

# Homocysteine and Its Disulfide Derivatives

## A Suggested Consensus Terminology

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In recent years, there has been an upsurge of interest in elevation of the plasma concentration of homocysteine and closely related metabolites as an independent risk factor for cardiovascular disease (reviewed, for example, in References 1 through 3). Homocysteine itself is a thiol- (sulfhydryl-) containing amino acid, but in normal human plasma and other tissues, a variety of related disulfide derivatives may be present. Different authors have written about these compounds and their effects by using differing terminologies. To promote clarity of meaning and to minimize uncertainty, perhaps even confusion, it is important that each article discussing these compounds either defines explicitly the terms and/or abbreviations used or cites a prior publication in which such definitions are provided. Optimally, a more uniform consensus terminology will be developed and adopted by the field. This article describes very briefly the structures of the relevant compounds and sets forth terms and abbreviations that, it is hoped, may provide a basis for such a consensus.

The word "homocysteine" was used first by Du Vigneaud and coworkers >65 years ago when they discovered this compound<sup>4</sup> and provided definitive proof that it had the structure of a thiol (ie, sulfhydryl) 4-carbon  $\alpha$ -amino acid:  $\text{HSCH}_2\text{CH}_2\text{CH}(\text{NH}_2)\text{COOH}$ .<sup>5</sup> The symmetrical disulfide of homocysteine was termed "homocystine," both names being

chosen to indicate that each carbon chain of these compounds contained 1  $-\text{CH}_2-$  group more than those of, respectively, cysteine and cystine (already well known by that time). Since then, homocysteine and homocystine have been the standard names, widely accepted, and extensively used in the chemical and biochemical literature.

Normal human plasma contains total concentrations of homocysteine and its derivative disulfides close to 10  $\mu\text{mol/L}$ , although there is some variation due to genetic factors, age, sex, menopausal status, and other physiological and lifestyle variables (detailed, for example, in Refsum et al<sup>6</sup>). Of this total, only  $\approx 1\%$  to 2% occurs as the thiol, homocysteine.<sup>7</sup> The remaining 98% is in the form of disulfides (the Figure). Perhaps 75% of the total is bound to protein through disulfide bonds with protein cysteines, mainly in albumin, whereas the remainder occurs in non-protein-bound forms: homocystine, homocysteine-cysteine disulfide, and more minor amounts of other mixed disulfides, eg, homocysteine-cysteinylglycine disulfide.<sup>7</sup> In patients with abnormally elevated total concentrations of these compounds, such as occur in the homocystinurias (examples specified below), the percentage contribution of the thiol homocysteine to the total of these forms in plasma rises, reaching 10% to 25% as the total reaches 150 to 400  $\mu\text{mol/L}$ .<sup>8</sup>

All of the disulfides in question may be cleaved by treatment with suitable reducing agents, yielding the thiol homocysteine. Most modern methods for measuring homocysteine and its disulfides rely on such a reductive treatment, followed by either determination of the resulting homocysteine or measurement of a suitable derivative of it, so that the amount measured is the sum of any homocysteine originally present plus that originally present as a disulfide. However, for some purposes, it is important to distinguish between these forms—for example, when considering the pathophysiological importance of the individual compounds. At a minimum, when writing about them, it is essential to be clear which were measured and what forms one has in mind when mentioning metabolic, physiological, or pathophysiological effects.

We list the relevant structures in the Table, together with definitions of each and suggested abbreviations that may be useful.

### Comments on the Abbreviations, Their Definitions, and Their Usage

#### "Hcy"

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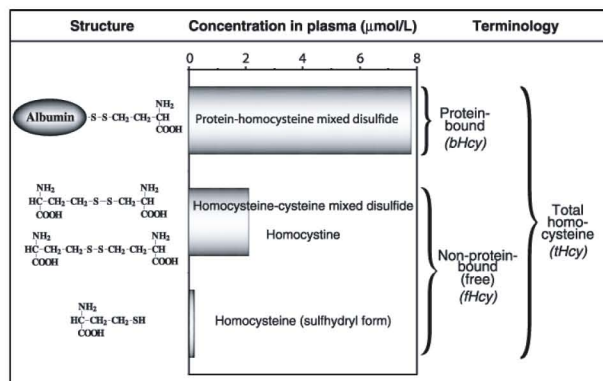
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Homocysteine and the major related disulfides in normal human plasma.

istry (IUPAC-IUB) Joint Commission on Biochemical Nomenclature for chemical compounds, "Hcy" is the abbreviation suggested for the structure  $\text{-SCH}_2\text{CH}_2\text{CH}(\text{NH}_2)\text{COOH}$ . Examples include AdoHcy for *S*-adenosylhomocysteine, Hcy-Hcy for homocystine, Hcy-Cys for homocysteine-cysteine mixed disulfide, and so forth. On the basis of the same convention, we suggest the abbreviation "HcyH" for the free thiol, homocysteine itself.

When used *within a text*, Hcy is a *generic* term that designates collectively not only homocysteine itself but also all compounds that will give rise to the thiol homocysteine after reductive cleavage of disulfide bonds. The latter compounds include homocystine, mixed disulfides of homocysteine with other thiols, and homocysteine moieties bound to protein by a disulfide bond. The term is useful when the particular chemical form is not known—for instance, in the statement "Hcy is a risk factor for cardiovascular disease" (but it is not certain whether HcyH, Hcy-Cys, or some other Hcy-containing form has the specified effect) or when it is not particularly important to identify the chemical form.

Use of "Hcy" may require definition of the relevant tissue preparation or sample (see the examples used with "tHcy" in the next paragraph). Equally important when relevant is the distinction between total, protein-bound, and non-protein-bound forms.

### "tHcy"

This abbreviation refers to the totality of homocysteine present after the quantitative reductive cleavage of all disul-

fide bonds in a sample. As noted above for Hcy, in general, a modifying adjective must specify the specimen source. Examples would include plasma tHcy, serum tHcy, whole-blood tHcy, hepatocyte tHcy, endothelial cell tHcy, urinary tHcy, and so forth. When defined as above, tHcy *does not include* material bound to protein by an amide linkage, such as may be formed by reaction of homocysteine thiolactone with protein lysine.<sup>9</sup> Such compounds are resistant to the reductive cleavage used to split disulfides.

### "fHcy"

This abbreviation may be used to signify "free Hcy" (alternatively termed "non-protein-bound Hcy" or "acid-soluble Hcy"). In each case, the prefix to Hcy will designate homocysteine together with its disulfide moieties other than those bound to protein. When used, the terms "fHcy" or "free Hcy" must be defined carefully to prevent confusion with the thiol homocysteine (because this compound contains what is often referred to as a "free" sulfhydryl group).

### "bHcy"

This abbreviation designates bound homocysteine (ie, homocysteine bound to protein by disulfide linkage).

## Terms That May Be Useful for Clinical Purposes

*Homocystinuria* (*NOT* homocysteinuria). We recommend that the term "homocystinuria" should be used exclusively for the inborn errors of metabolism characterized by markedly elevated concentrations of plasma or serum tHcy. Examples include severe deficiencies in the activity of cystathionine  $\beta$ -synthase<sup>10</sup> or methylenetetrahydrofolate reductase<sup>11</sup> or abnormalities in the transport or metabolism of cobalamin ( $\text{B}_{12}$ ).<sup>12</sup> To specify these abnormalities, it is generally sufficient merely to name the defect, eg, cystathionine  $\beta$ -synthase deficiency, cobalamin C disease, and so forth. Some authors may choose to include the word "homocystinuria." If that is done and a particular defect is being discussed, it is generally necessary to specify, for example, "homocystinuria due to deficient activity of cystathionine  $\beta$ -synthase." Read strictly, the term "homocystinuria" implies merely the presence of some homocysteine in the urine. This term was introduced in the early 1960s before sensitive quantitative assays for tHcy determination had been developed. Homocystinuria was then diagnosed by measuring Hcy in urine by using qualitative

### Homocysteine and Its Disulfide Derivatives: Terms, Definitions, and Abbreviations

Term/Name	Definition	Abbreviation
Thiol (including homocysteine)	A compound with a free SH group	RSH
Homocysteine	The thiol (sulfhydryl) compound	HcyH
Homocystine	The symmetrical disulfide	Hcy-Hcy
Homocysteine-cysteine mixed disulfide		Hcy-Cys
Homocysteine-RSH mixed disulfide		Hcy-SR
Hcy	HcyH and/or Hcy-SR (ie, thiol status not specified)	
Free Hcy	Non-protein-bound Hcy	fHcy
Bound Hcy	Protein-bound Hcy	bHcy
Total Hcy	fHcy + bHcy	tHcy

methods or by amino acid analysis that detected homocystine. In publications from the 1960s and 1970s, "homocystinuria" usually refers to the inborn errors but in some instances may reflect other conditions associated with severe elevations of tHcy, eg, vitamin B<sub>12</sub> deficiency (see Higginbottom et al<sup>13</sup>).

More recently, urine from normal volunteers has been shown to contain small amounts of tHcy (some 6 μmol/24 h).<sup>14</sup> Thus, the presence of abnormally elevated amounts of urinary homocystine in the genetic diseases in question might more accurately be termed "hyperhomocystinuria." However, the long-time use of "homocystinuria" in these situations and the frequent employment of a variety of similar terms that analogously designate abnormal elevation in urine of the compound in question—eg, glycosuria, pentosuria, and phenylketonuria—suggest that an attempt to convert "homocystinuria" to "hyperhomocystinuria" would at best be of questionable value.

**Hyperhomocysteinemia.** By definition, this is the presence of an abnormally elevated concentration of plasma or serum tHcy. Thus, it pertains not only to homocystine but also to all of its disulfide derivatives; ie, it designates "total homocystine." When and where this term is used, the relevant reference range should be defined explicitly. "Hyperhomocyst(e)inemia" and "homocyst(e)ine" are terms that have often been used as synonyms for "hyperhomocysteinemia" and for "tHcy" or "Hcy," respectively, as defined in this article. If these terms are used in the future, they should be defined specifically.

Some authors have defined the quantitative degree of hyperhomocysteinemia by use of terms such as "moderate, intermediate, and severe"<sup>15</sup> or "mild, intermediate, and severe."<sup>16</sup> Currently there is no agreement on the quantitative limits denoted by these terms, and it is imperative that authors using them provide the relevant concentration ranges.

**Normohomocysteinemia and hypohomocysteinemia.** These terms provide further descriptors of the concentration of tHcy in plasma or serum and have been used by some authors.<sup>17,18</sup> As above, an explicit statement of the quantitative limits implied should be provided if and when these terms are used.

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KEY WORDS: homocysteine ■ homocystine ■ disulfide ■ hyperhomocysteinemia ■ tHcy