Peptide modification by incorporation of $\alpha$-trifluoromethyl substituted amino acids

Review Article

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Summary. Metabolic stabilization of pharmacologically active peptides can be achieved by incorporation of sterically hindered non-natural amino acids, e.g. C$\alpha\alpha$-disubstituted amino acids. $\alpha$-Trifluoromethyl substituted amino acids, a subclass of C$\alpha\alpha$-disubstituted amino acids, also fulfill this requirement while featuring additional properties based on the electronic influence of the fluorine substituents.

This review summarizes the results concerning the stability of peptides containing $\alpha$-TFM amino acids towards proteolysis by $\alpha$-chymotrypsin. Furthermore, configurational effects of $\alpha$-TFMAla on the proteolytic stability of peptides are explained using empirical force field calculations. The influence of $\alpha$-TFMAla incorporation on the secondary structure of selected tripeptide amides is compared to the effects exerted by its fluorine-free analogue, aminoisobutyric acid.

Finally, results on metabolic stabilization and biological activity of modified thyrotropin releasing hormone are interpreted.

Keywords: $\alpha$-Trifluoromethyl substituted amino acids – $\alpha$-Chymotrypsin – Proteolytic stability – C$\alpha\alpha$-Disubstituted glycines – TRH

1 Introduction

Some major disadvantages for the application of peptides as pharmaceutics are their low bioavailability, their sensitivity to enzymatic degradation, and their low selectivity because of the considerable conformational flexibility leading to interactions with different receptors (Hölzemann, 1991; Miller et al., 1994; Gillmor and Cohen, 1993). Therefore, investigation of the biological properties and three-dimensional structure of peptides rich in the conformationally restricted C$\alpha\alpha$-disubstituted amino acids is of current
interest. Certain C<sup>a,a</sup>-dialkylated amino acids have been shown to impart well defined and predictable conformations to the peptide backbone (Rizo and Gierasch, 1992; Valle et al. 1991; Bindra and Kuki, 1994; Altmann et al., 1992). These amino acids, especially α-methylalanine (α-aminoisobutyric acid; Aib) are present in naturally occurring peptide antibiotics (Matha et al., 1992) conferring on them a stable helical secondary structure and thereby, ion transporting properties. Furthermore, peptides containing these residues tend to dramatically slow down degradation processes (Toniolo et al., 1991).

α-Trifluoromethyl substituted amino acids (α-TFM amino acids) constitute a special class of C<sup>a,a</sup>-disubstituted amino acids due to the unique electronic properties of fluorine substituents. Therefore, they are interesting building blocks for peptide synthesis (Sewald and Burger, 1995). A trifluoromethyl group in α-position of an amino acid exerts considerable polarization effects on the neighbouring substituents. This structural alteration influences the hydrolytic stability of peptides containing TFM amino acids resulting in retarded degradation by peptidases (Burger et al., 1993) and, consequently, in prolonged intrinsic activity. The often postulated quasi-isosterism between a methyl and a trifluoromethyl group is still controversial (Seebach, 1990). The steric requirement of a trifluoromethyl group seems to be closer to that of an isopropyl than a methyl group. Hence, upon incorporation of α-TFM amino acids, severe conformational restrictions are exerted on the peptide chain. Furthermore, due to the high electron density, the trifluoromethyl group is capable of participating in hydrogen bonding as a proton acceptor. In contrast to an α-methyl group this property enables α-TFM substituted peptides to interact additionally with enzyme or receptor subsites.

2 Proteolytic stability of α-TFM amino acid derivatives

2.1 Enzymatic hydrolysis of N-protected α-TFM amino acid esters

Proteases like subtilisin, α-chymotrypsin or papain accept α-TFM amino acid esters only to a very limited extent (Burger et al., 1993). Both the hydrolysis rate and the turnover decrease in the order Z-(α-TFM)Gly-OMe > Z-(α-TFM)Ala-OMe > Z-(α-TFM)Leu-OMe. Z-(α-TFM)Phe-OMe is not turned over at all (Fig. 1). These data exclude the application of proteases in the resolution of enantiomeric α-TFM amino acid derivatives except in the case of Z-(α-TFM)Gly-OMe (Koksch et al., manuscript in preparation).

2.2 Protease catalyzed peptide synthesis using α-TFM amino acid esters as substrates

Dipeptide esters with an N-terminal TFM amino acid are accepted as substrates by proteolytic enzymes (Fig. 1). For example, H-(α-TFM)Phg-Phe-OMe is hydrolyzed by α-chymotrypsin or subtilisin within short reaction times