamate using NADPH to form GSA. GSA is then converted to ALA by GSA-AT (2). Investigation of GluTR catalysis has been hampered by its low cellular concentration, difficulties with the overexpression of the respective gene from various species in Escherichia coli, and the tendency of the enzyme to aggregate and precipitate at high protein concentration (3, 4). Earlier characterizations with purified GluTR from barley, from the green alga Chlamydomonas reinhardtii, and from the bacteria Bacillus subtilis, Synecochystis sp. PCC 6803, and E. coli resulted in the description of highly variable molecular masses, specific activities, and catalytic properties (4–8).

Recently, characterization of barley GluTR expressed as a fusion protein with glutathione S-transferase indicated the participation of a heme cofactor in the catalysis of the pentameric enzyme (9, 10). Nevertheless, central questions concerning the exact enzymatic mechanism of GluTR and its structural basis remain to be answered. Here, we describe the utilization of recombinant GluTR from the extreme thermophilic archaeon Methanopyrus kandleri for the determination of the native molecular mass and the elucidation of essential structural features of the substrate and the cofactor NADPH for enzyme recognition. To achieve this, a new test system was established and a new GluTR-specific inhibitor was synthesized. Protein modification, site-directed mutagenesis, and enzymatic activity analysis were used to gain first insights into the molecular basis of GluTR catalysis. Evidence for an enzyme-localized thioester intermediate was obtained, allowing us to propose a mechanism for GluTR catalysis.

**EXPERIMENTAL PROCEDURES**

**Cloning of the M. kandleri hema Gene**

The ALA-uxotrophic E. coli hema strain GE1387 was transformed with a M. kandleri genomic library prepared in the vector pBluescript SK+, and complementing clones were identified as described before for the cloning of Methanobacterium thermoautotrophicum hema (11).

**Construction of the M. kandleri hema Expression Vector pMkhema**

A 1228-base pair fragment encoding the 404 amino acid residues of M. kandleri GluTR was amplified by polymerase chain reaction using the primer IM1 (5'-GGAGGGCATATTGGGAGGTCGCGC-3') and the primer IM2 (5'-CCCCTTCGGTACTAACGCCGTCGCGC-3'). The resulting polymerase chain reaction fragment was digested with NdeI and EcoRI (underlined in the primer sequences) and ligated into the appropriately digested vector pETs (Strategen, Heidelberg, Germany) to generate pMkhema.

**Site-directed Mutagenesis of M. kandleri hema**

To exchange amino acid residues of M. kandleri GluTR, the Quickchange$^{TM}$ Kit (Strategen, Heidelberg, Germany) was used according to the manufacturer’s instructions. The following oligonucleotides, with newly introduced codons underlined, were employed to generate the mutants indicated: for C68 GGAGGACCTGTTAGCCGTCGTTATCAC-
Bound proteins were eluted using a linear 100-ml gradient of 0–1 M onto a MonoQ HR 10/10 column previously equilibrated with buffer A. The protein solution (10 mg/ml) was loaded at a concentration of 5 mg of protein per ml of column volume. Fractions containing GluTR were pooled, dialyzed against buffer A, and concentrated to 7 mg/ml by ultrafiltration as described above. The protein showed a high degree of amino acid sequence identity to archaeal, bacterial, and plant GluTRs (data not shown).

For each employed condition served as background controls for spontaneous substrate hydrolysis. GluTR activity was measured by this first-order equation by a method described previously (16). This procedure was necessary since the limiting amounts of pure enzyme did not allow the excess of substrate required for classical Michaelis-Menten kinetics.

High Performance Liquid Chromatography (HPLC) Analysis of GluTR Product Formation

Standard assay mixtures were performed as outlined above with an incubation at 56 °C for 7 min. In most cases the reaction product GSA was further identified by its specific conversion into ALA using purified E. coli GluRS (17). Reaction products were analyzed on a Waters Bondapak™ C18 reversed phase column (3.9 × 150 mm, 125 Å pore size, 10 µm particle diameter) as described before (18). The radioactive GluTR substrate [14C]-Glu-tRNAGlu still contained residual amounts of free [14C]Glu. Under the employed separation conditions, the commercially available [14C]Glu (ICN Pharmaceuticals, Irvine, CA) appeared as a double peak at 3 and 4.5 min retention time, with a small third peak at 8 min retention time (Fig. 3).
using a T7 RNA polymerase-driven expression system. Recombinant GluTR was purified to apparent homogeneity as described under “Experimental Procedures”. Fig. 1 shows an SDS-polyacrylamide gel electrophoresis analysis of proteins contained in cell-free extracts prepared from E. coli BL21 (DE3) carrying pMkhemA before IPTG induction (lane 1), after IPTG induction (lane 2), after chromatography on Red Sepharose CL-GB (lane 3), after MonoQ (lane 4), and after Superdex 200 and protein concentration (lane 5). Lane M represents the dalton marker.

A Novel Substrate Depletion Assay for Testing GluTR Activity—Purified recombinant GluTR was used to establish a new GluTR assay based on substrate utilization measurements. The assay originates from the well established and frequently used aminocyl-tRNA synthetase assay, in which the product aminocyl-tRNA is recovered after the reaction by acid precipitation (15). However, due to the principle of substrate detection, real product formation was always verified using HPLC analysis. This was necessary when significant changes in supplied cofactors, inhibitors, or reaction conditions were introduced to the assay. Under the standard assay conditions, outlined under “Experimental Procedures”, M. kandleri GluTR showed a specific activity of 0.75 nmol h\(^{-1}\) mg\(^{-1}\) of protein. This specific activity was usually set to 100% activity, and other values obtained were related to that. A reaction temperature of 56 °C instead of the measured temperature optimum of 90 °C (see below) was used to stabilize the employed substrate glutamyl-tRNA. The heterologous substrate E. coli glutamyl-tRNA was routinely used, since the supply of the extremely limited. The specific activity measured with homologous substrate M. kandleri glutamyl-tRNA at 90 °C was only approximately 3–4 times higher compared with the routinely used conditions with E. coli glutamyl-tRNA at 56 °C (data not shown).

As shown in Fig. 2, recombinant M. kandleri GluTR efficiently utilized the E. coli glutamyl-tRNA substrate. Under the assay conditions at 56 °C, only slow substrate hydrolysis (approximately 10% after 5 min) was observed. The reduction of glutamyl-tRNA to the product GSA was visualized using HPLC analysis (Fig. 2, panel B). Addition of purified E. coli GSA-AT to the reaction mixture resulted in the almost complete conversion of GSA into ALA (Fig. 2, panel B).
Influence of Temperature, pH, and Ionic Strength on M. kandleri GluTR Activity and Stability—To determine the structural features of glutamyl-tRNA for GluTR recognition, we studied various factors affecting GluTR activity. Results showed that GluTR activity was significantly affected by temperature, pH, and ionic strength. At high temperatures, the enzyme lost activity. The optimal pH for GluTR activity was 7.0, and its activity was not significantly affected by ionic strength.

Metal Ions Are Not Required for M. kandleri GluTR Activity—To test the requirement of metal ions, we performed experiments with purified M. kandleri GluTR. Results indicated that metal ions were not required for GluTR activity, as there was no significant difference in activity between samples with and without metal ions.

Structural Features of the Substrate Glutamyl-tRNA for GluTR Recognition—To investigate the structural features of GluTR, we tested various inhibitors and substrates. Results showed that GluTR activity was inhibited by several inhibitors, including glutamycin and heme. The enzyme was also inhibited by synthetic inhibitors mimicking the intact chemical bond between glutamate and tRNA.

results and the calculated subunit molecular mass of 45,448 Da, we conclude that M. kandleri GluTR is a tetrameric protein.

| TABLE I | Catalytic properties of M. kandleri GluTR |
|---------|----------------------------------------|
| Additions | Concentration | GluTR activity (%) |
| None | 100 | 100 |
| L-Glutamate | 10.0 mM | 105 |
| D-Glutamate | 10.0 mM | 110 |
| Glutaric acid | 5.0 mM | 90 |
| 4,5-Dioxovaleric acid | 5.0 mM | 110 |
| 4,5-Diaminovaleric acid | 5.0 mM | 105 |
| Glutamate-1-semialdehyde | 1.0 mM | 50 |
| E. coli tRNA_{Glu} | 0.1 mM | 110 |
| M. kandleri tRNA_{Glu} | 0.1 mM | 95 |
| Glutamycin | 0.1 mM | 100 |
| Heme | 0.1 mM | 105 |
| FAD | 10.0 mM | 95 |
| PMN | 10.0 mM | 105 |
| Pyridoxal 5’-phosphate | 0.1 mM | 110 |
| EDTA | 10.0 mM | 95 |
| EGTA | 10.0 mM | 95 |
| 1,10-Phenanthroline | 5.0 mM | 110 |
| 2,2’-Dipyridyl | 5.0 mM | 95 |
| MgCl$_2$ | 10.0 mM | 110 |
| CaCl$_2$ | 10.0 mM | 90 |
| CoCl$_2$ | 1.0 mM | 90 |
| NiCl$_2$ | 5.0 mM | 95 |
| ZnCl$_2$ | 5.0 mM | 25 |
| PbCl$_2$ | 5.0 mM | 10 |
| PtCl$_4$ | 1.0 mM | 45 |
| K$_2$PtCl$_4$ | 1.0 mM | 10 |
| Iodoacetamide$^b$ | 1.0 mM | 70 |
| TPCK$^c$ | 0.1 mM | 10 |
| 5,5’-Dithiobis(2-nitrobenzoic acid)$^d$ | 0.1 mM | 70 |
| 1.0 mM | 10 |

$^a$ Indicated substances were pH adjusted and pre-incubated with purified M. kandleri GluTR under standard assay conditions as outlined under “Experimental Procedures” for 20 min before reactions were started with the addition of the substrate, radioactive E. coli glutamyl-tRNA.

$^b$ GluTR activity was measured using the substrate depletion assay as outlined under “Experimental Procedures.” Product formation was verified using HPLC analysis. GluTR specific activity obtained without additions was set to 100%, and all other measured GluTR specific activities were related to that.

$^c$ Purified M. kandleri GluTR was pre-treated for 45 min at room temperature with indicated amounts of modifying reagent or potential inhibitor. Residual reagent was removed from the assay by centrifugation through a G50 spin column under test conditions before activity determination.

$^d$ ND, not detectable.
dinucleotide phosphate, reduced form) converted the cofactor into a potential inhibitor with 50% inhibition at a concentration of 1 mM. Removal of the adenosine phosphate part (β-nicotinamide mononucleotide, reduced form) completely abolished cofactor utilization by the enzyme. Interestingly, flexibility for the localization of the phosphoryl group of the adenosine ribose between the 2′ (NADPH) and 3′ (3′NADPH) position was observed. However, as mentioned above, experiments using NADH demonstrated the absolute requirement for the presence of the phosphoryl group. These experiments showed an overall requirement for all the major determinants of NADPH for efficient recognition and utilization by GluTR. Tight NADPH coordination by the enzyme was concluded.

TABLE II

| Additions | Reducing agent | Pre-incubation temperature and time | Assay temperature | pH | GluTR activity |
|-----------|----------------|------------------------------------|-------------------|----|----------------|
| NADPH     | 56°C, 1 h      | 60                                 | 6.0°C             | 60 |               |
| NADPH     | 56°C, 1 h      | 65                                 | 6.0°C             | 65 |               |
| NADPH     | 56°C, 1 h      | 70                                 | 6.0°C             | 70 |               |
| NADPH     | 56°C, 1 h      | 95                                 | 6.0°C             | 95 |               |
| NADPH     | 56°C, 1 h      | 80                                 | 6.0°C             | 80 |               |
| NADPH     | 56°C, 1 h      | 95                                 | 6.0°C             | 95 |               |
| NADPH     | 56°C, 1 h      | 100                                | 6.0°C             | 100|               |
| NADPH     | 56°C, 1 h      | 70                                 | 6.0°C             | 70 |               |
| NADPH     | 56°C, 1 h      | 130                                | 6.0°C             | 130|               |
| NADPH     | 56°C, 1 h      | 170                                | 6.0°C             | 170|               |
| NADPH     | 90°C, 1 h      | 210                                | 6.0°C             | 210|               |
| NADPH     | 95°C, 1 h      | 150                                | 6.0°C             | 150|               |
| 1.0 M NaCl| NADPH          | 95°C, 1 h                          | 60                | 95 |               |
| 1.0 M NH4Cl| NADPH        | 95°C, 1 h                          | 60                | 95 |               |
| NADH      | 56°C, 1 h      | 100                                | 6.1°C             | 100|               |
| NHDP      | 56°C, 1 h      | ND                                 | 6.1°C             | ND |               |
| NM        | 56°C, 1 h      | ND                                 | 6.1°C             | ND |               |
| 3′-NADPH  | NADPH          | 10                                 | 6.1°C             | 10 |               |
| 5 mM NADH | NADPH          | 100                                | 6.1°C             | 100|               |
| 5 mM NM   | NADPH          | 100                                | 6.1°C             | 100|               |
| 5 mM 3′-NADPH | NADPH      | 100                                | 6.1°C             | 100|               |
| 1 mM NHDP | NADPH          | 50                                 | 6.1°C             | 50 |               |
| 5 mM NHDP | NADPH          | 20                                 | 6.1°C             | 20 |               |

a 2 mM NADPH, NADH, NHDP, NM, and 3′-NADPH were used. NADH, NHDP, NM, and 3′-NADPH were preincubated with GluTR for 10 min at 56 °C before assays were started.

b For the pH titration, the following buffer systems were used: 50 mM phosphate, 50 mM Na-BICINE, and 50 mM Na-HEPES (see footnotes c–g).

c All assays were performed in 5 times the usual assay volume for periods of 30 s to 2 min. GluTR activity was measured using the substrate depletion assay as outlined under “Experimental Procedures.” Product formation was verified using HPLC analysis. GluTR specific activity obtained without additions was set to 100%, and all other measured GluTR specific activities were related to that. Background controls without enzyme addition measuring substrate hydrolysis were performed for each employed condition and subtracted.

d During incubation at 85–95 °C, assays were overlaid with mineral oil to prevent evaporation.

e 50 mM phosphate.

f 50 mM Na-BICINE.

g 50 mM Na-HEPES.

h ND, not detectable.

FIG. 3. Structure of the GluTR inhibitor glutamycin compared with the 3′ end of glutamyl-tRNA.

GluTR Modification Using Iodoacetamide, TPCK, and 5,5′-Dithiobis(2-nitrobenzoic Acid) Inhibits Enzyme Activity—An initial proposal suggested the back reaction catalyzed by GAPDH as a mechanistic model for GluTR activity (2). GAPDH catalysis involves an active site cysteinyl residue, which forms a thioester with the substrate after NAD−-dependent oxidation (21). To analyze M. kandleri GluTR catalysis for a potential participation of active site-localized nucleophilic amino acid residues, chemical protein modification experiments were performed. As shown in Table I, treatment of M. kandleri GluTR with iodoacetamide, TPCK, and 5,5′-dithiobis(2-nitrobenzoic acid) totally abolished enzyme activity. In agreement with the observed heavy metal sensitivity, these results suggested the
presence of one or more nucleophilic cysteinyi residues involved in catalysis. Additionally, potential histidinyl residue involvement in enzyme activity was suggested.

**GluTR Possesses Glutamyl-tRNA Esterase Activity**—The enzyme efficiently utilized glutamyl-tRNA in the absence of NADPH. However, HPLC analysis of the resulting reaction products revealed the liberation of glutamate from the tRNA (Fig. 2). The catalytic rate of GluTR-dependent glutamyl-tRNA esterase activity observed in the absence of NADPH was comparable to the catalytic rate of the GluTR reductase activity in the presence of NADPH. Esterase activity is a reaction typical of other enzymes forming a covalent acyl-enzyme intermediate involving an active site cysteinyl residue like GAPDH, thiol proteinases, and aldehyde dehydrogenases (22–24).

**Mutation C48S Totally Abolishes GluTR Reductase and Esterase Activity**—To identify definitely the cysteinyl residues important in GluTR catalysis, all 5 cysteinyl residues of the enzyme located at positions 6, 42, 48, 90, and 393 were individually changed to serine residues. Only GluTR mutant C48S had completely lost its reductase and esterase activities (Table III). In agreement with this result, Cys-48 was identified as the only cysteinyl residue conserved in all known GluTR enzymes. All other mutants retained their full catalytic activity (Table III). These observations provided evidence for a role of Cys-48 as the active site nucleophile.

**The Role of His-84 in GluTR Catalysis**—Inhibition of *M. kandleri* GluTR by TPCK indicated a potential involvement of histidinyl residues in catalysis. In the case of cysteine proteinases, the formation of a Cys–His ion pair between the active site cysteinyl residue and a conserved histidinyl residue was found essential for the nucleophilic character of the catalytic cysteine. A conserved histidine of GAPDH acted as a base catalyst facilitating hydride transfer toward NAD\(^+\) (25). All known GluTR enzymes contain one completely conserved histidinyl residue, which is located at position 84 of *M. kandleri* GluTR. To distinguish between the potential functions of His-84, the two mutant GluTRs H84A and H84N were analyzed. GluTR-H84A had significantly reduced enzymatic activity indicating the general importance of His-84 for GluTR catalysis (Table III). The GluTR-H84N mutant still possessed 30% of wild type reductase activity and 15% of esterase activity. Analogous to the findings recently obtained for similar mutants of GAPDH these results make the participation of His-84 in Cys-48 reactivity enhancement very unlikely (25). The potential role of His-84 as a base catalyst in facilitating hydride transfer toward the activated glutamate will be subject to future investigations.

**An Enzymatic Mechanism for *M. kandleri* GluTR: A Proposal**—Based on the results obtained, we postulate that in analogy to the formal back reaction of GAPDH the nucleophilic Cys-48 of GluTR attacks the α-carbonyl group of glutamate activated by an ester linkage to tRNA\(^{Glu}\). Subsequently, a highly reactive enzyme-localized thioester is formed and free tRNA\(^{Glu}\) is released. Direct hydride transfer from NADPH, potentially facilitated by His-84, leads to the formation and the release of GSA and NADP\(^+\). In the absence of NADPH, the thioester is hydrolyzed by GluTR esterase activity and glutamate is released (Fig. 4).

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REFERENCES

1. Jordan, P. M. in New Comprehensive Biochemistry (Neuberger, A., and van Deenen, L. L. M., eds) Vol. 19, pp. 1–66, Elsevier, Amsterdam
2. Jahn, D., Verkamp, E., and Soll, D. (1992) Trends Biochem. Sci. 17, 215–218
3. Verkamp, E., Jahn, M. Jahn, D., Kumar, A. M., and Soll, D. (1992) J. Biol. Chem. 267, 8275–8280
4. Schröder, I., Hederstedt, L., Kannangara, C. G., and Gough, S. (1992) Biochem. J. 281, 843–850
5. Chen, M.-W., Jahn, D., O’Neill, G. P., and Soll, D. (1990) J. Biol. Chem. 265, 4058–4063
6. Jahn, D., Michelsen, U., and Soll, D. (1991) J. Biol. Chem. 266, 2542–2548
7. Rieble, S., and Beale, S. I. (1991) J. Biol. Chem. 266, 9740–9745
8. Pontoppidan, B., and Kannangara, C. G. (1994) Eur. J. Biochem. 255, 529–537
9. Vothknecht, U. C., Kannangara, C. G., and von Wettstein, D. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 9287
10. Vothknecht, U. C., Kannangara, C. G., and von Wettstein, D. (1996) Phytocchemistry 47, 513–519
11. Hungerer, C., Weiss, D. S., Thauer, R. K., and Jahn, D. (1996) Bioorg. Med. Chem. 4, 1089–1095
12. Frankenber, N., Heinz, D., and Jahn, D. (1999) Biochemistry, in press
13. Proulx, M., and Lapointe, J. (1985) Methods Enzymol. 113, 50–54
14. Chen, M.-W., Jahn, D., Schon, A. O’Neill, G. P., and Soll, D. (1990) J. Biol. Chem. 265, 4054–4057
15. Hok, P., and Soll, D. (1985) Methods Enzymol. 113, 55–59
16. Chapman, K. T., Kopka, J. E., Durett, P. J., Eser, C. K., Lanza, T. J., Izquierdo-Martín, M., Niedzwiecki, L., Chang, B., Harrison, R. K., Kus, D. W., Lin, T. Y., Stein, R. L., and Hagmann, W. K. (1993) J. Med. Chem. 36, 4293–4301
17. Ilag, L. L., Jahn, D., Eggertsson, G, and Soll, D. (1991) J. Bacteriol. 173, 3408–3413
18. Mau, Y.-H., Wang, W.-Y., Tamura, R. N., and Chang, T.-E. (1987) Arch. Biochem. Biophys. 255, 75–79
19. Kannangara, C. G., Andersen, R. V., Pontoppidan, B., Willows, R. D., and von Wettstein, D. (1994) Ciba Found. Symp. 180, 3–25
20. Haile, D. J., Rouault, T. A., Harford, J. B., and Klausner, R. D. (1990) J. Biol. Chem. 265, 12786–12789
21. Habenicht, A. (1997) Biol. Chem. 378, 1413–1419
22. Boschi-Muller, S., and Branlant, G. (1999) Arch. Biochem. Biophys. 363, 259–266
23. Nagradowa, N. K., and Schmalhausen, E. V. (1998) Biochemistry (Mosc.) 63, 504–515
24. Perrotich, J., Nicholas, H., Wang, B. C., Lindahl, R., and Hempel, J. (1999) Protein Sci. 8, 137–146
25. Talfournier, F., Colloch, N., Mornon, J. P., and Branlant, G. (1998) Eur. J. Biochem. 252, 447–457