Homocysteine Thiolactone: Metabolic Origin and Protein Homocysteinylation in Humans1,2

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ABSTRACT Homocysteine thiolactone, an intramolecular thioester of homocysteine, is synthesized by methionyl-tRNA synthetase in an error-editing reaction that prevents translational incorporation of homocysteine into proteins. The synthesis of thiolactone occurs in all human cell types investigated. An increase in homocysteine levels leads to elevation of thiolactone levels in human cells. In cultured human cells and in human serum, homocysteine thiolactone reacts with proteins by a mechanism involving homocysteinylation of protein lysine residues. The homocysteinylation leads to protein damage. A calcium-dependent homocysteine thiolactonase, tightly associated with HDL in human serum, may prevent protein damage by detoxifying thiolactone. J. Nutr. 130: 377S–381S, 2000.

KEY WORDS: • homocysteine thiolactone • homocysteine • protein homocysteinylation • HDL-associated thiolactonase • calcium • atherosclerosis

Although homocysteine (Hcy)3 thiolactone was obtained by chemical synthesis in the 1930s (Baernstein 1934, Riegel and Du Vigneaud 1935), the first indication of its biological significance came almost 50 years later with the discovery of enzymatic conversion of Hcy to Hcy thiolactone in error-editing reactions of some aminoacyl-tRNA synthetases (AARS) in vitro (Jakubowski and Fersht 1981) and in vivo (Jakubowski 1990). Hcy thiolactone reacts easily with proteins. Protein damage caused by homocysteinylation may underlie the involvement of Hcy in human pathologies such as vascular disease (Jakubowski 1997).

Mechanisms of homocysteine thiolactone synthesis

In all cell types, from bacterial to human, Hcy is metabolized to Hcy thiolactone by methionyl-tRNA synthetase (MetRS) (Jakubowski 1990, 1991, 1995 and 1997, Jakubowski and Goldman 1993). Because Hcy thiolactone forms at the active site of MetRS, the synthesis of thiolactone increases with an increase in the Hcy/Met ratio. Two other synthetases, LeuRS and IleRS, can also convert Hcy to the thiolactone under some conditions in bacteria (Jakubowski 1995). The mechanism of Hcy thiolactone synthesis involves a two-step reaction driven by the hydrolysis of ATP (Jakubowski and Fersht 1981). In the first step (Eq. 1), a carboxyl group of Hcy is activated by ATP, forming a MetRS-bound homocysteinyl adenylate.

$$\text{MetRS} + \text{Hcy} + \text{ATP} \rightleftharpoons \text{MetRS} \cdot \text{Hcy} \sim \text{AMP} + \text{PPi} \ (1)$$

In the second step (Eq. 2), the side chain thiolate of Hcy displaces the AMP group from the activated carboxyl group of Hcy, forming Hcy thiolactone as a product. The energy of the anhydride bond of Hcy-AMP is conserved in the intramolecular thioester bond of Hcy thiolactone.

$$\text{NH}_2 \quad \text{O} \quad \text{SH} \quad \text{AMP} \quad \text{H} \quad \text{H} \quad \text{S} \quad \text{O}$$

Hcy thiolactone is synthesized by human endothelial cells (Table 1), fibroblasts, breast cancer cells (Table 2), HeLa cells, as well as by normal BALB/c 3T3 and transformed RAG mouse cells (Jakubowski and Goldman 1993). MetRS mutants of Chinese hamster ovary cells, defective in the Met binding site of the enzyme, are also defective in Hcy thiolactone synthesis (Jakubowski and Goldman 1993). Methionine inhibits synthesis of Hcy thiolactone in rodent and human cells (Jakubowski, unpublished data). Because of its mostly neutral character at physiologic pH (pK = 7.1; Anderson and Packer 1974), Hcy thiolactone diffuses through cell membranes and accumulates in the culture medium (Fig. 1).

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3 Abbreviations used: AARS, aminoacyl-tRNA synthetase; DTT, dithiothreitol; Hcy, homocysteine; HUVEC, human umbilical vein artery endothelial cells; MetRS, methionyl-tRNA synthetase; PTH, phenylthiohydantoin.

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Elevation of Hcy levels leads to enhanced synthesis of the thiolactone

Under normal metabolic conditions, synthesis of Hcy thiolactone in human cells is low because intracellular concentrations of Hcy are relatively low (Table 1). However, if Hcy levels are increased because of a reduction in its transmethylation and/or transsulfuration, the synthesis of Hcy thiolactone is enhanced. For example, in the absence of folate, human fibroblasts, breast cancer cells and endothelial cells accumulate Hcy and synthesize large amounts of the thiolactone (Table 2). Thiolactone levels can reach as much as 60% of the metabolized Hcy in extreme cases of intercellular Hcy elevation, such as observed in human endothelial cells maintained on Hcy in Met-free media deprived of vitamin B-12 and folate (Table 2).

Metabolism of Hcy thiolactone in human cell cultures and serum

As shown in Figure 1, Hcy thiolactone undergoes the following two major reactions in human cells and serum in vitro: 1) protein homocysteinylolation at lysine residues (Jakubowski 1997, 1999a and 1999b); and 2) enzymatic hydrolysis to Hcy by calcium-dependent Hcy thiolactonase, a component of HDL (Jakubowski 1999a). Preliminary experiments suggest that protein homocysteinylolation may occur in humans. For example, small amounts of Hcy are present in acid hydrolyzates of dithiothreitol (DTT)-treated human serum proteins from normal subjects; more Hcy is recovered from serum proteins from homocysteinuric subjects (Table 3).

Enzymatic hydrolysis of thiolactone to Hcy also occurs in vivo; this was shown by means of dietary supplementation or injection of Hcy thiolactone as a source of Hcy in laboratory animals. Although Hcy thiolactone is eliminated rapidly from blood and cells (the half-life of exogenous Hcy thiolactone is 1 h or less; Donahue et al. 1974, Dudman and Wilken 1981, Dudman et al. 1991, Jakubowski 1997), small amounts of Hcy are present in human serum proteins (Table 3). Because of its reactivity, thiolactone is unlikely to be detected in vivo.
In human serum, about half of the exogenous thiolactone incorporated into protein is released as free Hcy after reduction with DTT. The other half represents Hcy attached via an amide bond between its carboxyl group and the amino group of a protein lysine residue (Fig. 2).

**Protein homocysteinylilation**

After acid hydrolysis, a small amount of [35S]Hcy was recovered from protein of cultured cells incubated with [35S]Met. More Hcy was recovered from protein when human cells were treated with aminopterin, an antifolate drug that inhibits methionine synthase (Jakubowski 1997). In recent experiments with [35S]Met-labeled human umbilical vein endothelial cells (HUVEC), in which methionine synthase was severely inhibited by deprivation of vitamin B-12 and folate, Hcy incorporation into protein represented up to 36% of Hcy metabolized to Met (Table 2). When HUVEC were labeled with [35S]Hcy, incorporation of Hcy into protein represented up to 65% of Hcy metabolized to Met (Table 2). Hcy was present in both cellular and extracellular proteins (Fig. 3, Table 1). Data suggest that Hcy incorporation into protein is post-translational, reflecting facile homocysteinylilation of protein lysine residues by Hcy thiolactone (Fig. 2). Indeed, phenylthiohydantoin (PTH)-(S-carboxymethyl)homocysteine was recovered from tissue culture proteins subjected to carboxymethylation and Edman degradation (Jakubowski, unpublished data). Translational incorporation of Hcy into protein is unlikely because AARS do not aminoacylate tRNA with Hcy (Jakubowski 1999c and 1999d).

Reactions of Hcy thiolactone with protein lysine residues are robust under physiologic conditions (Table 4). In human serum incubated with the thiolactone, protein homocysteinylilation is a major reaction, which could be observed with as little as 10 nmol/L thiolactone. Individual proteins are homocysteinylated at rates proportional to their lysine contents.
The thiolactonase requires calcium for activity and stability, and is associated with the HDL fraction of serum lipoproteins (Jakubowski 1999a). Examples of time courses of protein homocysteinylation are shown in Figure 4. Homocysteinylation results in protein damage, manifested as loss of function. For example, methionyl-tRNA synthetase (Fig. 5) and trypsin (Jakubowski 1999b) are inactivated by homocysteinylation. Lysine oxidase, an important enzyme responsible for post-translational modification essential for the biogenesis of connective tissue matrices, is also inactivated irreversibly by Hcy thiolactone (Liu et al. 1997).

**HDL-associated Hcy thiolactonase in human serum**

Hcy thiolactone is hydrolyzed to Hcy in human serum by a single enzyme, Hcy thiolactonase, which is present at a concentration of ~50 μg/mL or ~1 μmol/L (Jakubowski 1999a). The thiolactonase requires calcium for activity and stability, and is associated with the HDL fraction of serum lipoproteins (Table 5). The enzyme is inhibited noncompetitively by isoleucine (Ki = 2 mmol/L) and penicillamine (Ki = 0.2 mmol/L).

**Summary: role of homocysteine thiolactone in human disease**

Elevated levels of Hcy are an independent risk factor for cardiovascular disease in humans (e.g., Jacobsen 1998). Available data suggest that Hcy can be harmful to human cells because of its metabolic conversion to Hcy thiolactone, a reactive thioester. This conversion occurs in all human cell types, including endothelial cells. When methionine synthase activity is inhibited by folate or vitamin B-12 deprivation, almost all Hcy is converted to thiolactone. Subsequent inadvertent homocysteinylation of cellular and extracellular proteins by Hcy thiolactone might lead to impaired function. The metabolic conversion of Hcy to Hcy thiolactone, the reactivity of the thiolactone toward proteins and resulting protein damage might explain some pathologic consequences of elevated Hcy levels, including atherosclerosis. The tight association of Hcy thiolactonase with HDL in serum could contrib-
ute to the protective role of HDL in the human vascular system.

**LITERATURE CITED**


