Metabolism of Homocysteine-thiolactone in Plants*

Hieronim Jakubowski§§§ and Andrzej Guranowski‡

From the §Department of Microbiology and Molecular Genetics, University of Medicine and Dentistry of New Jersey (UMDNJ)-New Jersey Medical School, International Center for Public Health, Newark, New Jersey 07103, ¶Institute of Bioorganic Chemistry, Polish Academy of Sciences, 61-704 Poznań, Poland, and |Department of Biochemistry and Biotechnology, Agricultural University, 60-637 Poznań, Poland

Received for publication, November 20, 2002, and in revised form, December 17, 2002
Published, JBC Papers in Press, December 20, 2002, DOI 10.1074/jbc.M211819200

Editing of the amino acid homocysteine (Hcy) by certain aminocetyl-tRNA synthetases results in the formation of an intramolecular thioester, Hcy-thiolactone. Here we show that the plant yellow lupin, Lupinus luteus, has the ability to synthesize Hcy-thiolactone. The inhibition of methylation of Hcy to methionine by the antifolate drug aminopterin results in greatly enhanced synthesis of Hcy-thiolactone by L. luteus plants. Methionine inhibits the synthesis of Hcy-thiolactone in L. luteus, suggesting involvement of methionyl-tRNA synthetase. Consistent with this suggestion is our finding that the plant Oryza sativa methionyl-tRNA synthetase, expressed in Escherichia coli, catalyzes conversion of Hcy to Hcy-thiolactone. We also show that Hcy is a component of L. luteus proteins, most likely due to facile reaction of Hcy-thiolactone with protein amino groups. In addition, L. luteus possesses constitutively expressed, highly specific Hcy-thiolactone-hydrolyzing enzyme. Thus, Hcy-thiolactone and Hcy bound to protein by an amide (or peptide) linkage (Hcy-N-protein) are significant components of plant Hcy metabolism.

Homocysteine (Hcy)-thiolactone, a cyclic thioester of Hcy, was discovered by serendipity almost 70 years ago as a by-product of the digestion of methionine with hydriodic acid, a procedure used then for the determination of protein methionine (1). The discovery of an error editing reaction of aminoacyl-tRNA synthetases, in which Hcy is converted to Hcy-thiolactone, highlighted the biological significance of Hcy-thiolactone (2). Hcy-thiolactone is synthesized by methionyl-tRNA synthetase (MetRS) in bacterial (3–6), yeast (6–8), and mammalian, including human, cells (9–17). Isoleucyl-tRNA synthase and leucyl-tRNA synthase, in addition to MetRS, synthesize Hcy-thiolactone from exogenous Hcy, at least in bacteria (5). Hcy-thiolactone forms in a two-step reaction driven by the hydrolysis of ATP (2). In the first step, MetRS catalyzes reaction of Hcy with ATP, which leads to the formation of a MetRS-bound homocysteinyl adenylate.

MetRS + Hcy + ATP→MetRS·Hcy~AMP + PP;

REACTION 1

In the second step, MetRS catalyzes the reaction of the side chain thiolate of Hcy, which displaces the AMP moiety from the activated carboxyl group of Hcy; Hcy-thiolactone is a product of this reaction (REACTION 2).

rea

Hcy-thiolactone, an intramolecular thioester of Hcy, is relatively stable and has a half-life of about 25 h under physiological conditions of pH and temperature (10). Because the pK of its amino group is unusually low at 7.1 (18), Hcy-thiolactone freely diffuses through cellular membranes and accumulates in extracellular fluids (3–17). A characteristic ultraviolet absorption spectrum with a maximum at 240 nm allows facile detection and quantification of Hcy-thiolactone in biological samples (4, 5, 19). Like all thiocetates, Hcy-thiolactone is chemically reactive. For example, Hcy-thiolactone forms adducts with protein (Hcy-N-protein), in which the carboxyl group of Hcy is linked by an amide bond with ε-amino group of a protein lysine residue (10, 15, 20). The modification by Hcy-thiolactone results in protein damage (12–17, 20).

Although its role in cell physiology is largely unknown, Hcy-thiolactone has been suggested to be a positive effector of the stationary phase response in Escherichia coli (21) and is also likely to be involved in the regulation of methionine synthase gene expression in E. coli (6).

In humans, Hcy-thiolactone is likely to play a role in cardiovascular disease due to its ability to form Hcy-N-protein, which leads to protein damage (10–17, 20). A protein component of high-density lipoproteins, Hcy-thiolactone, detoxifies Hcy-thiolactone, thereby minimizing formation of Hcy-N-protein in humans (22, 23).

Whether Hcy-thiolactone is present and how it is metabolized in plants was unknown. Here we report that Hcy-thiolactone and Hcy-N-protein are components of Hcy metabolism in yellow lupine (Lupinus luteus). We also show that Hcy-thiolactone is synthesized by rice (Oryza sativa) methionyl-tRNA synthetase and degraded by a highly specific yellow lupine Hcy-thiolactone hydrolase.
**Materials and Methods**

**^{35}S-Sulfur Amino Acids**—Carrier-free L-{^35}S-Met, from Amersham Biosciences, was supplemented with unlabeled methionine (Sigma-Aldrich) to a specific activity of 2.0 ml of 1 m M L-threonine and 50 (20,000 Ci/mol) was prepared by digestion of L-{^35}S-Met with hydroxide acid (1) as described previously (24). L-{^35}S-Hcy was prepared by hydrolysis of L-[^{35}S]Hcy-thiolactone with 0.1 m NaOH at 37 °C for 15 min. The preparations of [^{35}S]Met and [^{35}S]Hcy were confirmed to be free of Hcy-thiolactone (< 0.1%).

**Seed Germination and 2°-Labeling Conditions**—Yellow lupine (L. luteus, var. Juno) seeds were germinated at 21 °C on cellulose paper towels soaked with deionized sterile water. On the 6th day, the roots were removed, and the seedlings were transferred into 50 ml of M9 minimal medium supplemented with 1 m M thiourea and 50 (20,000 Ci/mol) was prepared by digestion of L-[^{35}S]-Met with hydroxide acid (1) as described previously (24). L-[^{35}S]-Hcy was prepared by hydrolysis of L-[^{35}S]Hcy-thiolactone with 0.1 m NaOH at 37 °C for 15 min. The preparations of [^{35}S]Met and [^{35}S]Hcy were confirmed to be free of Hcy-thiolactone (< 0.1%).

**Preparation of L. 2° Extracts**—Yellow lupine hypocotyls or cotyledons were then shifted to 30 °C for 15 min using isopropanol/ethyl acetate/water/ammonium formate-methanol (2:1, v/v). Organic layers were reextracted with 0.1 M phosphate, and Hcy-thiolactone was extracted with 4 volumes of chloroform.

**Lycopodium**—Lycopodium 2°-labeled protein was dissolved in phosphate buffer, pH 7.5, using a mortar and pestle. The extracts were centrifuged at 30,000 g for 30 min using 1.2 M para-nitrophenyl phosphate (paraoxon) was measured spectrophotometrically using ε = 1,300 m M−1 cm−1 at 270 nm for phenol and ε = 13,000 m M−1 cm−1 at 412 nm for p-nitrophenol, respectively. Hydrolysis of diethyl p-nitrophenyl phosphate (paraoxon) was measured spectrophotometrically using ε = 15,000 m M−1 cm−1 at 412 nm for p-nitrophenol (22).

In experiments in which utilization of other (thio)esters (10 mm) by Hcy-thiolactonase was tested, potential substrates and products were separated by TLC and visualized by staining with ninhydrin or under UV. With all potential substrate-product pairs, complete separation was achieved on cellulose plates (Analtech) using 1-butanol/acetic acid/pH 7.5, respectively, 1 m M 2-mercaptoethanol, and 5% nitrophenyl phosphate (22).

**Determination of Hcy-thiolactone in L. 2°—**The plant yellow lupine hypocotyls or cotyledons were then shifted to 30 °C for 15 min using isopropanol/ethyl acetate/water/ammonium formate-methanol (2:1, v/v). Organic layers were reextracted with 0.1 M phosphate, and Hcy-thiolactone was extracted with 4 volumes of chloroform.

**Measurement of Hcy-thiolactone Synthesis by Cation Exchange HPLC**—Metabolism of Hcy-thiolactone in Plants

**Enzyme Assays**—Unless indicated otherwise, incubations were carried out at 37 °C in 0.1 M potassium-Hepes buffer (pH 7.2). Hcy-thiolactonase activity was determined by following the formation of [^{35}S]Hcy from [^{35}S]Hcy-thiolactone. Hcy was isolated on TLC and by its sensitivity to lupine Hcy-thiolactonase or NaOH. The detection limit was 5 mmol/mg of Hcy-thiolactone.

**HPLC Chromatography**—HPLC analyses were carried out using a cation exchange PolySULFOETHYL Aspartamide column (2.1 × 200 mm, 5 μm, 300 A) from Polyclc, Inc. and System Gold HPLC instrumentation from Beckman-Coulter as described previously (19, 26).

**Results.**—The hypocotyls and cotyledons were then shifted to 30 °C for 15 min using isopropanol/ethyl acetate/water/ammonium formate-methanol (2:1, v/v). Organic layers were reextracted with 0.1 M phosphate, and Hcy-thiolactone was extracted with 4 volumes of chloroform. Hcy-thiolactone was confirmed by its co-migration with an authentic Hcy-thiolactone (60 mg), eluting at 0.35 M KCl, were concentrated by ammonium formate-methanol (2:1, v/v). A soluble fraction was separated by gel filtration on a Superdex 200 column equilibrated with Buffer C. Hcy-thiolactonase activity was determined by following the formation of [^{35}S]Hcy from [^{35}S]Hcy-thiolactone. Hcy was isolated on TLC and by its sensitivity to lupine Hcy-thiolactonase or NaOH. The detection limit was 5 mmol/mg of Hcy-thiolactone.

**Purification of Hcy-thiolactone Hydrolase**—All steps were carried out at 4 °C. Buffer A, B, or C containing 10, 20, or 50 mM potassium phosphate (pH 6.8), respectively, 1 mM EDTA, and 5% glycerol was used. Yellow lupine seed (L. luteus, var. Juno) meal (100 g) was extracted with 300 ml of Buffer A. Protein (9,030 mg) in crude extract, obtained by centrifugation at 20,000 × g for 30 min, was fractionated with ammonium sulfate. Protein (2,320 mg) precipitated between 0–35% ammonium sulfate saturation was collected by centrifugation, dissolved in 5 ml of Buffer B, and extensively dialyzed against Buffer B. Dialysate was clarified by centrifugation and applied on DEAE-Sephacel column. The column was washed with 5 volumes of Buffer B and eluted with a KCl gradient in Buffer B. Protein fractions with Hcy-thiolactonase activity (60 mg), eluting at 0.3–0.35 m KCl, were concentrated by ammonium sulfate precipitation, dissolved in 5 ml of Buffer C, and further purified by gel filtration on a Superdex 200 column equilibrated with Buffer C. The enzyme eluted from the gel filtration column as a protein with a molecular mass of 55 kDa. Active fractions (0.7 mg of protein) were applied on a hydroxyapatite column equilibrated with Buffer A and eluted with a gradient of 10–100 mM phosphate, pH 6.8, in Buffer A. Active fractions, eluted at 75 mM phosphate, were concentrated, dialyzed against Buffer A, and stored at −20 °C. The Hcy-thiolactonase preparation (0.2 mg of protein) had a specific activity of 536 mmol/mg/h and was purified 25,000-fold. The purified enzyme preparation showed several protein bands on SDS-PAGE.
RESULTS

Synthesis of Hcy-thiolactone in Yellow Lupine Seedlings Increases upon Depletion of Tetrahydrofolate—In plants, Hcy is synthesized de novo from sulfate and also as a by-product of cellular methylation reactions (Fig. 1) (28, 29). Three pathways of further Hcy metabolism are utilized to different extents by living organisms: methylation to methionine, trans-sulfuration to cysteine, and conversion to Hcy-thiolactone. In plants, Hcy is further metabolized by methylation to methionine by a methyltetrahydrofolate-dependent methionine synthase (Fig. 1, MS) (29) or by S-methyl-methionine-dependent Hcy S-methyltransferase (29–31). Trans-sulfuration of Hcy to cysteine, present in fungi and mammals, is absent in plants (29). MetRS-dependent metabolism of Hcy to Hcy-thiolactone, present in bacteria, yeast, and mammalian cells (15–17), was not known to be present in plants.

To determine whether metabolism of Hcy to Hcy-thiolactone occurs in plants, yellow lupine seedlings were examined for the presence of Hcy-thiolactone and Hcy-thiolactone hydrolase. Before extraction of Hcy-thiolactone, 6-day-old seedlings were maintained for additional 60 h on water in the absence and presence of the antifolate drug aminopterin (25 mM), which indicated that cellular folate pools have a relatively long half-life in this plant (33).

Because of its mostly neutral character under physiological pH (18), Hcy-thiolactone is expected to diffuse out from lupine seedlings. Indeed, we have found that Hcy-thiolactone was excreted from seedlings grown in the presence of aminopterin. When yellow lupine seedlings were labeled with [35S]Met in the presence of 0.1 and 1 mM methionine, levels do not increase in the presence of sulfonamide, an inhibitor of de novo folate synthesis (32), suggests that endogenous methyltetrahydrofolate pools in lupine seedlings are not significantly depleted during growth. This suggestion is consistent with a study of one carbon fluxes in Arabidopsis thaliana, which indicated that cellular folate pools have a relatively long half-life in this plant (33).

MetRS Is Involved in the Synthesis of Hcy-thiolactone in Plants—To determine whether plant MetRS metabolizes Hcy to Hcy-thiolactone, rice MetRS was expressed in E. coli BL21 harboring pET/MOsΔC, a plasmid bearing the rice MetRS gene under the control of the lac promotor (25). The rate of Hcy-thiolactone synthesis in the culture of E. coli BL21/pET/MOsΔC increased about 3-fold upon induction with isopropyl-β-D-thiogalactopyranoside (Fig. 4), which indicates that rice MetRS catalyzes the synthesis of Hcy-thiolactone. Supplementation of growth medium with methionine resulted in inhibition of Hcy-thiolactone synthesis in these cultures, as expected (Fig. 4).

To determine whether MetRS is involved in Hcy-thiolactone synthesis in plants in vivo, yellow lupine seedlings were maintained in aminopterin in the presence of increasing concentrations of methionine. In the presence of 0.1 and 1 mM methio-
Figure 4. Hcy-thiolactone synthesis in E. coli cells harboring a plasmid containing rice MetRS gene under the control of the lac promotor. The synthesis of Hcy-thiolactone was monitored after induction of the expression of rice MetRS with 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG); as a control, the synthesis of Hcy-thiolactone was also monitored in noninduced culture (C). In other control experiments, Hcy-thiolactone was measured in isopropyl-β-D-thiogalactopyranoside-induced (●) and noninduced (○) cultures supplemented with 0.2 mM methionine.

Table I
Levels of Hcy metabolites in hypocotyls of yellow lupine seedlings

| Antifolate drug treatment | Hcy | Hcy-thiolactone | Hcy-N-protein |
|--------------------------|-----|----------------|--------------|
| None, control            | 4.3 ± 0.6 | <0.6 | <0.06 (±0.2%) |
| Aminopterin, 25 μM       | 245 ± 20  | 49.5 ± 8 | 0.47 ± 0.16 (5.2%) |
| Trimethoprim, 0.1 mM     | 4.3 ± 0.6 | <0.6 | <0.06 (±0.2%) |

Note: Values in parentheses show [35S]Hcy-N-protein relative to [35S]Met-protein determined from experiments in which plant seedlings were labeled with 1.5 μM [35S]Met (20,000 Ci/mol), and metabolites were analyzed by two-dimensional TLC as described under “Materials and Methods.”

Methionine inhibits the synthesis of Hcy-thiolactone in yellow lupine seedlings

| Methionine supplementation | Lupine tissue Hcy-thiolactone μM |
|---------------------------|---------------------------------|
| 0                         | 55.9 ± 6.0                     |
| 0.01                      | 57.7                            |
| 0.1                       | 33.5                            |
| 1.0                       | 3.3                             |

Note: The concentrations were determined from Hcy-thiolactone measurements using HPLC.

Values in parentheses show 5% Hcy-N-protein relative to [35S]Met-protein in the absence and presence of aminopterin, respectively (Table III).

Hcy-thiolactone Hydrolase Metabolizes Hcy-thiolactone in Yellow Lupine Plants—To determine whether plants have the ability to metabolize Hcy-thiolactone, yellow lupine seedlings were maintained on 0.75 μM [35S]Hcy-thiolactone in water. The seedlings metabolized 60% and 100% [35S]Hcy-thiolactone after 7 and 24 h, respectively. Analysis of plant extracts showed that [35S]Met was a major metabolite derived from [35S]Hcy-thiolactone after 24 h (data not shown). In the absence of yellow lupine seedlings, Hcy-thiolactone was stable under the experimental conditions utilized (half-life > 3 days). Because Hcy-thiolactone is unlikely to be metabolized without ring opening, its fast metabolism suggests that Hcy-thiolactone-hydrolyzing enzyme is present in plants.

Indeed, when crude extracts from yellow lupine seeds were incubated with [35S]Hcy-thiolactone, it was hydrolyzed to [35S]Hcy at a rate of 0.21 μmol/mg/h. This level of Hcy-thiolactone-hydrolyzing activity is 3.7-fold higher than the level present in human serum (22). Lupine Hcy-thiolactonase activity, measured in extracts from cotyledons, did not change significantly after seed germination and growth up to 6 days (data not shown).

The Hcy-thiolactonase activity, precipitated from crude extracts of yellow lupine seed meal with 35% ammonium sulfate, was further purified by anion exchange chromatography on DEAE-Sephalac, gel exclusion chromatography on Superdex, and absorption chromatography on hydroxylapatite. At all steps of purification procedure, a single peak of Hcy-thiolactonase activity was observed, suggesting that a single enzyme was responsible for Hcy-thiolactone hydrolysis in yellow lupine. The specific activity of the purified Hcy-thiolactonase preparation, 536 μmol/mg/h, was 25,000-fold greater than that measured in crude extracts. The plant Hcy-thiolactonase preparation exhibited 7-fold higher specific activity than pure human Hcy-thiolactonase.

Examination of the substrate specificity showed that, in addition to l-Hcy-thiolactone, the purified enzyme also hydrolyzed α-aminocyl esters and thioesters (Table IV). For example, thioesters of methionine, such as Met-S-CoA and Met-S-DTT, and methionine methyl esters were hydrolyzed. Esters of other α-aminic acids, such as methyl esters of alanine, cysteine, phenylalanine, tryptophan, and lysine, were also hydrolyzed. D-Hcy-thiolactone and D-forms of α-aminocyl esters were hydrolyzed up to 20-fold less efficiently than the l-forms.

l-Homoserine-lactone was also a substrate. However, N-acetyl-d,l-Hcy-thiolactone was not hydrolyzed. Esters of β-aminic acids, such as β-Ala methyl ester, esters and thioesters of acetic acid, such as O-acetyl-l-serine and acetyl-S-CoA, and γ-methyl ester of glutamic acid were not hydrolyzed. In contrast to human Hcy-thiolactonase/paraoxonase, the plant enzyme did not hydrolyze non-natural aryl esters, such as phenyl acetate and p-nitrophenyl acetate, or the organophosphate paraoxon (Table IV).

The substrate specificity studies indicate that lupine Hcy-
The plant Hcy-thiolactone-hydrolyzing enzyme exhibits selectivity of an α-aminoacyl-(thio)ester hydrolase. The plant Hcy-thiolactone-hydrolyzing enzyme eluted from a Superdex gel filtration column as a 55-kDa protein. The human Hcy-thiolactonase/paraoxonase does not require calcium for activity, whereas the human Hcy-thiolactonase/paraoxonase does (22, 23). Our present work shows that yellow lupine plants possess a novel Hcy-thiolactone-hydrolyzing enzyme. For example, the plant enzyme exhibited a broad pH optimum, from pH 6 to pH 8, whereas the human Hcy-thiolactonase/paraoxonase does (22, 23). Although both enzymes hydrolyze Hcy-thiolactone, they differ in their ability to hydrolyze other (thio)esters. For example, whereas the plant enzyme hydrolyzes α-aminoacyl (thio)-

### TABLE III

| Growth conditions | [35S]Hcy-N-protein, cpm | [35S]Met-protein, cpm | [35S]Hcy-N-protein/Met-protein, % |
|------------------|-------------------------|-----------------------|----------------------------------|
| Control          | 162 ± 14                | 1930 ± 66             | 8.3                              |
| + Aminopterin    | 259 ± 16                | 297 ± 23              | 87.2                             |

### TABLE IV

| Compound                     | Relative activity of plant Hcy-thiolactonase | Relative activity of human Hcy-thiolactonase* |
|------------------------------|----------------------------------------------|-----------------------------------------------|
| L-Hcy-thiolactone            | 100                                          | 100                                           |
| D-Hcy-thiolactone            | 6                                            | 24                                            |
| L1-Hcy-thiolactone           | 50                                           | 46                                            |
| N-acetyl-D.L-Hcy-thiolactone | n.d.                                         | n.d.                                          |
| L-Hse-lactone                | ++                                           | n.d.                                          |
| L-Methionyl-S-CoA            | +++                                          | n.d.                                          |
| Acetyl-S-CoA                 | –                                            | n.d.                                          |
| L-Methionine-D-thioetheriol   | ++                                           | n.d.                                          |
| L-Methionine methyl ester    | ++                                           | n.d.                                          |
| D-Methionine methyl ester    | ++                                           | n.d.                                          |
| L-Phenylalanine ethyl ester  | n.d.                                         | n.d.                                          |
| L-Tryptophane methyl ester   | ++                                           | n.d.                                          |
| L-Lysine methyl ester        | +                                            | n.d.                                          |
| L-Cysteine methyl ester      | ++                                           | n.d.                                          |
| L-Alanine methyl ester       | ++                                           | n.d.                                          |
| β-Alanine methyl ester       | –                                            | n.d.                                          |
| γ-Glu methyl ester           | –                                            | n.d.                                          |
| O-Acetyl-serine              | –                                            | –                                             |
| Phenyl acetate               | 280,000                                      | 4,000                                         |
| p-Nitrophenyl acetate        | –                                            | 330                                           |

* Data from Ref. 22.

** Symbols + or – indicate that a compound is hydrolyzed or is not hydrolyzed, respectively.

n.d., not determined.

**DISCUSSION**

This work demonstrates a novel aspect of Hcy metabolism in plants: synthesis and degradation of Hcy-thiolactone in the plant yellow lupine (Fig. 1). In the synthetic pathway, Hcy is converted to Hcy-thiolactone by the plant MetRS. In the degradation pathway, Hcy-thiolactone is hydrolyzed to Hcy by a unique plant Hcy-thiolactonase hydrolase.

Hcy-thiolactone, a product of an error-editing reaction of MetRS in bacteria, yeast, and mammalian, including human, cells (2–17), has not been reported in plants before. As shown here, Hcy-thiolactone can be a significant component of sulfur amino acid pools in yellow lupine, particularly when the conversion of Hcy to methionine is limited by the antifolate drug aminopterin. Our results suggest that the synthesis of Hcy-thiolactone in plants is catalyzed by MetRS. The demonstration of Hcy-thiolactone synthesis also in plants supports a conclusion that Hcy editing during selection of amino acids for protein synthesis is most likely universal (15–17).

Hcy-thiolactone reacts easily with protein lysine residues under physiological conditions (20). This reaction is responsible for the presence of Hcy in endothelial cell proteins (11–15) and, most likely, in human blood proteins (26). Our data demonstrate that Hcy is also present in yellow lupine proteins. When methylation of Hcy to methionine synthase was inhibited by the antifolate drug aminopterin, Hcy-N-protein became a major metabolite of Hcy in yellow lupine seedlings. The presence of Hcy-N-protein in mammals (26) and plants suggests that Hcy-N-protein is likely to be a component of Hcy metabolism in multicellular organisms.

Incorporation of Hcy into protein mediated by Hcy-thiolactone is known to result in protein damage (14–17, 20). Because of this, the ability to detoxify Hcy-thiolactone is essential for biological integrity, particularly in multicellular organisms. Indeed, specific Hcy-thiolactone hydrolase/paraoxonase, tightly associated with high density lipoprotein, exists in mammals, including humans (22, 23). Our present work shows that yellow lupine plants possess a novel Hcy-thiolactone-hydrolyzing enzyme. The plant Hcy-thiolactonase is different from the human Hcy-thiolactone-hydrolyzing enzyme. For example, the plant Hcy-thiolactonase does not require calcium for activity, whereas the human Hcy-thiolactonase/paraoxonase does (22, 23). Although both enzymes hydrolyze Hcy-thiolactone, they differ in their ability to hydrolyze other (thio)esters. For example, whereas the plant enzyme hydrolyzes α-aminoacyl (thio)-
esters, the human enzyme does not (Table IV). On the other hand the plant enzyme does not hydrolyze phenyl and \( p \)-nitrophenyl esters of acetic acid or the organophosphate paraoxon. These artificial esters are very good substrates of the human enzyme (Table IV).

In conclusion, our findings show that two novel pathways of Hcy metabolism are utilized by the plant \( L. \) \textit{luteus}: 1) metabolic conversion of Hcy to Hcy-thiolactone, a fundamental editing reaction in protein synthesis, which appears to be conserved in all living organisms; and 2) hydrolysis of Hcy-thiolactone to Hcy, which thus far has been documented in multicellular organisms such as mammals and plants.

\textbf{Acknowledgments}—We thank S. Harvey Mudd (National Institutes of Health, Bethesda, MD) for comments on plant sulfur metabolism, Marc Mirande for a clone of rice MetRS, and Elżbieta Starzynska for help in purification of plant Hcy-thiolactonase.

\textbf{REFERENCES}

1. Baernstein, H. D. (1934) \textit{J. Biol. Chem.} 106, 451–456
2. Jakubowski, H. & Fersht, A. (1981) \textit{Nucleic Acids Res.} 9, 3105–3117
3. Jakubowski, H. (1990) \textit{Proc. Natl. Acad. Sci. U. S. A.} 87, 4504–4508
4. Gao, W., Goldman, E. & Jakubowski, H. (1994) \textit{Biochemistry} 33, 11528–11535
5. Jakubowski, H. (1995) \textit{J. Biol. Chem.} 270, 17672–17673
6. Jakubowski, H. & Goldman, E. (1992) \textit{Microbiol. Res.} 96, 412–429
7. Jakubowski, H. (1991) \textit{EMBO J.} 10, 593–598
8. Senger, B., Despons, L., Walter, P., Jakubowski, H. & Fasiolo, F. (2001) \textit{J. Mol. Biol.} 311, 205–216
9. Jakubowski, H. & Goldman, E. (1993) \textit{FEBS Lett.} 317, 237–240
10. Jakubowski, H. (1997) \textit{J. Biol. Chem.} 272, 1935–1941
11. Jakubowski, H., Zhang, L., Bardeguez, A. & Ariv, A. (2000) \textit{Circ. Res.} 87, 45–51
12. Jakubowski, H. (2000) \textit{J. Nutr.} 130, (suppl.) 377–381
13. Jakubowski, H. (2001) \textit{J. Nutr.} 131, (suppl.) 2983–2987
14. Jakubowski, H. (2001) \textit{Biomed. Pharmacother.} 55, 443–4477
15. Jakubowski, H. (2001) in \textit{Homozygote in Health and Disease} (Carmel, R. & Jacobsen, D. W., eds), pp. 21–31, Cambridge University Press, Cambridge, United Kingdom
16. Jakubowski, H. (2001) in \textit{Encyclopedia of Life Sciences}, Vol. 18, pp. 441–449, Nature Publishing Group, London
17. Jakubowski, H. (2003) in \textit{The Aminoacyl-tRNA Synthetases} (Iba, M., Cusack, S. & Franchlyn, C., eds, Landes Biosciences, Georgetown, TX, in press
18. Anderson, H. F. & Packer, J. E. (1974) \textit{Int. J. Radiat. Phys. Chem.} 6, 33–46
19. Jakubowski, H. (2002) \textit{Anal. Biochem.} 308, 112–119
20. Jakubowski, H. (1999) \textit{FASEB J.} 13, 2277–2283
21. Goodrich-Blair, H. & Kolter, R. (2000) \textit{FEBS Lett.} 491, 117–121
22. Jakubowski, H. (2000) \textit{J. Biol. Chem.} 275, 3957–3962
23. Jakubowski, H., Ambrosius, W. & Pratt, J. H. (2001) \textit{FEBS Lett.} 491, 35–39
24. Jakubowski, H. (2000) \textit{J. Biol. Chem.} 275, 21813–21816
25. Kaminska, M., Denizia, M., Kerjan, P, Barciszewski, J. & Mirande, M. (2000) \textit{EMBO J.} 19, 6907–6912
26. Jakubowski, H. (2002) \textit{J. Biol. Chem.} 277, 30425–30428
27. Jakubowski H. (1998) \textit{Biochemistry} 37, 5147–5153
28. Giovanelii, J., Mudd, S. H. & Datko, A. H. (1995) \textit{Plant Physiol.} 70, 555–560
29. Giovanelii, J. (1987) Methods Enzymol. 143, 419–426
30. Mudd, S. H. & Datko, A. H. (1990) \textit{Plant Physiol.} 93, 623–630
31. Ranocha, P., McNeil, S. D., Ziemak, M. J., Tarezyiska, M. C. & Hanson, A. D. (2001) \textit{Plant J.} 25, 574–584
32. Prabhu, V., Chatson, K. B., Lui, H., Abrams, G. D. & King, J. (1998) \textit{Plant. Physiol.} 116, 137–144
33. Cai, X. Y., Jakubowski, H., Redfield, B., Zaleski, B., Brot, N. & Weisbach, H. (1992) \textit{Biochem. Biophys. Res. Commun.} 182, 651–658