IN SILICO EVALUATION FOR BIOLOGICAL ACTIVITY OF DIHOXAMIC ACIDS AS METALLOPROTEINASE INHIBITOR

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Summary

Matrix metalloproteinases (MMP) represent a class of structural and functional kindred enzymes that are involved in altering the natural compounds of the extracellular matrix. MMP are Zn and Ca dependent enzymes that are intracellular synthesized as zymogens that can be inhibited by 4 classes of natural inhibitors called TIMPs (tissue inhibitor for matrix metalloproteinases) Material and methods. The structures of the proposed inhibitors were recomposed in Hyperchem and the MMP molecular structures were taken from ProteinDataBank (PDB codes 1QIB and 1CK7 for MMP2, 1L6J and 1GKC for MMP9 respectively). Specific Molecular Docking software (Autodock 4.0) was user for docking procedures and energy binding calculations, while and graphic representations were performed by PyMol v.0.98. Results. Following docking procedures and energy binding calculations, performed on enzymes with included/excluded Zn ion from the catalytic site, we have obtained values similar to the known and clinically tested synthetic inhibitors, as batimastat. Molecular docking was performed in 8 cases, by coupling two dihydroxamic compounds ADH and ATDH consecutively on MMP2 and MMP9. Each of the two enzymatic structures was considered with/without the Zn ion in order to investigate the importance of this ion for the activation/inactivation procedure. Conclusions. Molecular docking allows the dihydroxamic products evaluation as potential inhibitors for the matrix metalloproteinases with specific catalytic Zn ions., Regarding the inhibition constants experimentally (in silico) determined, they may play an important role in locking the substrate access to the catalytic site of the enzyme, impeding its overreaction during pathological processes

Keywords: in silico study, molecular docking, metalloproteinase, dihydroxamic acid.

Introduction

Matrix metalloproteinases (MMP) represent a class of structural and functional kindred enzymes that are involved in altering the natural compounds of the extracellular matrix (Shapiro SD, 2000; Woessner Jr, 1999). MMP are Zn and Ca dependent enzymes that are intracellular synthesized as zymogens that can be inhibited by 4 classes of natural inhibitors called TIMPs (tissue inhibitor for matrix metalloproteinases) (Brew et al., 2000). Even if MMP plays important roles in physiological processes, their overexpression plays also crucial roles in pathological processes as multiple sclerosis, arthritis, Alzheimer disease and especially in cancer and metastasis (Fang L. et al., 2009; Galvão et al., 2009; Korpos et al., 2009; Ando, et al., 2009; Gentner et al., 2009).

According to the substrate specificity and primary sequence similarities, the members of this enzyme family can be grouped into five subfamilies: gelatinases (MMP-2, -9), which cleave denatured collagen, elastin, and type IV and V collagens; collagenases (MMP1,-8,-13), which cleave native collagen; stromelysins (MMP3,-10,-11), which may cleave proteoglycans; membrane-type MMPs (MMP14,-15,-16,-17), which are associated with activation of pro-MMPs (Rajeshwar, 2007).
From the structural point of view, MMP consist of four distinct domains: N-terminal pro-domain, catalytic domain, hinge region and C-terminal hemopexin-like domain. The latter may be responsible for the substrate recognition as well as for interaction with TIMPs. The first MMP structure in complex with a synthetic inhibitor was described by Lovejoy (Lovejoy et al., 1994). The MMP catalytic site is characterized by the presence of a Zn atom together with a conserved zinc binding motif, HExxHxxGxxH. In order for a molecule to become an effective inhibitor of the MMP it must show a functional group (e.g., hydroxamic acid, carboxylic acid, and sulphydryl, etc.) capable of attaching to the catalytic zinc atom, at least one functional group which provides a hydrogen bond interaction with the enzyme backbone, and one or more side chains which undergo effective van der Waals interactions with the enzyme subsites. Some synthetic and natural inhibitors bind in the same way to several MMPs, thus deducing that the inhibition mechanisms can be somehow similar. From these inhibitors, compounds with the hydroxamate zinc binding group may be the most popular inhibitors for MMPs such as MMP1, MMP2, MMP3 and MMP-9. The crystal structures of hydroxamate inhibitors complexed with MMPs have revealed that the catalytic zinc is pentacoordinated with three histidine nitrogens in MMP2 and two hydroxamate oxygens in inhibitor (fig.1) (Spurlino et al., 1994).

Gelatinase A (MMP2) is an unique member of metalloproteinase family while it is expressed by numerous cell types, shows an ubiquitous distribution and present an activation mechanism that differs from the other related enzyme family members. Together with progelatinase B (proMMP9) they are usually isolated as complexes with TIMP2 and TIMP1 respectively. Progelatinase A binds TIMP2 specifically but not TIMP1. The interaction between progelatinase and TIMP2 can mediate the gelatinase A activity at cell surface. MMP2 is involved, as most MMP, in regulating cell responses as proliferation, adhesivity or migration.

The MMP substrate specificity and the inhibitor specificity are directly dependent on the the catalytic domain structure; however, hemopexin domain is required for binding the collagen substrate (Knäuper et al., 1997). The catalytic domain of MMP9 includes two zinc ions and five calcium ions. The active-site zinc ion and the structural zinc ion are placed in similar location with the Zn ions in the catalytic domain of MMP2 (PDB codes 1QIB and 1CK7). Two calcium ions are located similarly in both the intact and truncated (1CK7) structures of MMP2. A third calcium ion is present in the MMP9 and MMP2 catalytic domain but is missing in the intact MMP2 structure. The final model for the wild-type complex comprises 159 out of 163 residues; two Zn and five Ca ions; one inhibitor molecule and 54 water molecules per protein molecule (Rowsell et al., 2002).

The purpose of the present paper was to investigate the binding affinity between two dihydroxamic compounds, ADH – adipoyl dihydroxamic acid and ATDH – tereftaloyl dihydroxamic acid and two members of the MMP family, MMP2 and 9 (gelatinases A and B). Testing the binding abilities by molecular docking (in silico study) represents a preliminary compulsory step before experimental in vitro and in vivo testing for these compounds.
Materials and methods

The structures of the proposed inhibitors were recomposed in Hyperchem and the MMP molecular structures were taken from ProteinDataBank (PDB codes 1QIB and 1CK7 for MMP2, 1L6J and 1GKC for MMP9 respectively). Specific Molecular Docking software (Autodock 4.0) was used for docking procedures and energy binding calculations, while graphic representations were performed by PyMol v.0.98.

Dihydroxamic acids were manually built and optimized in Hyperchem software. PDB file 1L6J contain the whole sequence for MMP9 from which we have selected for the docking study only the catalytic domain. These domains were superposed over the theoretical model for MMP9 (PDB code 1LKG) by Swiss PBD viewer application. The chosen residues were 29-444 for the N-terminal domain that includes also the catalytic site for MMP9 and residues 513-707 for the hemopexinic domain. For this docking study we have chosen the whole molecule, even if previous studies insist in docking only on catalytic site. We have performed docking on the overall molecules, while the conformational change following the inhibitor binding supposes 3D changes in the whole molecule.

Working algorithm. From PDB code 1MMB we have selected and extracted only MMP3, in a separate file. The other molecule in the 1MMB file was BB-94 (batimastat), a potent MMP inhibitor; it was rebuilt in Hyperchem, with the same optimization parameters as the dihydroxamic inhibitors to be tested.

Then we have performed docking by Autodock 3.0.5 for a rebuilt BB-94 to the enzyme extracted from 1MMB (here MMP3). The results have shown free coupling energies similar for the rebuilt MMP3-BB-94 complex in Hyperchem with those of the initial 1MMP complex, \( E = -7.84 \text{ kcal/mol} \) and the inhibition constant \( K_I = 1.78\times10^{-6} \).

Results

Following docking procedures and energy binding calculations, performed on enzymes with included/excluded Zn ion from the catalytic site, we have obtained values similar to the known and clinically tested synthetic inhibitors, as batimastat.

Molecular docking was performed in 8 cases, by coupling two dihydroxamic compounds ADH and ATDH consecutively on MMP2 and MMP9.

Each of the two enzymatic structures was considered with/without the Zn ion in order to investigate the importance of this ion for the activation/inactivation procedure. The values for the free binding energy are shown in table 1.

At the same time we have represented the structures bound to the investigated inhibitors.

We have used Pymol software to show optima conformations for ADH bound to MMP2 in the absence of Zn ion (fig. 3) and in the presence of Zn ion, respectively (fig.4).
Table 1. Free binding energy values and inhibition constant (Ki) for dihydroxamic (ADH and ATDH) – MMP binding

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<tr>
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<th>MMP2</th>
<th>MMP9</th>
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<tr>
<td></td>
<td>ADH</td>
<td>ATDH</td>
</tr>
<tr>
<td>Zn²</td>
<td>-6.98</td>
<td>-6.77</td>
</tr>
<tr>
<td>E kcal/mol</td>
<td></td>
<td></td>
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<tr>
<td>Ki</td>
<td>7.64 × 10⁻⁶</td>
<td>1.08 × 10⁻⁵</td>
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Figures 3 and 4 depict ADH structures bound to MMP2 in the absence and presence of Zn ion. Figure 5 represents the electrostatic potential for MMP2 catalytic pocket in bound state with ATDH.

We have observed the difference for inhibitor position that is obviously influenced by the Zn atom presence.

In figures 5 and 6 we depict ATDH structures bound to MMP2 in the absence and presence of Zn ion. In figure 6 we have represented the electrostatic potential for MMP2 catalytic pocket in bound state with ATDH.

The inhibitor position difference is obviously influenced by the Zn ion presence but the variability is slightly reduced compared to ADH-MMP2 complex.

Figures 7 and 8 represent the ADH placement in the MMP9 catalytic site. Docking was performed for the whole molecule and not only for the catalytic site.
Figures 9 and 10 represent the ATDH placement in the MMP9 catalytic site.

**Figure 7.** ADH bound to the MMP9 catalytic site (no catalytic Zn ion).

**Figure 8.** ADH bound to catalytic site of MMP9 (with Zn ion, no Zn ion CPK code from figure 7)

**Figure 9.** ATDH bound to the MMP9 catalytic site (no catalytic Zn ion).

**Figure 10.** ATDH bound to catalytic site of MMP9 (with Zn ion, no Zn ion CPK code from figure 9)

**Conclusions**

Molecular docking allows the dihydroxamic products evaluation as potential inhibitors for the matrix metalloproteinases with specific catalytic Zn ions.

The structures that were investigated in this study are included in the inhibitors group that occupies the catalytic site without Zn ion coordination.

Regarding the inhibition constants experimentally (in silico) determined, they may play an important role in locking the substrate access to the catalytic site of the enzyme, impeding its overreaction during pathological processes.

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