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Cysteine

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Abbreviations.

AEMTS,Aminoethyl methanethiosulphate; BSS, ADH, Alcohol dehydrogenase; BSS, benzyl succinate synthase; Cys, Cysteine; $FADH_2$, Flavin adenine dinucleotide (reduced form); GR, Glutathione reductase; GSH, Glutathione (reduced); GSSG, Glutathione disulphide (oxidized); GSH /GSSG, Glutathione/Glutathione disulphide couple; GADPH, Glyceraldehydes 3-phosphate dehydrogenase; NAD^+, Nicotinamide adenine dinucleotide; NADH, Nicotinamide adenine dinucleotide (reduced); $NADP^+$, Nicotinamide adenine dinucleotide phosphate; NADPH, Nicotinamide adenine dinucleotide phosphate (reduced); Nox, NADH oxidase; Npx , NADH peroxidase; PFL, Pyruvate formate kinase; RNRase, Ribonucleotide reductase; Trx, Thioredoxin; TrxR, Thioredoxin reductase.

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Abstract

Cysteine is a sulfur containing non-essential amino acid. It is found in diverse array of organic molecules. Thiols, thiolates, thiyl radicals, disulfides, disulfides-S-oxides , seleno disulfides, sulfenic, sulfinic and sulfonic acids found in biological sytems have cysteiene residues in their structure. Cysteine is a component of many enzymes. Most importantly, glutathione which plays a vital role in cellular redox mechanisms,contains cysteine. Studies suggest the key role of cysteine in the redox activity of cells. The reduced form of cysteine is found to be involved in nucleophilic substitution and tranfer reactions involving electrons, protons, metal ions, hydrogen atoms, oxygen atoms and hydrides.This review focuses on the redox behaviour of cysteine.

Introduction

The cysteine residue is present in most proteins and is often an integral component of protein tertiary structure.Within extracellular proteins, cysteines are frequently involved in disulphide bonds, where pairs of cysteines are oxidised to form a covalent bond that stabilises the protein structure. In the intracellular environment, their sulfydryl side-chain is excellent for binding to metals, such as zinc. Cysteines are also very common in protein active and binding sites.Cysteine is stored in the body as cystine. The sulfide group present in cysteine impart it a unique redox behaviour. This review addresses the unique redox behaviour of cysteine.

Chemical structure of cysteine.

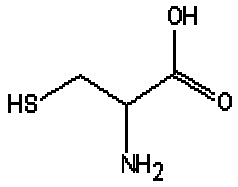


Figure 1.Chemical structure of cysteine [3].

Cysteine ($C_3H_7NO_2S$) is neutral, polar and found on the surface of proteins. An amino acid with a thiol side chain ($HS\text{-}CH_2\text{-}$), it plays a special role in protein structure due to covalent disulphide links. The ability of sulfur to occur in up to 10 different oxidation states *in vivo* leads to a range of cysteine modification in peptides and proteins.

Cysteine metabolism

The sulfur for cysteine synthesis comes from the essential amino acid methionine. While cysteine readily oxidizes in air to form the disulfide cysteine, the cells contain little, if any free cystiene,

because the ubiquitous reducing agent glutathione effectively reverses the formation of cystine by a non-enzymatic reduction reaction [7].

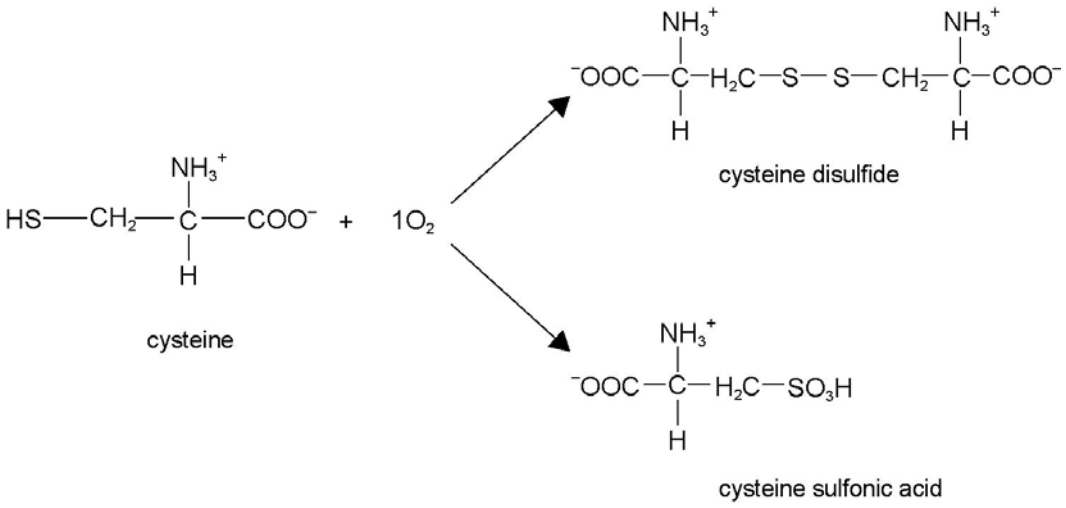
There are three pathways for cysteine catabolism. The simplest, but least important pathway runs by liver desulfurase and produces hydrogen sulfide and pyruvate. The more important catabolic pathway is by cytochrome-P_{450}-coupled dioxygenase that oxidizes the cysteine sulfhydryl to sulfinate, producing the intermediate cysteine sulfinate. The glucogenic product pyruvate is formed from cysteine sulfinate by transamination, followed by the removal of SO_3^-. Other than protein, the most important product of cysteine metabolism is the bile salt precursor, which is used to form the bile acid conjugates taurocholate and taurochenodeoxycholate [7].

Redox reactions of cysteine.

Cysteine thiols or sulfhydryl (S-H) groups are the most chemically reactive sites in proteins at physiological conditions[4].The oxidation of two cysteine (S-H) bonds to form the cystine disulphide linkage(S-S) is a key step in redox chemistry.

Oxidation of cysteine.

Cysteine residues are oxidised to disulphides that can be further oxidised to form sulfenic ,sulfenic and sulfonic derivatives. Scheme 1 illustrates the reaction of cysteine with singlet oxygen to form cysteine disulphide and cysteine sulfonic acid.



Scheme 1: The reaction of cysteine with singlet oxygen to form cysteine disulphide and cysteine sulfonic acid.

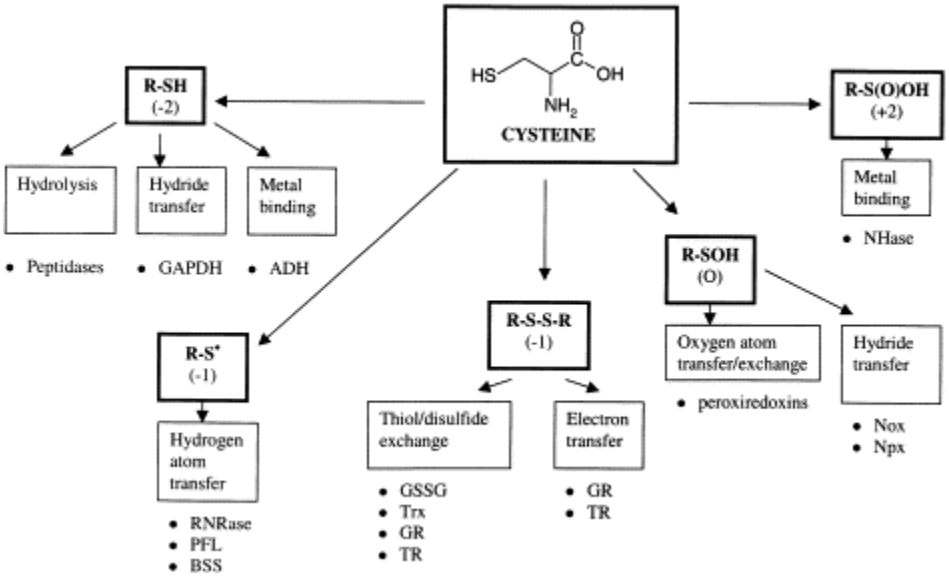
Csteine reacts with nitric oxide to produce nitrosothiol derivatives.The S-nitrosothiol derivatives formed by the reaction of cysteine with nitric oxide ($^{\bullet}NO$) might be important in the storage and /or transport of nitric oxide *in vivo*.

$$Cys\text{-}SH + {}^{\bullet}NO \rightarrow Cys\text{-}S\text{-}NO + H^{+} + e^{-} \quad (1)$$

$$Fe^{3+} + NO \rightarrow [Fe^{2+}\text{-}NO^{+}] \quad (2)$$

$$[Fe^{2+}\text{-}NO^{+}] + RSH \rightarrow Fe^{2+} + RSNO + H^{+} \quad (3)$$

The following schema 2, focus on the unique reactivity of cysteine and represents the variety of transformations the thiol group can undergo *in vivo.*



Scheme 2: The oxidation states, properties, reactivity and occurrence of different cysteine modifications *invivo* is shown in this schema**.** Oxidation states of sulfur are given for R in oxidation state +1. Cysteine based redox reactions are classified in this schema as thiol/disulphide exchange reactions(eg.,in thioredoxin (Trx)),electron-transfer reactions(e.g.,in glutathione reductase(GR)),thiol/thiyl hydrogen radical transfer reactions(eg.,in ribonucleotide reductase(RNRase)),oxygen atom transfer–redox couples(e.g.,in NADH oxidase(Nox) and NADH peroxidase(Npx)),and different hydride transfer reactions(e.g., in Npx and glyceraldehydes 3-phosphate dehydrogenase(GADPH) [1].

Thiol / disulphide exchange reactions.

These are *in vivo* substitution reactions,with cysteine acting as nucleophile. These reactions are key in maintaining the cellular redox balance by the GSH /GSSG redox-couple. This kind of exchange reactions is particularly important in the catalytic cycle of glutathione reductase (GR) and thioredoxin reductase (TrxR) [1].

$$GSSG + NADPH + H^+ \xrightarrow{GR} 2\,GSH + NADP^+ \qquad (4)$$

Electron transfer reactions.

Electron transfer to disulfides by enzymes like glutathione reductase and thioredoxin reductase helps to link the two different redox-systems of electron transfer and nucleophilic exchange [1]. In thioredoxin reductase and similar enzymes, cysteine appears in pairs of the type Cys-x-x-Cys and reducing power is transferred through electron transfer which affects cycling between disulphide and sulfhydryl forms [2].This mechanism is important in glutathione reductase (GR) where the reduction of glutathione disulphide (GSSG) is brought about by cysteine. This leads to the formation of glutathione(GSH) and an intramolecular disulphide at the active site of GR. This disulphide is rereduced by electron transfer from $FADH_2$ [1].

Radical formation and transfer reactions.

The thiyl radicals are formed by the homolytic fission of disulphides , including the disulfide linkages in proteins [3] .

$$\text{Cys-S-S- Cys} \rightarrow \text{cysteine-S}^{\bullet} + \text{S}^{\bullet}\text{-cysteine.} \qquad (5)$$

The thiyl radical also can be formed by long-range one electron transfer from the thiol (with the subsequent loss of H^+) or by short range hydrogen atom abstraction from the thiol.The side groups of cysteine reacts rapidly with $HO^{\bullet}$ to form thiyl radicals. The thiol/thiyl radical couple occurs in important human enzymes such as ribonucleotide reductase (RNRase),pyruvate formate kinase(PFL) and benzyl succinate synthase(BSS) [1].

Oxygen atom tranfer.

Sulfur acids are formed in this process of oxygen atom transfer from oxidising species to cysteine . Sulfenic acids are found in proteins such as glutathione reductase and human peroxiredoxin(Pox).

$$CysS^{\bullet} + O_2 \rightarrow CysSOO^{\bullet} \quad (6)$$

$$CysSOO^{\bullet} + CysSH \rightarrow CysSO^{\bullet} + CysSOH \quad (7)$$

Sulfenic acid (Cys-SOH) formation is reversible and is considered to have important roles in signal transduction,oxygen metabolism and transcriptional regulation.

Indirect redox activities of cysteine:

Cysteine facilitates the catalysis of many oxidoreductases either as a ligand for catalytic metal ions or as a hydride transfer facilitator.Hydride transfer from substrate to NAD^+ is found in dehyrogenases like alcohol dehydrogenase.Alcohol dehydrogense contains a $zinc^{2+}$/ sulphur complex that catalyses the oxidation of ethanol to acetaldehyde. Zinc (Zn^{2+}) is bound by two cysteines and a histidine and binding of alcohol substrate by its OH- group completes the zinc coordination and effectively locks the alcohol into position for hydride transfer to NAD^+ , hence oxidising the alcohol without changes in redox state of zinc or sulphur [1]. The active site of cysteine not only facilitates effective catalyses, it also makes the enzymes sensitive to oxidation and metal poisoning. Cysteine also was found to act as a ligand for catalytic metal ions like iron,nickel, copper, manganeese and molybdenum [1].

Radioprotective action of cysteine

The radioprotective action of cysteine thiols is brought about by its reaction with radiation induced reactive radicals to form less reactive thiyl radicals [5].

Determination of cysteine

A method to detect the simultaneous detection of cysteine and cystine in proteins has been developed using 2-aminoethyl methanethiosulphate (AEMTS). In this method , the sulfhydryl groups of the cysteine residues are first blocked with AEMTS and subsequent treatment with performic acid. This procedure quantitatively converts all cysteine residues to cysteic acid. This can be quantitatively analysed [6].

Conclusion:

This review has addressed the complexity of cysteine's thiol group *in vivo.*The amino acid's various redox-transformations, its ability to strongly coordinate transition metal ions, its nucleophilic nature and facile reaction with electrophiles makes it one of the interesting amino acids to study. A better understanding of the redox chemistry of this unique amino acid will provide plenty of possibilities for future research.

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