Available online at www.sciencedirect.com
ScienceDirect
Mendeleev Commun., 2017, **27**, 157–159
Mendeleev Communications

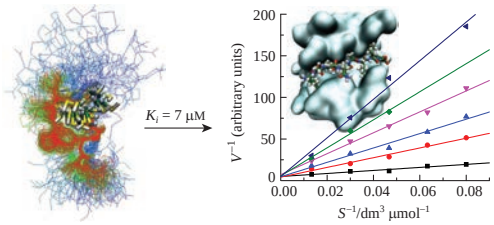
Molecular mechanism of interactions between MMP-2 and its oligopeptide-based inhibitors

Maria G. Khrenova,[a,b] Ilya D. Solovyev,[a,b] Grigory D. Lapshin[b] and Alexander P. Savitsky*[b]

[a] *Department of Chemistry, M. V. Lomonosov Moscow State University, 119991 Moscow, Russian Federation*
[b] *A. N. Bach Institute of Biochemistry, Research Center of Biotechnology of the Russian Academy of Sciences, 119071 Moscow, Russian Federation. Fax: +7 495 954 2732; e-mail: apsavitsky@inbi.ras.ru*

DOI: 10.1016/j.mencom.2017.03.017

Taking matrix metalloproteinase MMP-2 as an example, we demonstrate that the rational design of oligopeptide-based inhibitors by molecular modeling should involve both a study of interactions in the active sites of the target enzyme and the conformational dynamics of the oligopeptide in solution.



Matrix metalloproteinases (MMPs) belong to a wide class of secreted or membrane-associated zinc-dependent endopeptidases and comprise more than 20 members in a human organism. Under normal physiological conditions, MMPs play important roles in tissue remodeling and other processes associated with the metabolism of extracellular matrix proteins. Overactivated MMPs promote invasion and assist in the progress of certain types of cancer. Thus, the development of the specific inhibitors of MMPs is an important problem to be solved.[1–3] The most promising strategy comes from oligopeptide-based inhibitors, which interact with both the Zn^{2+} of the active site of MMP and various binding sites of the substrate-binding cleft.[4] Among the oligopeptide-based inhibitors, the β-amyloid precursor protein-derived inhibitory peptide (APP-IP) exhibits a low value of IC_{50} = 30 nM.[5] It is tightly bound to the substrate-binding cleft of the MMP-2 in the opposite N to C direction compared with the natural substrate, and its aspartic acid coordinates the zinc cation of the active site.[6] Recent molecular modeling studies revealed a reaction mechanism in the active site of MMP-2[7–11] and suggested certain ways to obtain oligopeptide-based inhibitors by introducing functional groups into natural substrates that can interact with the zinc cation of the active site.[12–14]

Here, we apply modern molecular modeling tools to study the mechanism and the origin of the inhibitory properties of the APP-IP and its derivatives. We change aspartate of APP-IP to serine and cysteine that can form coordination bonds with Zn^{2+}. We focus on both the conformational dynamics of these compounds in solution and the mechanism of inhibition in the active site of MMP-2. We complement this study with the experimental evaluation of the suggested inhibitors.

In the steady state kinetic experiments,[†] we measured the initial rates and obtained the value of IC_{50} = 32±3 nM for the APP-IP, which was in a good agreement with a published value.[5] For the APP-IP-Asp6Cys inhibitor, the nonlinear regression analysis of the dependence of reaction rate on substrate concentration confirms competitive manner and results in K_i = 7±1 μM (Figure 1), which is two orders of magnitude greater than K_i = 40 nM for the competitive inhibition described for the full-length APP.[15] The APP-IP-Asp6Ser did not exhibit inhibition activity in a range of concentrations up to 10^{-4} M.

We applied modern molecular modeling tools including QM/MM, QM/MM-MD and classical MD approaches[12] to study the origin of differences in the inhibitory properties of APP-IP and its Asp6Cys and Asp6Ser mutants. First, we propose that all enzyme-inhibitor (EI) complexes are formed structurally in the

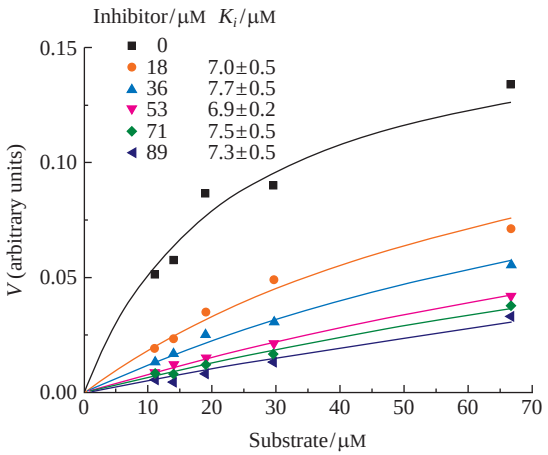


Figure 1 Fluorogenic substrate cleavage by MMP-2. The initial velocities are obtained at various concentrations of APP-IP-Asp6Cys inhibitor and substrate.

[†] *Enzymatic experiments*. Kinetic measurements were performed in a buffer solution containing 50 mM Tris-HCl (pH 7.5), 0.2 M NaCl, 10 mM $CaCl_2$, 50 μM $ZnSO_4$ and 0.05% Brij-35 as suggested in ref. 16. The fluorescence signals were measured in quartz microcells with an optical pathlength of 1.5 mm (Hellma, USA) at an excitation wavelength of 328 nm (slit width, 10 nm) and an emission wavelength of 365 nm (slit width, 20 nm) using a Cary Eclipse fluorescence spectrometer equipped with a thermostatic cuvette holder at 22 °C. The fluorescence intensity was recorded at regular intervals of 2 s for 300 s; *i.e.*, 150 spots were obtained in a single measurement. The peptide inhibitor APP-IP (Ile-Ser-Tyr-Gly-Asn-Asp-Ala-Leu-Met-Pro) and its Asp6Cys and Asp6Ser mutants were synthesized at the Branch of the Institute of Bioorganic Chemistry of the Russian Academy of Sciences (Pushchino, Moscow region, Russia).

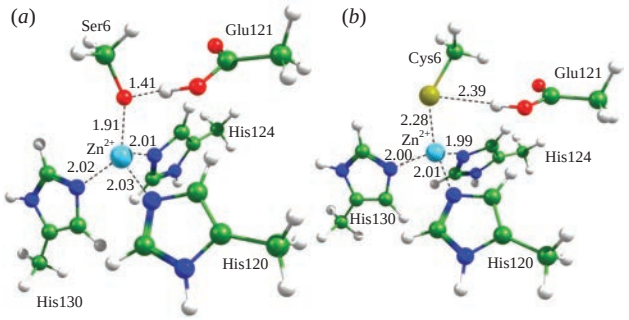


Figure 2 Equilibrium geometry configurations of the MMP-2 complexes with (*a*) APP-IP-Asp6Ser and (*b*) APP-IP-Asp6Cys. Distances are shown in Å.

same manner as the MMP-2-APP-IP complex. We manually replaced the Asp6 of APP-IP by cysteine and serine residues in neutral form; the carboxylate group of catalytic Glu121 (notations are used from the crystal structure PDB ID: 3AYU) was considered in the deprotonated form. In the QM/MM-MD simulation, the proton from the thiol group of the APP-IP-Asp6Cys transferred simultaneously to the carboxylate group of Glu121; the same was observed in the APP-IP-Asp6Ser, the proton from the alcohol group of serine transferred to Glu121. To further confirm this observation, the same initial structures were optimized at a higher level QM/MM protocol PBE0-D3/cc-pvdz/AMBER and gave qualitatively same results (Figure 2). Zn^{2+} has four ligands in the coordination sphere: three histidines from the enzyme and the functional group of the sixth amino acid (cysteine or serine) of the inhibitor, similarly to that observed in the QM/MM and QM/MM-MD simulations of MMP-2-APP-IP complex.[12]

To further explore the properties of APP-IP and its derivatives, we performed molecular dynamics simulations of oligopeptides in aqueous solution. We performed 100 ns runs for each system at 300 K in NPT ensemble with a 1 fs integration timestep. The APP-IP is very flexible, as can be found from both the RMSD and MD trajectories visualization. Contrary, the APP-IP-Asp6Cys and APP-IP-Asp6Ser demonstrate two and three types of conformations, respectively. Ramachandran plots indicate that the main differences are in the Asn5 and Ile8 residues. The φ,ψ distributions are similar for the APP-IP and the APP-IP-Asp6Cys, whereas those differ significantly for the APP-IP-Asp6Ser. On a plot corresponding to the Asn5, one can find only one type of conformations for the APP-IP-Asp6Ser, whereas there are definitely two sets for two others. The dynamics of the Ile8 residue indicates that the major set of conformations of the APP-IP-Asp6Ser exists in the region that is empty for two other oligopeptides. These two residues are important in the binding process as Asn5 is placed in the part of the oligopeptide where the main chain has a loop important for the orientation of the sixth residue that coordinates Zn^{2+}. Ile8 interacts with the aromatic rings of the His84, Tyr73 and Phe86 of the protein binding site, and certain conformations may disturb these contacts. It is likely that the absence of the inhibition of MMP-2 by APP-IP-Asp6Ser can be explained in terms of the preferable conformations of the oligopeptide in solution that are not favorable for binding to the enzyme.

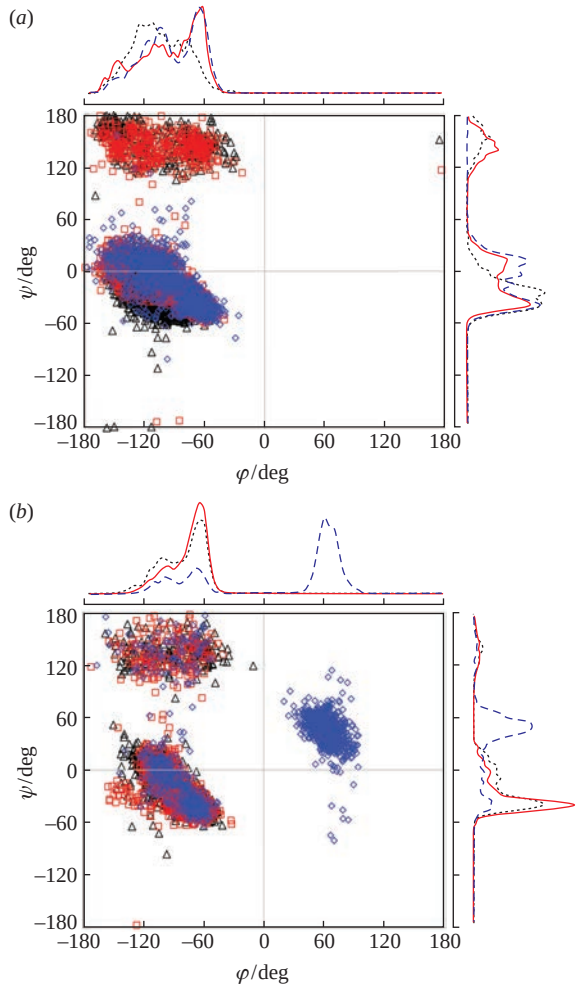


Figure 3 Ramachandran plots of the dynamics of (*a*) Asn5 and (*b*) Ile8; each dot corresponds to the MD trajectory frame. Red solid lines and red squares correspond to the APP-IP, blue dashed lines and blue diamonds to the APP-IP-Asp6Ser, black dotted lines and black triangles to the APP-IP-Asp6Cys.

To sum up, we performed experimental studies and provided a theoretical interpretation of the inhibitory properties of oligopeptides derived from the β-amyloid precursor protein-derived inhibitory peptide (APP-IP). We demonstrated that both conformational dynamics in solution and the mechanism of interactions in the active site of the target enzyme should be considered in the rational design of oligopeptide-based inhibitors.

We are grateful to Professor A. V. Nemukhin for valuable advice. This work was supported by the Russian Science Foundation (grant no. 15-14-30019). We acknowledge the use of supercomputer resources of the M. V. Lomonosov Moscow State University[18] and the Joint Supercomputer Center of the Russian Academy of Sciences.

The fluorogenic substrate FlS (DNP-Pro-Leu-Gly~Met-Trp-Ser-Arg-OH) (DNP = dinitrophenyl) from Calbiochem MerckMillipore (USA) was preliminarily dissolved in DMSO as described earlier.[16] DMSO has an inhibitory effect on MMP;[17] thus, its concentration in the reaction mixture was decreased to the lowest possible value (0.02–0.1%). The concentrations of FlS [ε(378 nm) = 17300 dm^3 mol^{-1} cm^{-1}][16] and inhibitors [ε(275nm) = 1400 dm^3 mol^{-1} cm^{-1}, corresponding to the tyrosine] were determined spectrophotometrically. The 150 μM FlS solution was prepared by dilution with a reaction buffer by a factor of 400. A 2.5 μl portion of enzyme solution was added to 10 μl of a buffer or inhibitor solution. After incubation for 15 min (30 min in case of APP-IP inhibitor), we added 10 μl of the substrate to achieve a final concentration from 10 to 80 μM; the enzyme concentration (3 nM) remained constant in all experiments. The concentrations of the inhibitors varied from 0 to 0.45 μM for the APP-IP, from 0 to 10 μM for the APP-IP-Asp6Cys and from 0 to 100 μM for the APP-IP-Asp6Ser. All of the experiments were performed with freshly prepared solutions.

References

1 R. E. Vandenbroucke and C. Libert, *Nat. Rev. Drug Discov.*, 2014, **13**, 904.
2 C. E. Brinckerhoff and L. M. Matrisian, *Nat. Rev. Mol. Cell Biol.*, 2002, **3**, 207.

3 C. M. Overall and C. López-Otín, *Nat. Rev. Cancer*, 2002, **2**, 657.
4 M. W. Ndinguri, M. Bhowmick, D. Tokmina-Roszyk, T. K. Robichaud and G. B. Fields, *Molecules*, 2012, **17**, 14230.
5 S. Higashi and K. Miyazaki, *J. Biol. Chem.*, 2003, **278**, 14020.
6 H. Hashimoto, T. Takeuchi, K. Komatsu, K. Miyazaki, M. Sato and S. Higashi, *J. Biol. Chem.*, 2011, **286**, 33236.
7 T. Vasilevskaya, M. G. Khrenova, A. V. Nemukhin and W. Thiel, *J. Comput. Chem*., 2015, **36**, 1621.
8 V. Pelmenschikov and P. E. M. Siegbahn, *Inorg. Chem*., 2002, **41**, 5659.
9 N. Díaz, D. Suárez and E. Suárez, *Proteins*, 2010, **78**, 1.
10 T. Vasilevskaya, M. G. Khrenova, A. V. Nemukhin and W. Thiel, *J. Comput. Chem*., 2016, **37**, 1801.
11 T. Vasilevskaya, M. G. Khrenova, A. V. Nemukhin and W. Thiel, *Mendeleev Commun*., 2016, **26**, 209.
12 M. G. Khrenova, A. P. Savitsky, I. A. Topol and A. V. Nemukhin, *J. Phys. Chem. B*, 2014, **118**, 13505.
13 M. G. Khrenova, A. V. Nemukhin and A. P. Savitsky, *J. Phys. Chem. B*, 2014, **118**, 4345.
14 M. G. Khrenova, I. D. Solovyev, V. N. Azev, G. D. Lapshin and A. P. Savitsky, *Mendeleev Commun.*, 2016, **26**, 207.
15 K. Miyazaki, M. Hasegawa, K. Funahashi and M. Uneda, *Nature*, 1993, **362**, 839.
16 S. Netzel-Arnett, S. K. Mallya, H. Nagase, H. Birkedal-Hansen and H. E. Van Wart, *Anal. Biochem*., 1991, **195**, 86.
17 G. Murphy, Q. Nguyen, M. I. Cockett, S. J. Atkinson, J. A. Allan, C. G. Knight, F. Willenbrock and A. J. Docherty, *J. Biol. Chem.*, 1994, **269**, 6632.
18 Vl. V. Voevodin, S. A. Zhumatiy, S. I. Sobolev, A. S. Antonov, P. A. Bryzgalov, D. A. Nikitenko, K. S. Stefanov and Vad. V. Voevodin, *Otkrytye Sistemy*, 2012, no. 7, 36 (in Russian).

Received: 8th August 2016; Com. 16/5024