Biosynthesis of Food Constituents: Peptides – a Review

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Abstract

Velíšek J., Kubec R., Cejpek K. (2006): **Biosynthesis of food constituents: Peptides – a review**. Czech J. Food Sci., **24**: 149–155.

This review article gives a brief survey of principal pathways that lead to the biosynthesis of most important peptides occurring in foods. Glutathione, selected plant γ-glutamyl peptides, and animal histidine dipeptides are included in this review.

Keywords: biosynthesis; glutathione; γ-glutamyl peptides; *S*-alk(en)ylcysteine sulfoxides; phytochelatins; histidine dipeptides; carnosine; anserine; balenine

Peptides display a wide variety of biological functions and many of them have remarkable physiological properties. Some are widely distributed in nature and found in many different organisms, whereas others are only of a restricted occurrence. For example, peptides function as neurotransmitters and hormones and thus control many physiological processes. Furthermore, the toxic principles of some plants and mushrooms, insect, snake, and spider venoms are usually peptides.

Peptides are biosynthesised by ribosomal and nonribosomal (multi-functional enzyme) processes from a wide range of amino acids. Ribosomal peptide biosynthesis leads to enzymes, peptide hormones, and many other physiologically active peptides. Nonribosomal peptide biosynthesis is responsible for the formation of glutathione, histidine-containing dipeptides of skeletal muscles, peptide toxins, and peptide antibiotics. The mechanisms of these processes are, however, beyond the scope of this review.

Many structures biosynthesised by ribosomal processes are additionally modified. Glycopeptides are produced by adding sugar residues via *O*-glycoside linkages to the hydroxyls of serine and threonine residues or via *N*-glycoside linkages to the amino group of asparagine. Phosphopeptides and phosphoproteins have the hydroxyl group of serine or threonine esterified with phosphoric acid. Many peptides contain a pyroglutamic (glutiminic) acid residue at the *N*-terminus, which is a consequence of glutamic acid intramolecular cyclisation between the γ-carboxyl and the α-amino group. The *C*-terminal carboxylic acids may also frequently be converted to an amide.

Nonribosomal processes synthesise many natural peptides occurring in foods. These peptides are formed by a sequence of enzyme-controlled reactions. During these processes, each amino acid (often not encoded by DNA) is added (after activation by conversion into AMP esters) as a result of the specificity of the enzyme involved.

Partly supported by the Ministry of Education, Youth and Sports of the Czech Republic, Project MSM 6046137305.

Unusual amino acids are often formed as a result of an elimination reaction from proteinogenic amino acids. For example, 2-aminobutanoic (α-aminobutyric) acid is formed by decarboxylation of glutamic acid, dehydroalanine (2-aminoprop-2-enoic acid, 2-aminoacrylic acid, anhydroserine) is generated by elimination of H_2S from cysteine or by elimination of water from serine, and threonine yields (*Z*)-dehydrobutyrine, also called (*Z*)-aminobut-2-enoic acid (2-aminocrotonic acid or anhydrothreonine), by dehydration. Except of activating the amino acid and catalysing formation of the peptide, these multi-functional enzymes may possess activities responsible e.g. for racemisation of L-amino acids into D-amino acids and cyclisation of the amino acids at both termini of a linear peptide chain (Dewick 2002).

1 γ-Glutamyl peptides

1.1 Glutathione

Glutathione (γ-L-glutamyl-L-cysteinylglycine, GSH) is present in high concentrations in most living cells, being the major reservoir of non-protein sulfur. In animal cells, its levels range from 300 to 1500 mg/kg and lower levels occur in plants and microorganisms. For example, wheat flour contains glutathione at a level of 10–15 mg/kg (Velíšek 2002). GSH biosynthesis is similar in all living organisms.

GSH is synthesised from its constituent amino acids by two ATP-dependent reactions catalysed by γ-glutamylcysteine synthetase (EC 6.3.2.2) and glutathione synthetase (EC 6.3.2.3). In the first step, γ-L-glutamyl-L-cysteine is formed from L-glutamic acid and L-cysteine. During the second step, glycine is added to the C-terminal of γ-glutamylcysteine, forming GSH (Figure 1) (Mendoza-Cózatl *et al.* 2005).

Because of its unique redox and nucleophilic properties, GSH serves in bio-reductive reactions (Figure 2) and as an important line of defence against reactive oxygen species, heavy metals, xenobiotics, as well as pharmaceuticals (glutathione conjugates such as mercapturic acids resulting from detoxification processes). For example, in wheat flour, L-ascorbic acid is oxidised to L-dehydroascorbic acid (via L-monodehydroascorbic acid), which becomes a co-substrate of glutathione dehydrogenase (EC 1.8.5.1), which reduces dehydroascorbic acid back to ascorbic acid using GSH.

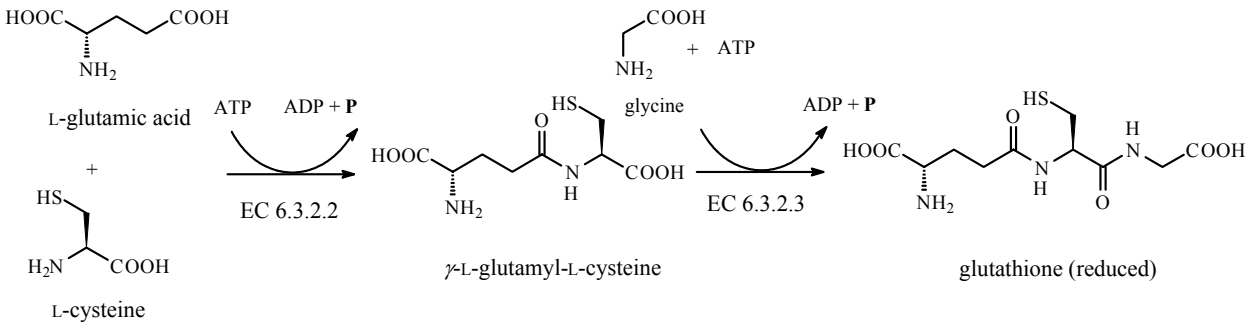


Figure 1

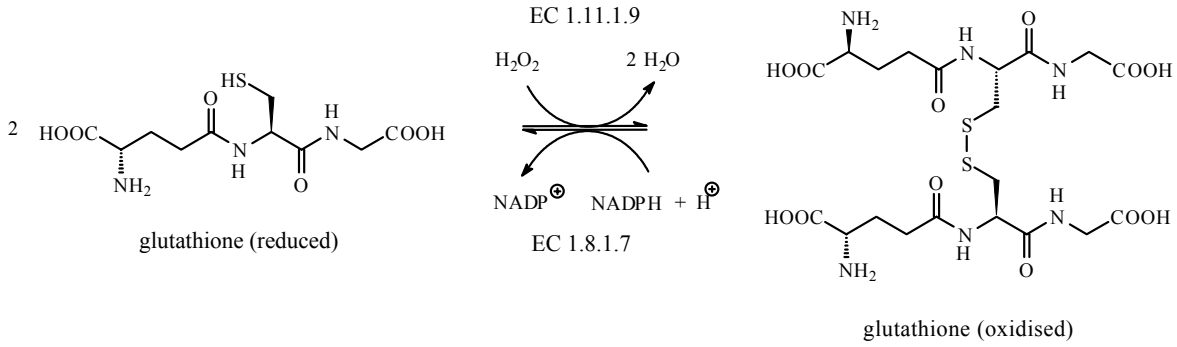


Figure 2

In fruits, L-ascorbate peroxidase (EC 1.11.1.11) catalyses oxidation of ascorbic acid by H_2O_2 to monodehydroascorbic acid and further to dehydroascorbic acid. Dehydroascorbic acid can be reduced back to ascorbic acid by GSH. Glutathione peroxidase (EC 1.11.1.9), a protein containing a selenocysteine residue, decomposes H_2O_2 (as well as steroid and lipid hydroperoxides) in biological membranes. The enzyme uses GSH, glutathione (reduced), which is oxidised to GSSG, glutathione (oxidised). GSH is then regenerated, e.g. by the action of NADPH-dependent glutathione reductase (EC 1.8.1.7) (KEGG).

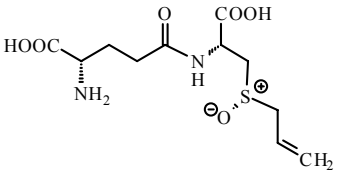
1.2 Other γ-glutamyl peptides

Although γ-glutamyl peptides are well represented in many organisms, their roles and properties are often not well understood yet (Velíšek 2002; Velíšek *et al.* 2005). In some cases, plant γ-glutamyl peptides play a role in the transport of amino acids across the membranes, protect plant cells (phytochelatins) against phytotoxic heavy metals, or they may function as storage compounds of nitrogen and sulfur (in *Allium* and *Brassica* species), and may also represent a significant pool of bioactive organoselenium compounds (Lancaster & Shaw 1989, 1991; Shaw *et al.* 2005).

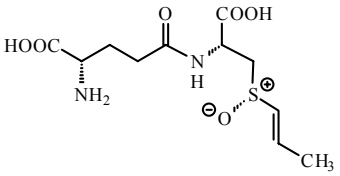
In *Allium* species, more than 20 γ-glutamyl peptides have been isolated, such as γ-glutamyl *S*-alk(en)ylglutathiones, γ-glutamyl-*S*-alk(en)ylcysteines, and γ-glutamyl-*S*-alk(en)ylcysteine sulfoxides (Jones *et al.* 2004). Nine of these peptides occur as intermediates in the biosynthesis of *S*-alk(en)ylcysteine sulfoxides (Velíšek *et al.* 2005), including γ-glutamyl-*S*-allylcysteine sulfoxide in garlic. (*E*)-γ-Glutamyl-*S*-(prop-1-en-1-yl)cysteine sulfoxide and *S*-(2-carboxypropyl)glutathione occur in onion (Figure 3). In pre-bulbing onions these two peptides were found below 50 mg/kg fresh weight and at bulbing accumulated to the levels of 2100 and 400 mg/kg resh weight, respectively. These levels were maintained throughout storage and decreased by 50% during sprouting (Lancaster & Shaw 1991).

γ-Glutamyl peptides are formed in reactions catalysed by the enzyme L-γ-glutamyltransferase (EC 2.3.2.2). This enzyme catalyses the splitting of the γ-glutamyl linkages in γ-glutamyl peptides (e.g. GSH) and the transfer of the glutamate moiety to amino acids, peptide acceptors or water (Shaw *et al.* 2005). For example, this enzyme catalyses the reaction in which glutathione (GSH, acting as a donor) reacts with an acceptor amino acid yielding the corresponding dipeptide (γ-glutamyl amino acid) (Figure 4). Such γ-glutamyl peptides may be formed by the reaction of GSH with the ethylene precursor, i.e. 1-aminocyclopropane-1-carboxylic acid, in tomato (Martin & Slovin 2000) and hypoglycin A in the ackee fruit, respectively (Velíšek *et al.* 2005).

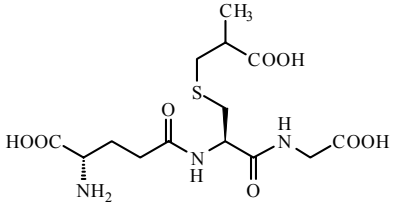
One of two possible biosynthetic pathways leading to *S*-alk(en)ylcysteine sulfoxides in *Allium* vegetables (Velíšek *et al.* 2005) proceeds via *S*-alk(en)ylation of the cysteine in glutathione (GSH), followed by transpeptidation to remove the glycyl moiety, oxidation to cysteine sulfoxide by oxidases, and finally a removal of the glutamyl



γ-glutamyl-*S*-allyl-L-cysteine sulfoxide



(*E*)-γ-glutamyl-*S*-(prop-1-en-1-yl)-L-cysteine sulfoxide



S-(2-carboxypropyl)glutathione

Figure 3

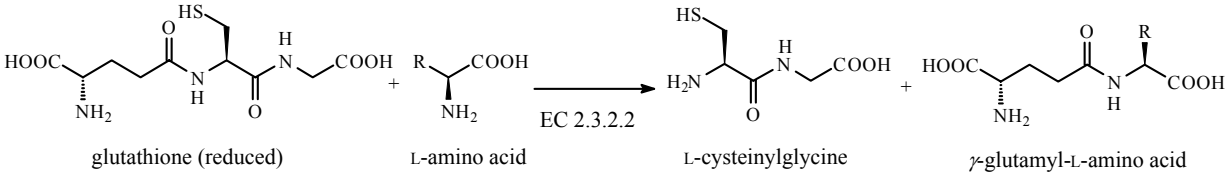


Figure 4

group to yield *S*-alk(en)ylcysteine sulfoxide. Alternatively, *S*-alk(en)ylcysteines may also be formed by the removal of the glutamic acid moiety from the dipeptide. Oxidation of these *S*-substituted cysteine derivatives then yields *S*-alk(en)ylcysteine sulfoxides (Jones *et al.* 2004; Hughes *et al.* 2005). The oxidation to the sulfoxides is carried out stereospecifically by enzymes with a broad substrate specificity (Jones *et al.* 2004) (Figure 5).

The biosynthesis of (R_C,S_S)-*S*-methylcysteine sulfoxide (methiin) requires methylation of the cysteine in glutathione by a suitable methyl donor to give *S*-methylglutathione. The biosynthesis of (R_C,S_S)-*S*-allylcysteine sulfoxide (alliin), (R_C,S_S)-*S*-propylcysteine sulfoxide (propiin), and (R_C,R_S)-(*E*)-*S*-(prop-1-en-1-yl)cysteine sulfoxide (isoalliin) is proposed to occur similarly but with participation of another alk(en)yl donors. The proposed pathway requires *S*-(2-carboxypropyl)glutathione as the precursor (Jones *et al.* 2004). The alk(en)yl side-chains in alliin and isoalliin are derived from the *S*-2-carboxypropyl group through decarboxylation and oxidation and that of propiin by reduction as indicated in Figure 6. It is believed that

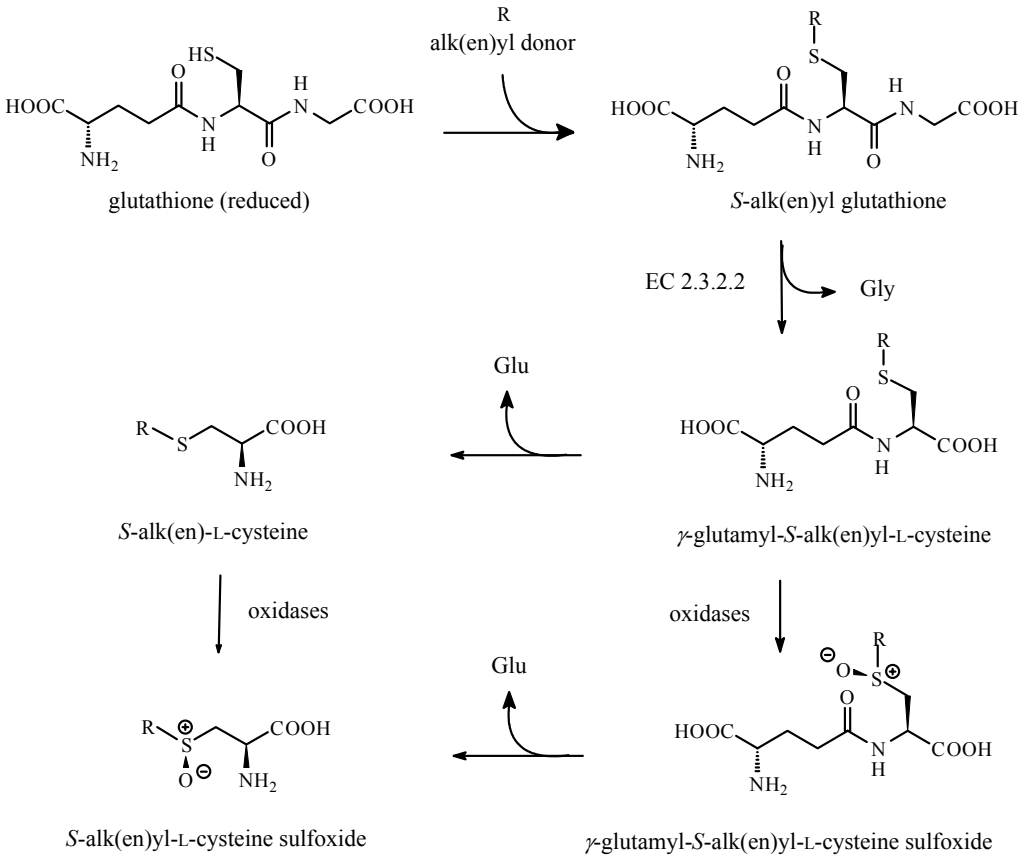


Figure 5

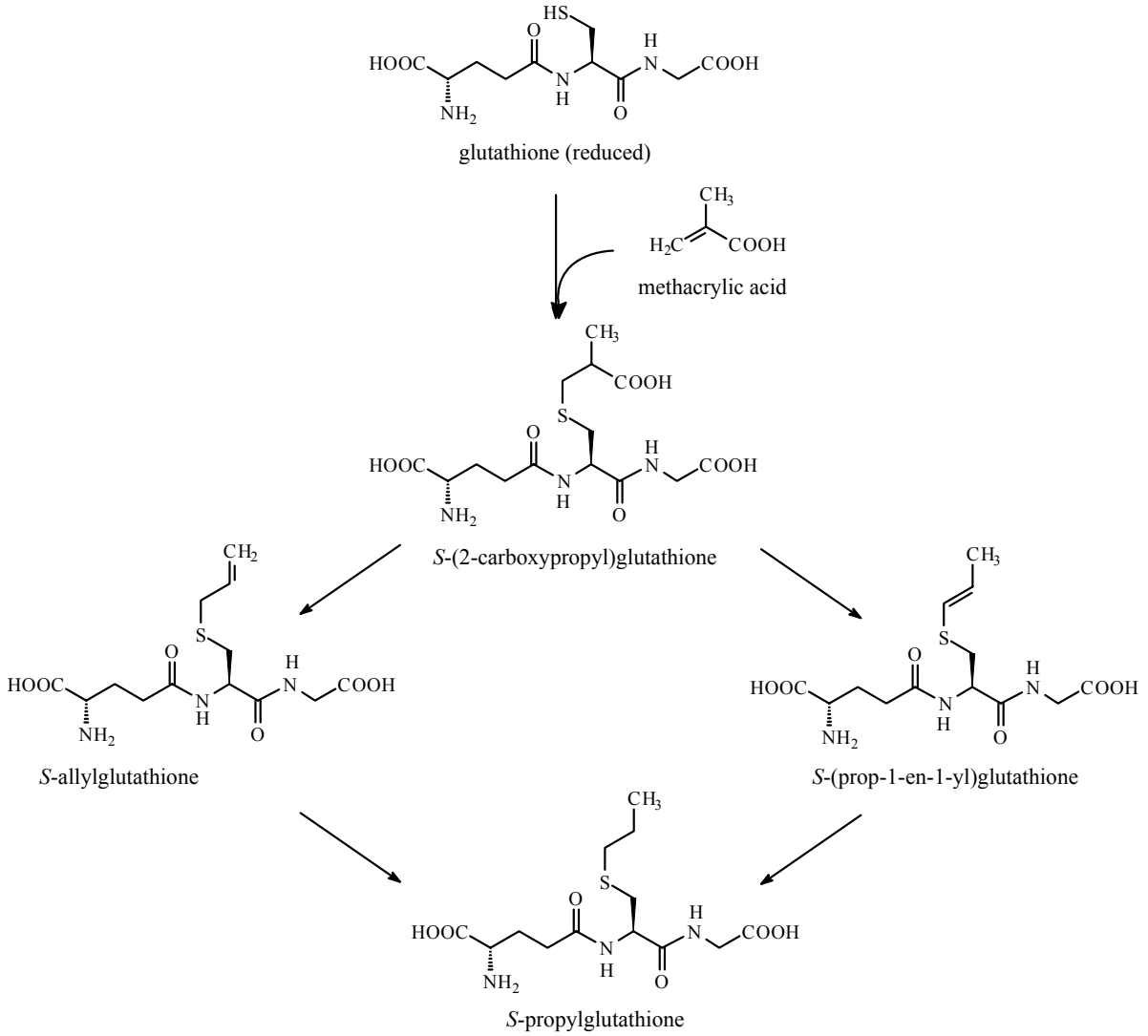


Figure 6

S-(2-carboxypropyl)glutathione is formed by the reaction of the cysteine residue in glutathione with methacrylic acid[1].

2 Histidine dipeptides

2.1 Carnosine and anserine

The histidine dipeptides carnosine (*β*-alanyl-L-histidine), anserine (*β*-alanyl-L-1-methylhistidine), balenine or ophidine (*β*-alanyl-L-3-methylhistidine), and homocarnosine (*γ*-aminobutyryl-L-histidine) occur in vertebrate tissues[2]. They show certain physiological properties such as buffering and antioxidant capacity, activity as neurotransmitter substances, and vasodilatory action. They also act as modulators of some enzymes and may play a role in the metabolism of copper. In meat, they are related to the sensory perception and generation of some meat flavour compounds.

[1]*In vivo*, methacrylyl-CoA is an intermediate in valine catabolism (Zolman *et al.* 2001).

[2]The level of carnosine in tissues is controlled by a number of enzymes transforming carnosine into related compounds, such as carcinine (by decarboxylation), *N*-acetylcarnosine (by acetylation), and anserine or balenine (by methylation) or into the corresponding amino acids by hydrolysis. Hydrolysis is mainly due to tissue carnosinase (EC 3.4.13.3) and anserinase (EC 3.4.13.5). These enzymes act on carnosine, anserine, and homocarnosine, and to a lesser extent on some other aminoacyl-L-histidine dipeptides (Pegova *et al.* 2000).

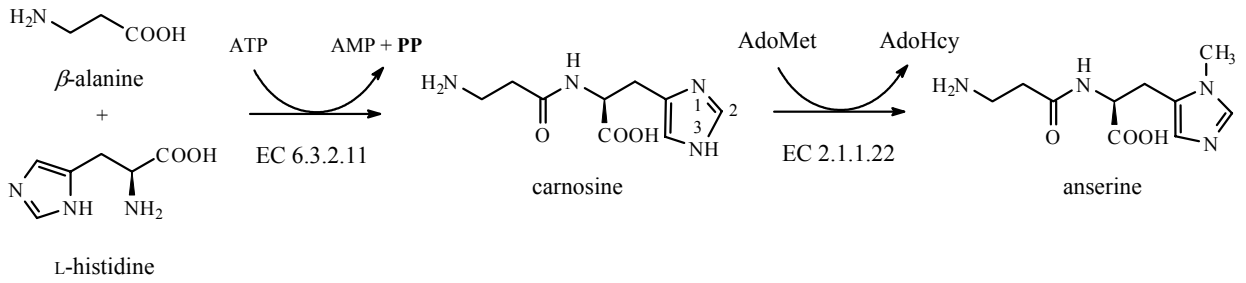


Figure 7

Anserine is the predominant dipeptide in birds, while mammal tissues contain higher amounts of anserine and carnosine, except humans that only have carnosine. Balenine is usually present in low amounts except in snakes and marine mammals, such as whales, where it is the predominant histidine dipeptide (Aristoy *et al.* 2004). For example, pork meat contains 1000–3400 mg/kg carnosine, 70–160 mg/kg anserine, and 180 mg/kg balenine (Velíšek 2002).

Carnosine is synthesised from β-alanine and L-histidine by carnosine synthetase (EC 6.3.2.11). The same enzyme catalyses the formation of homocarnosine from γ-aminobutyric (4-aminobutanoic) acid and histidine. The methylation of carnosine with *S*-adenosylmethionine (SAM or AdoMet) is catalysed by carnosine *N*-methyltransferase (EC 2.1.1.22) and yields anserine and *S*-adenosyl-L-homocysteine (SAH or AdoHcy) (Figure 7).

EC (Enzyme Commission) numbers and some common abbreviations

EC (Enzyme Commission) numbers, assigned by IUPAC-IUBMB, were taken from KEGG: Kyoto Encyclopedia of Genes and Genomes, http://www.biologie.uni-hamburg.de. At physiological pH some groups will be ionised, but in pictures the unionised forms are depicted to simplify the structures, to eliminate the need for counter-ions, and to avoid the mechanistic confusion.

AdoHcy	*S*-adenosyl-L-homocysteine (SAH)
AdoMet	*S*-adenosyl-L-methionine (SAM)
ADP	adenosine 5´-diphosphate
ATP	adenosine 5´-triphosphate
CoA	coenzyme A as a part of a thioester
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
P	phosphoric acid
PP	diphosphoric acid

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Received for publication June 23, 2005
Accepted after corrections September 26, 2005

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