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Oxoethylene derivative of the natural substrate as an inhibitor of matrix metalloproteinase MMP-2

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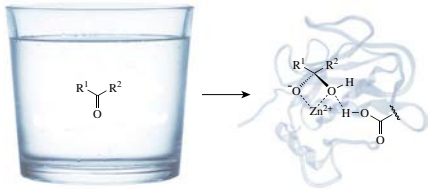
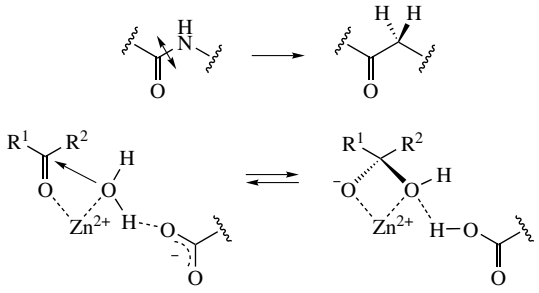
A mimetic of a natural substrate of the matrix metalloproteinase MMP-2, in which the amide group with the scissile peptide bond is replaced with the oxoethylene moiety, inhibits the enzyme.



Matrix metalloproteinases (MMP) play an important role in a balance of the extracellular matrix composition. In particular, excess catalytic activity of MMP-2 promotes metastasis growth and tissue degradation.[1–4] One of the most promising approaches to develop novel inhibitors is related to the synthesis of the oligopeptide compounds or their mimetics. Those can be normally metabolized in living organisms unlike small organic molecules and interact with a large amount of binding sites increasing specificity to a certain type of MMP.[5–7] Here we present an experimental evaluation of the idea suggested from our molecular modeling simulations.[8] If substitute the cleaving amide fragment of oligopeptide substrate, proposed for MMP-2,[9] with the oxoethylene (oxoethane-1,2-diyl) group, the obtained mimetic will act as inhibitor of MMP-2. Ketone group is relatively inert in aqueous solution, but when bound to the active site of MMP-2 the C=O bond is polarized and carbonyl carbon atom is ready for the nucleophilic attack (Scheme 1). Catalytic water molecule of the active site, that normally attacks carbonyl atom of the scissile amide fragment, forms the covalent bond with the carbon atom of the ketone group transforming it to the *gem*-diol form. The latter coordinates Zn^{2+} of the active site by its functional group formed *in situ*.



Scheme 1 Replacement of the amide group with oxoethylene one and ketone hydration in the active site of MMP-2.[8]

MMP-2 cleaves peptide bond between glycine and leucine in the oligopeptide Gly-Pro-Gln-Gly~Leu-Ala-Gln. We synthesized[†] oligopeptide mimetic that contained pseudodipeptide with the oxoethylene fragment instead of Gly~Leu peptide bond (Scheme 2).

During the kinetic experiments[‡] we varied concentrations of both the substrate and inhibitor, the enzyme concentration remained constant (Figure 1).

[†] *Synthesis of the inhibitor*. Compound **4** was prepared from three major building blocks: tripeptides **1** and **3** and keto amino acid **2**. The Nα-Boc (*Nα-tert*-butoxycarbonyl) group in **3** was removed with trifluoroacetic acid (TFA). The resulting trifluoroacetate salt was coupled with **2** either using PyBOP (benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate) as a coupling reagent or utilizing the corresponding pentafluorophenyl ester prepared from **2** with an aid of F-complex.[10] The Nα-Boc group in the resulting pseudopentapeptide was deprotected with TFA. The obtained trifluoroacetate salt was coupled to tripeptide **1** using the conditions that are known to induce low level of racemization at glutamine residues in model peptides.[11] Finally, the Nα-Boc group in the resulting pseudooctapeptide was removed again with an aid of TFA. The structure of the trifluoroacetate salt of **4** was proved by ^{1}H and ^{13}C NMR spectra. The ^{13}C NMR spectrum exhibited two signals (at 205.9 and 205.6 ppm) for the ketone carbonyl groups of synthesized diastereomeric pseudopeptides. Compound **2** was prepared following the reported method.[12] We synthesized oligopeptide mimetic with the oxoethylene fragment instead of Gly~Leu peptide bond and tested its inhibitory properties.

[‡] *Enzymatic experiments*. Kinetic measurements were performed using active MMP-2, fluorogenic substrate MCA-Pro-Leu-Ala-Nva-Dpa-Ala-Arg [MCA = (7-methoxycoumarin-4-yl)acetyl; Nva = L-norvaline; Dpa = = 3-(2,4-dinitrophenyl)-L-2,3-diaminopropionyl] produced by Calbiochem Merck Millipore, BSA and Brij-35 produced by Sigma-Aldrich and commercially available buffer components. Reaction buffer contained 50 mM Tris-HCl, pH 7.5, 0.2 M NaCl, 10 mM $CaCl_2$, 50 μM $ZnSO_4$, 0.1% BSA. The time dependences of product fluorescence intensity (excitation at 325 nm, emission at 393 nm) were measured in the thermostatted microcells Hellma (USA) at 22 °C using fluorescence spectrophotometer Varian Cary Eclipse (USA). Reaction curves were analyzed by the classical initial rates method. We calculated values K_m = 9±0.5 μM and V_{max} = = 0.21±0.02 a.u. s^{-1} for the substrate–enzyme system using non-linear regression analysis. K_m and V_{max} values in the presence of an inhibitor (0–15 mM) were obtained similarly. Inhibition constant was determined from the nonlinear fitting of the rate dependence on the inhibitor concentration. All calculations were performed using Origin Microcal software.

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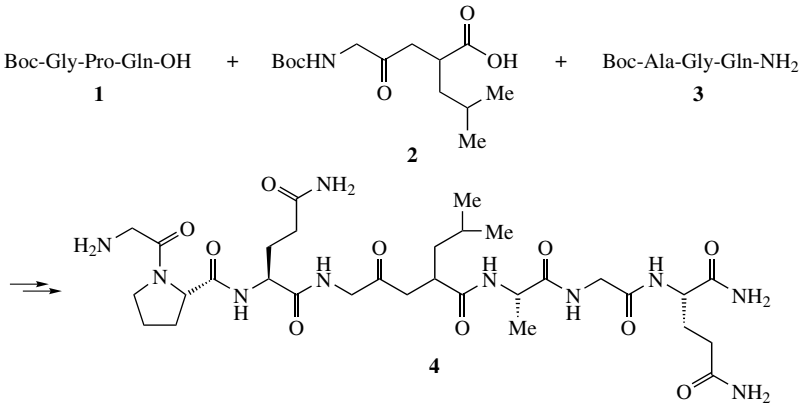

Scheme 2

The inhibition constant K_i = 5×10^{-3} M obtained from the experiments is comparable to that of the parent oligopeptides Gly-Pro-Gln-Gly~Leu-Ala-Gln (K_m = 7.3×10^{-3} M).[9] It can be explained on the basis of the molecular modeling results.[8] If we compare equilibrium geometry configurations of the ES and EI complexes, the only difference is one hydrogen bond that is present in ES (between NH group of the scissile fragment and the enzyme) and absent in EI as this group is replaced with CH_2 group; therefore, EI complex should be a couple of kcal mol^{-1} less favorable than ES. Upon EI formation, ketone is hydrated and EI complex with newly formed ketal decreases the energy of the system by ~3–4 kcal mol^{-1} according to our calculations.[8]

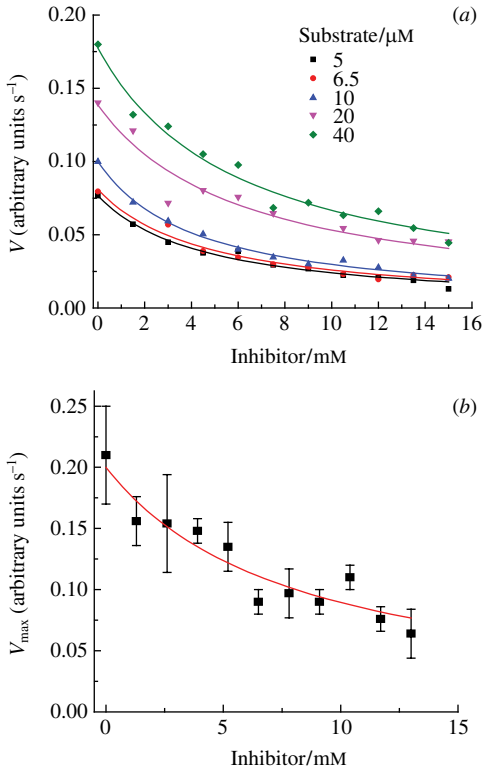

Figure 1 (*a*) *V* measured at different substrate and inhibitor concentrations and (*b*) V_{max} dependence on the inhibitor concentration obtained from the Michaelis curves.

Accordingly, the K_m and K_i values should be comparable as observed in the experiments.

In summary, we synthesized the mimetic of the natural substrate of MMP-2 with the amide group containing the Gly~Ile peptide bond replaced by the oxoethylene moiety. This compound inhibits MMP-2 with the K_i ~ 5×10^{-3} M, that is close to the K_m value of the oligopeptide substrate.[9] We demonstrated that this approach can be used to design novel inhibitors of the matrix metalloproteinases.

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