Conformationally restricted formyl-methionyl tripeptide chemoattractants

Claudio Toniolo[a,*], Marco Crisma[a], Giovanni Valle[a], Gian Maria Bonora[a], Stefano Polinelli[a], Elmer L. Becker[b], Richard J. Freer[c] and Padmanabhan Balaram[d]

[a]*Biopolymer Research Center, CNR, Department of Organic Chemistry, University of Padova, Via Marzolo 1, I-35131 Padova, Italy*
[b]*Department of Pathology, University of Connecticut Health Center, Farmington, CT 06032, U.S.A.*
[c]*Department of Pharmacology, Medical College of Virginia, Richmond, VA 23298, U.S.A.*
[d]*Molecular Biophysics Unit, Indian Institute of Science, Bangalore 560012, India*

Introduction

The tripeptide CHO-L-Met-L-Leu-L-Phe-OH is known to induce chemotaxis and selective release of lysosomal enzymes in neutrophils. The extended β-sheet conformation has been proposed as the receptor-bound conformation from spectroscopic analyses in solution. More recently, the X-ray diffraction structure of the bioactive derivative CHO-L-Met-L-Leu-L-Phe-OMe indicated the preference of the tripeptide for an 'open' folded conformation in the crystal state, helical at the central Leu residue but extended at the terminal Met and Phe residues. In this communication, we describe the results of a systematic, detailed biological study (using the release of the neutrophil granule enzyme β-glucosaminidase) and conformation analysis (using X-ray diffraction and ^{1}H NMR) of the tripeptide CHO-L-Met-Xxx-L-Phe-OMe (Xxx = Aib, Ac_3c, Ac_5c, Ac_6c, and Ac_7c) and their Boc-protected synthetic precursors as well. The α-amino acids dialkylated at the α-carbon listed above are known to be conformationally restricted and to favor strongly intramolecularly H-bonded forms of the β-bend type.

Results and Discussion

The N^{α}-formylated Ac_5c, Ac_6c, and Ac_7c analogs are approximately 2-, 5-, and 7-fold more active than the prototypical CHO-L-Met-L-Leu-L-Phe-OH ($ED_{50} = 1.3\text{–}3.5 \times 10^{-10}$). Conversely, the Aib and Ac_3c analogs are 4- and 150-fold less active, respectively. Thus, in general, the conformational restrictions in the CHO-L-Met-Xxx-L-Phe-OMe peptides are compatible with quite high activity. In addition, the activity increases as the hydrophobic pocket in the receptor for position 2 is more completely occupied. In the N^{α}-Boc-protected tripeptides, a marked fall in activity is observed.

* To whom correspondence should be addressed.

Our crystal-state X-ray diffraction analysis indicates that the peptide backbone of Boc-L-Met-Xxx-L-Phe-OMe (Xxx = Aib and Ac_5c) adopts a β-bend conformation at the -L-Met-Xxx- sequence stabilized by an intramolecular H-bond between the Phe NH and the Boc C=O groups. The ϕ,ψ values for the L-Met and Xxx residues are in reasonable agreement with those expected for a type-II β-bend conformation. The L-Phe residue is semi-extended. The only relevant conformational difference between the two structures is found in the L-Met side-chain disposition, (t, t, g^-) in the Aib tripeptide while (t, t, g^+) in the Ac_5c analog.

The involvement of the C-terminal (Phe) NH group of the tripeptides in intramolecular H-bonding in $CDCl_3$ solution (concn. 2×10^{-3} M) was determined on the basis of the modest variation in chemical shift experienced upon addition of DMSO to the $CDCl_3$ solution. Conversely, the behavior of the NH resonances of the Met and Xxx residues strongly favors the conclusion that these NH groups are solvent-accessible. In the different NOE spectra, obtained by irradiation of either the Xxx NH or the Met $C^{\alpha}H$ resonance, significant intensity enhancements are observed on the Met $C^{\alpha}H$ and Xxx NH resonances, respectively. These findings support the view that in $CDCl_3$ solution these peptides are folded in an intramolecularly H-bonded type-II β-bend conformation, as observed in the crystal state.

Our results establish that the conformationally restricted, β-bend forming formyl-methionyl tripeptides incorporating, at position 2, an α-carbon dialkylated residue, are able to induce granule enzyme secretion from rabbit peritoneal neutrophils. Therefore, this type of folded conformation may allow highly favorable interaction with the neutrophil formylpeptide receptor. The significant enhancements in activity observed by an increase of the bulkiness of the side chain of central residue indicates that peptide–receptor interactions involving this specific site are important modulators of biological effects.

Evidence for the tendency of the highly active CHO-L-Met-L-Leu-L-Phe-OH and its methyl ester to adopt the type-II β-bend conformation has not been found so far, either in the crystal state or in solution. In this connection, the possibility that the interaction of the formylpeptides with their neutrophil receptor involves either multiple sites or an induced-fit mechanism is one that cannot be ignored in view of the present findings and of the published results.