Insights into matriptase-2 substrate binding and inhibition mechanisms

Eva Maurer[a], Mihiet T. Sisay[a], Marit Stirnberg[a], Torsten Steinmetzer[b], Jürgen Bajorath[c] and Michael Gütschow[a]

[a] University of Bonn, Pharmaceutical Institute, Pharmaceutical Chemistry 1, An der Immenburg 4, 53121 Bonn
[b] University of Marburg, Pharmaceutical Institute, Pharmaceutical Chemistry, Marbacher Weg 6, 35032 Marburg
[c] University of Bonn, Bonn-Aachen International Center for Information Technology, Dahlmannstr. 2, 53113 Bonn
eva.maurer@uni-bonn.de

1. Introduction

Matriptase-2 belongs to the family of type II transmembrane serine proteases, representing an emerging class of cell surface proteolytic enzymes, and is predominantly expressed in the liver [1]. Physiologically, matriptase-2 was identified to be a key regulator in iron homeostasis. It was shown that matriptase-2 suppresses enzyme activity to be a key regulator in iron homeostasis. It was shown that matriptase-2 suppresses the presence of the catalytic terminal end of the protein. Physiologically, matriptase-2 was identified predominantly expressed in the liver [1]. Physiologically, matriptase-2 was identified to be a key regulator in iron homeostasis. It was shown that matriptase-2 suppresses enzyme activity.

2. Active site-mutated matriptase-2 variants

To obtain insights into the structural differences of the active sites of matriptase-2 and matriptase and the resulting enzyme-ligand interactions, four important amino acids of matriptase-2 (His[303], Glu[12], Ala[30], Leu[209]) were mutated to the equivalent residues in matriptase (Figure 1).

These matriptase-2 variants were obtained by site-directed mutagenesis. HEK cells were transfected with cDNA encoding wild-type or the mutated forms of matriptase-2 with a Myc-tag at the C-terminal end of the protein. Immunoblot analysis using Anti-c-Myc antibody showed the presence of the catalytic domain of approximately 30 kDa in the conditioned media, and the full length form of 120 kDa in membrane fractions of all mutants (Figure 2).

3. Matriptase-2 inhibitors

The inhibition of matriptase-2 by dipetide amides 1-4 has recently been reported [5]. Compounds 1 and 3 share the 4-amidinobenzylamine group, which is replaced by 2-aminoethyl-5-chlorobenzylamine in compounds 2 and 4. This part of the molecule is expected to bind to the S1 pocket and the D-arginine (in 1 and 2) or D-cyclohexylalanine moiety (in 3 and 4) to the S3/S4 pocket of matriptase-2.

4. Kinetic parameters

The replacement of Glu[12] by Tyr in MT2W205 [9] resulted in an overall decrease of the inhibitory effect caused by compounds 1-4. Concerning MT2W205, the potency of 1 and 3 was slightly improved compared to MT2WT (WT, wild-type). In MT2W205 variants, the inhibitory effect of compounds 3 and 4 decreased compared with MT2WT [9]. The simultaneous introduction of Ser at position Ala[30] and Asp at position Leu[209] caused similar results. The additional replacement of Glu[12] for Tyr in MT2A757S/L785D further decreased the affinity of all four compounds towards MT2E712Y/L785D.

5. Molecular modelling

The variant MT2W205 was 4-12 fold more strongly inhibited by compounds 1-4 than the wild-type enzyme. The inhibitory potency of 3 and 4, both containing a D-cyclohexylalanine, increased when comparing the mutated variant with MT2WT. The model indicates that the cyclohexyl group is directed towards the upper part of the S3/S4 pocket forming hydrophobic interactions with Thr783 and the introduced Phe665 (Figure 4), leading to improved inhibitory activity of 3 and 4.

References and Acknowledgement


This work was supported by Deutsche Forschungsgemeinschaft, SFB645. E.M. was supported by a fellowship award from Bayer HealthCare Pharmaceuticals.