Mimicking Natural Inhibitor Design While Improving Selectivity

Jean Gaffney[1], Netta Sela-Passwell[1], Raghavendra Kikkeri[2], Orly Dym[3], Haim Rozenberg[3], Raanan Margalit[3], Rina Arad-Yellin[3]
Miriam Eisenstein[4], Ori Brenner[5], Tsipi Shoham[3], Tamar Danon[6], Abraham Shanzer[2] & Irit Sagi[1]

Weizmann Institute of Science: Department of Biological Regulation[1], Department of Organic Chemistry[2], Department of Structural Biology[3], Department of Chemical Support[4], Veterinary Resources[5], Department of Molecular Cell Biology[6]

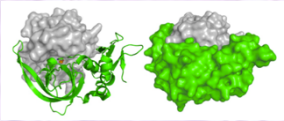
1. Introduction

The matrix metalloproteinases (MMPs) are a class of zinc dependent endopeptidases that are responsible for the degradation of the extracellular matrix. They have been implicated cancer, metastasis, angiogenesis, tumor growth as well as in inflammatory diseases such as rheumatoid arthritis. The MMPs are inhibited *in-vivo* by two mechanisms. The first is inhibition by the endogenous tissue inhibitors of metalloproteinases (TIMPs). There are four TIMPs which are responsible for inhibition of over two dozen MMPs. The second mechanism of inhibition is by the prodomain of the MMPs. MMPs are secreted as inert zymogens. The prodomain must be cleaved to generate the active enzyme. By taking clues from nature, our lab has sought to design novel inhibitors with increased specificity and stability. The first way is TIMP-like antibodies that are generated to recognize the catalytic zinc active site. These metallobodies effectively target the *in vivo* activity of the MMPs by mimicking the mechanism used by TIMPs (1). In addition, we explore the mechanism of inhibition of the prodomain of MT1-MMP. Here we see, the prodomain is specific for MT1-MMP, when tested against a panel of other MMPs.

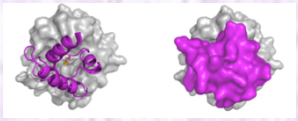
2. Natures Way of Inhibiting the MMPs

Natural inhibitor design of the MMPs utilizes both the zinc in the active site and the surrounding protein surface. MMPs are tightly regulated by their endogenous inhibitors (TIMPs) (2). Their activity is also regulated by their prodomain, which must be cleaved for enzyme activation. Our work looks towards nature, to mimic these interactions, and to improve the selectivity and stability of these designs.

Inhibition by TIMP

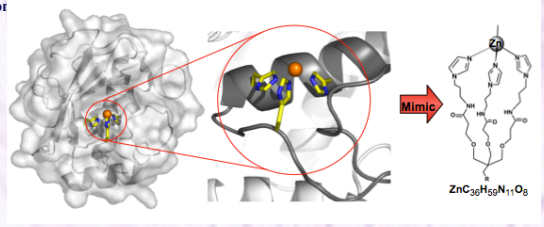


Inhibition by Prodomain

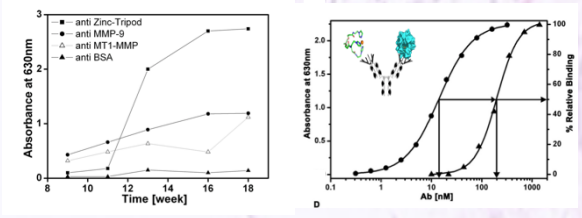


3. Production of TIMP-Like Inhibitory Antibodies (Metallobodies) Produced by Novel Immunization Strategy

Mice were immunized with the recombinant enzyme, followed by a synthetic zinc based molecule, known as zinc-tripod. Zinc tripod is a small, synthetic, organic metal-ligand molecule-tris imidazole zinc complex which was designed to mimic structural and chemical motifs of the catalytic machinery in the active site of the MMPs. The hypothesis was presenting Zn-tripod as a B cell epitope would induce an immune response against the synthetic zinc imidazole motif and initiate the production of mor



Above shows the crystal structure of MMP-9, a detailed view of the active site of MMP-9 and the Zinc-tripod molecule mimicking the MMP active site used in the injection scheme.

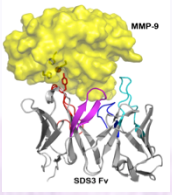


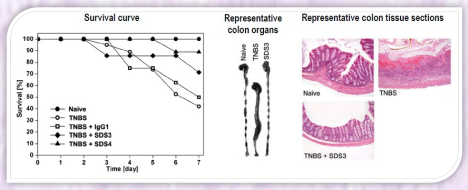
The goal is to generate antibodies that recognize both the synthetic mimicry Zn-tripod as well as the catalytic enzyme. Serum antibody titers from mice immunized with Zn-tripod against Zn-tripod–BSA, MMP-9 catalytic domain or BSA control antigens. MT1-MMP was used as a negative control. Binding curve of SDS3 (K_d = 200 ± 20 nM) and SDS4 (K_d = 20 ± 5 nM) mAb binding to MMP-9. Error bars represent the standard deviation of representative experiments done in triplicate.

4. Novel Metallobodies Show Selectivity for the Gelatinases and Demonstrate Therapeutic Activity in an IBD Mouse Model

TIMP-like metallobodies show novel enhanced selectivity for the gelatinases as compared to other proteases. A docking model of SDS3 Fv to MMP-9 catalytic domain (yellow) suggests direct contact with the catalytic zinc ion (orange sphere) as well as the proteinase surface loops is shown below. The convex edge of the MMP-9 substrate binding site inserts into the wide cleft at the rim of the antibody combining site, making contacts with the SDS3 CDRs L1 and H3, located near the center of this site. In vivo results in an irritable bowel disease model demonstrate the therapeutic potential of these novel antibodies.

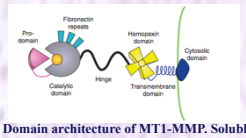
MMP	SDS3 IC_{50} [μM]	SDS4 IC_{50} [μM]
MMP-9	1±0.1	0.07±0.008
MMP-2	1.4±0.16	0.056±0.006
MMP-14	14±0.75	1.42±0.13
MMP-7	NI	NI
MMP-12	NI	NI
MMP-1	NI	NI
TACE	NI	NI



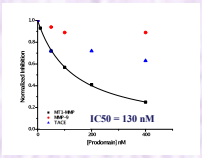


4. Preliminary Design and Stabilization of MT1-MMP Prodomain Indicates Selective Inhibition

The prodomain of the MMPs are naturally selective inhibitors (3,4). Here we work towards improving the stability of this reagent for use as a specific inhibitor. Here we expressed a stable MT1-MMP prodomain construct in *E.coli* and tested the selectivity of the prodomain against other proteases. As shown below, there is little effect of the prodomain of MT1-MMP on inhibition of MMP-9 and TACE. All experiments were performed using a fluorogenic peptide substrate.



Domain architecture of MT1-MMP. Soluble prodomain overexpressed in *E. coli*.



Inhibition of MT1-MMP, MMP-9 and TACE by the prodomain of MT1-MMP.

6. Conclusions

1. Our lab has been successful in the design of novel selective function blocking antibodies, known as metallobodies. SDS3 and SDS4 have shown selectivity for MMP-2 and MMP-9. The results show that immunization with a small synthetic active site mimic results in developing antibodies that are targeted to the catalytic metal-protein cleft. These antibodies bind the metalloenzyme target through interactions between both the metal motif and the enzyme surface. This mechanism is similar to that seen with the TIMPs.

2. In a second route to inhibition of the MMPs, we explored the inhibition of catalytic MT1-MMP by its prodomain, which was expressed recombinantly in *E. coli*. Here we demonstrated the prodomain of MT1-MMP is specific for MT1-MMP.

7. References

1. Sela-Passwell, N. A., Kikkeri, R., Dym, O., Rozenberg, H., Margalit, R., Arad-Yellin, R., Eisenstein, M., Brenner, O., Shoham, T., Danon, T., Shanzer, A., and Sagi, I. (**2012**) Antibodies targeting the catalytic zinc complex of activated matrix metalloproteinases show therapeutic potential, *Nature Medicine* 18, 143-147.
2. Sela-Passwell, N., Trahtenherts, A., Kruger, A., and Sagi, I. (**2011**) New opportunities in drug design of metalloproteinase inhibitors: combination between structure-function experimental approaches and systems biology, *Expert Opinion on Drug Discovery* 6, 527-542.
3. Golubkov, V. S., Cieplak, P., Chekanov, A. V., Ratnikov, B. I., Aleshin, A. E., Golubkova, N. V., Postnova, T. I., Radichev, I. A., Rozanov, D. V., Zhu, W. H., Motamedchaboki, K., and Strongin, A. Y. (**2010**) Internal Cleavages of the Autoinhibitory Prodomain Are Required for Membrane Type 1 Matrix Metalloproteinase Activation, although Furin Cleavage Alone Generates Inactive Proteinase, Journal of Biological Chemistry 285, 27726-27736.
4. Golubkov, V. S., Chernov, A. V., and Strongin, A. Y. (**2011**) Intradomain Cleavage of Inhibitory Prodomain Is Essential to Protumorigenic Function of Membrane Type-1 Matrix Metalloproteinase (MT1-MMP) in Vivo, *Journal of Biologica Chemistry* 286, 34215-34223.