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A study on the protection of methionine and the reduction of methionine sulfoxide in methionine-containing analogues of the growth-modeling factor Gly-His-Lys

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ABSTRACT

The protection of methionine and the reduction of methionine sulfoxide in methionine-containing analogues of Gly-His-Lys are described. The peptides were synthesized by a solid-phase method using the standard Fmoc procedure. Simultaneous deprotection of the peptide side chain and liberation from the resin were achieved using an appropriate TFA cocktail. The TFA cocktail was selected to minimize oxidation of the methionine residue. The reversible method of methionine oxidation was also studied. Selective identification and quantitative analysis of the methionine-containing peptides are based on an LC–MS study.

Growth-modeling factor (Gly-His-Lys) is an extremely interesting tripeptide with a long history. Gly-His-Lys was firstly co-isolated with copper and iron ions and was described in 1973 by Pickart.[1] The natural sequence of the tripeptide is localized in extracellular matrix proteins, especially in the α-II-chain of human collagen or in the chain of the secreted protein called SPARC. Gly-His-Lys is liberated from protein sequences by proteolytic enzymes during soft tissue injury. The tripeptide as a growth-modeling factor appears to play a physiological role in wound-healing regenerative processes, mainly by stimulating the effects of fibroblasts that participate in collagen synthesis.[2,3] It is also strongly involved in both anti-inflammatory and antioxidant activities.[3–6]

Accordingly, Gly-His-Lys has been successfully investigated as an agent with other small peptides in skin disease therapy and skin care products.[4,6,7] Interest in these fields has increased in the last few years and has resulted in the design of new peptidic compounds. The hydrophilic nature of small peptides was modeled to obtain Gly-His-Lys analogues with stability, solubility, potency, bioavailability, toxicity, complexation, and transdermal delivery system properties.[4] All the mentioned parameters are able to increase the possibility of the applications of the peptides. Moreover, newly designed analogues can intensify the antimicrobial properties and the infection protection ability of Gly-His-Lys, as very desirable features. Gly-His-Lys is known to be involved in the inhibition of bacterial growth in in vitro studies.[8] It was reported in these studies that relatively high levels of the peptide were used. Interestingly, in in vivo studies, strong antibacterial action against *Pseudomonas*, *Staphylococcus*, and *Streptococcus* spp. was observed at lower dosage, and the therapeutic effect was explained by the ability of Gly-His-Lys to increase expression of defensin antimicrobial genes.[9]

In spite of the wide range of its biological activities, numerous different modifications of the peptide chains have been reported to date. A very interesting group of Gly-His-Lys modifications is sequences containing other residues instead of histidine. It was confirmed that under physiological conditions in human plasma the long sequences containing the Gly-His-Lys fragment possess low stability, but the most crucial factor is a peptide bond between His and Lys. However, His is a residue strongly involved in the chemical nature of enzymes, and because of the participation of the imidazole ring in acid–base catalysis, the peptide bond is very susceptible to proteolytic damage.[4]

In the literature, there are many descriptions of new analogues of natural tripeptides modified at the second position of the peptide chain. Most consist of unnatural amino acids such as D-His, azaHis (1,2,3-triazole), L-4,5,6,7-tetrahydro-1*H*-imidazo[4,5-*c*]pyridine-6-carboxylic acid (Spi), or L-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (Tic).[4,10,11] Exchanging the His residue gives promising results for the improvement of the biological activity and stability of the designed compounds, but does not impart

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