European Journal of Medicinal Chemistry 66 (2013) 153–160

Contents lists available at SciVerse ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Exploring the space of histidine containing dipeptides in search of novel efficient RCS sequestering agents

Giulio Vistoli [a,*], Danilo De Maddis [a], Valentina Straniero [a], Alessandro Pedretti [a], Marco Pallavicini [a], Ermanno Valoti [a], Marina Carini [a], Bernard Testa [b], Giancarlo Aldini [a]

[a] Dipartimento di Scienze Farmaceutiche, Università degli Studi di Milano, Via Mangiagalli, 25, I-20133 Milano, Italy
[b] Dept of Pharmacy, Lausanne University Hospital (CHUV), Rue du Bugnon, CH-1011 Lausanne, Switzerland

ARTICLE INFO

Article history:
Received 16 October 2012
Received in revised form
25 February 2013
Accepted 10 May 2013
Available online 30 May 2013

Keywords:
Carnosine
Reactive carbonyl species
Carbonyl scavengers
4-Hydroxy-2-nonenal
Histidine nucleophilicity
Diastereoisomeric dipeptides

ABSTRACT

The study reports a set of forty proteinogenic histidine-containing dipeptides as potential carbonyl quenchers. The peptides were chosen to cover as exhaustively as possible the accessible chemical space, and their quenching activities toward 4-hydroxy-2-nonenal (HNE) and pyridoxal were evaluated by HPLC analyses. The peptides were capped at the C-terminus as methyl esters or amides to favor their resistance to proteolysis and diastereoisomeric pairs were considered to reveal the influence of configuration on quenching. On average, the examined dipeptides are less active than the parent compound carnosine (βAla + His) thus emphasizing the unfavorable effect of the shortening of the βAla residue as confirmed by the control dipeptide Gly-His. Nevertheless, some peptides show promising activities toward HNE combined with a remarkable selectivity. The results emphasize the beneficial role of aromatic and positively charged residues, while negatively charged and H-bonding side chains show a detrimental effect on quenching. As a trend, ester derivatives are slightly more active than amides while heterochiral peptides are more active than their homochiral diastereoisomer. Overall, the results reveal that quenching activity strongly depends on conformational effects and vicinal residues (as evidenced by the reported QSAR analysis), offering insightful clues for the design of improved carbonyl quenchers and to rationalize the specific reactivity of histidine residues within proteins.

1. Introduction

Reactive carbonyl species (RCSs) are electrophilic molecules which react with nucleophilic groups in proteins yielding oxidative-based non-enzymatic protein adducts [1–3]. These covalent adducts can be subdivided into two major classes depending on the source of the reactive species. Advanced glycation end products (AGEs) are generated by sugars or sugar derivatives including dicarbonyl derivatives such as glyoxal (GO), methylglyoxal (MGO), and 3-desoxyglucosone (3-DG), while advanced lipidoxidation end products (ALEs) are formed by the oxidative reactions of lipids comprising α,β-unsaturated carbonyls such as 4-hydroxy-2-nonenal (HNE), 4-oxo-2-nonenal (ONE) and acrolein (ACR) [4,5]. AGEs and ALEs are involved in oxidative cellular damage through different mechanisms [6] including protein dysfunction, protein oligomerization and fibrillogenesis [7], altered signal transduction, immune response [8] and activation of the receptor for AGEs (RAGE) which is a type I transmembrane glycoprotein of the immunoglobulin superfamily of cell surface receptors [9]. AGEs and ALEs have been widely accepted as biomarkers for oxidative-based diseases [10]. Moreover, taking into account the most recent studies reporting that AGEs and ALEs are involved in the pathogenesis of several diseases, including diabetes and arteriosclerosis, they are now also considered as promising targets for therapeutic intervention. This is promoting the design of carbonyl scavengers able to trap RCSs converting them into nontoxic and easily excretable derivatives so inhibiting protein carbonylation and all downstream pathways [11,12].

Although clinical investigations are still very limited, several *in vitro* and *in vivo* animal studies demonstrated that carnosine (βAla-His), an endogenous dipeptide found in millimolar concentrations in some tissues such as brain, heart and skeletal muscles [13,14], is able to detoxify RCS, inhibiting AGEs and ALEs formation and restraining oxidative-based diseases. The mechanism by which carnosine prevents AGEs and ALEs formation is still under investigation and the involvement of multiple molecular mechanisms should be considered, given that the formation of AGEs and ALEs can involve different reactions and several catalysts including

* Corresponding author. Tel.: +39 02 19349; fax: +39 02 19359.
E-mail address: Giulio.Vistoli@unimi.it (G. Vistoli).

0223-5234/$ – see front matter © 2013 Elsevier Masson SAS. All rights reserved.
http://dx.doi.org/10.1016/j.ejmech.2013.05.009

transition metals [15,16]. Nevertheless, there is enough evidence to indicate that carnosine acts by a direct quenching mechanism, at least for α,β-unsaturated aldehydes as clearly demonstrated by two independent groups [17,18].

As shown by Fig. 1, carnosine reacts with α,β-unsaturated aldehydes through a multi-step mechanism involving the initial formation of a reversible unsaturated imino intermediate followed by the key intramolecular Michael addition between the histidine imidazole ring and the acceptor β-carbon atom. The imino intermediate acts as an intramolecular catalyst promoting a stable approach between the two reacting centers, thus explaining why a mixture of the two separate amino acids (βAla + His) does not possess a significant quenching activity. Similarly, the reversible nature of the imino intermediate can explain why carnosine selectively quenches reactive α,β-unsaturated carbonyls without stably trapping physiological carbonyl compounds [19].

Despite these promising results, the beneficial activity of carnosine is limited in humans due to its unfavorable pharmacokinetic profile. Indeed, carnosine is actively absorbed by peptide transporters (hPepT1) [20] but immediately hydrolyzed in human plasma to its constituent amino acids by specific dipeptidases such as serum carnosinase (CN1) [21]. Considering the therapeutic interest of carnosine, it comes as no surprise that many analogs have been reported in the last few years. As recently reviewed [22], these derivatives have been designed with a view to A) increasing quenching activity, B) maintaining the mentioned selectivity, and C) ensuring a satisfactory oral bioavailability by preventing carnosinase-catalyzed hydrolysis while preserving active absorption by hPepT1. A critical analysis of all reported carnosine analogs [22], combined with computational studies involving serum carnosinase and peptide transporters [23–25], revealed that the carboxyl terminus and the β-alanine carbon skeleton can be largely modified to improve pharmacokinetic profile without detrimentally affecting quenching activity. Notably, C-terminus capping is a well-known strategy to enhance plasma stability by hampering peptide recognition by all hydrolases, thus suggesting that such a modification should prevent the hydrolytic effects of both specific carnosinases and nonspecific peptidases [26]. Conversely, the primary amino group and the imidazole ring are mandatory for quenching activity and cannot be modified without markedly altering efficiency and/or selectivity. However, it is worth observing that all proposed derivatives modified at the β-alanine residue were produced by replacing it with other β-amino acids, while the possibility of replacing β-alanine with proteinogenic α-amino acids has never been examined comprehensively.

On these bases, the present study was undertaken to investigate the quenching activity of proteinogenic histidine-containing dipeptides exploring as exhaustively as possible their accessible chemical space. Since the carnosine carboxyl group is not required for carbonyl scavenging and its modifications can improve the resulting pharmacokinetic profile (see above), the dipeptides were prepared with the C-terminus capped by a methyl ester or a primary amido group so as to study dipeptides which should be still recognized by peptide transporters and resistant to proteolysis. Moreover, the study considered diastereoisomeric pairs of dipeptides produced by alternating the absolute configuration of the histidine residue with a view to revealing the effect of configuration on quenching activity. Indeed, while the two carnosine enantiomers have the same quenching activity [27], a result easily explainable since two enantiomers must possess the same chemical reactivity, the introduction of a second chiral center should allow diastereoisomeric differences useful for a better understanding of the precise scavenging mechanism.

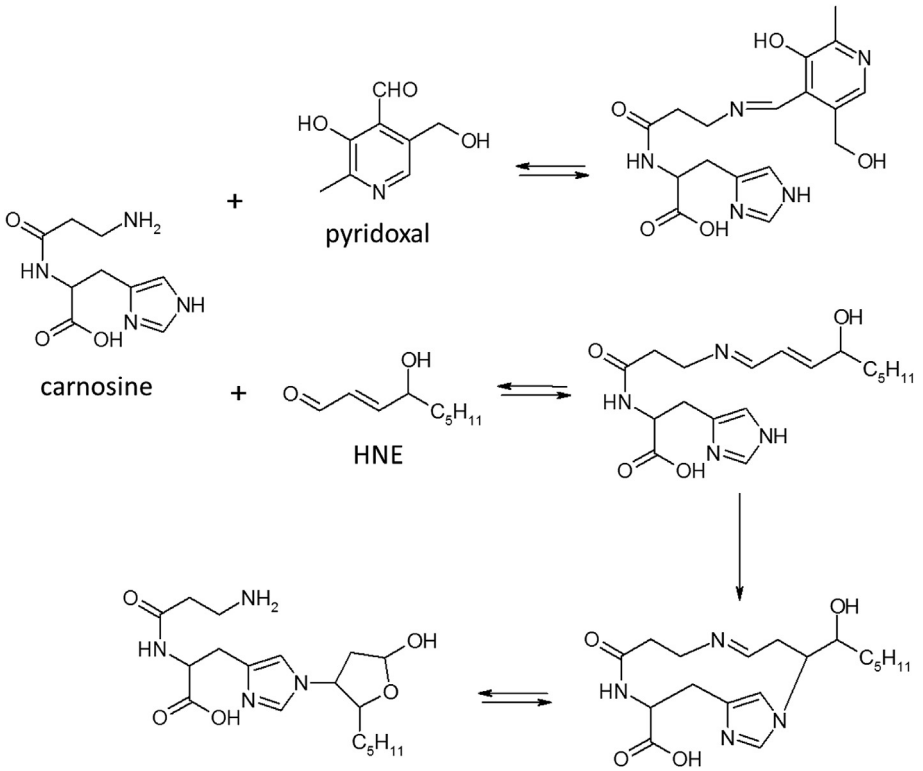

Fig. 1. Quenching mechanisms of carnosine with HNE and pyridoxal. One may note that quenching of pyridoxal involves a reversible condensation which yields the corresponding imine adduct, whereas HNE quenching occurs through a multi-step mechanism involving firstly the reversible imine intermediate, then the key intramolecular Michael addiction and lastly the hydrolysis of imine group to give the final hemiacetal adduct.