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# MMP-2: MOLECULE IDENTIFICATION AND STRUCTURE ANALYSIS

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## ABSTRACT

The upregulation of MMPs can cause several types of diseases. The cancer is main deadly disease caused by the MMPs. Different types of MMPs which are secreted by body itself to maintain the some useful activities. But the imbalance can cause some harmful effects. So to inhibit the activity of MMPs there are several inhibitors are also secreted in body itself. But sometimes these secreted inhibitors are insufficient in quantity and needed from outside as the drug. Several MMP inhibitors are available in nature as natural inhibitors and some synthetically derived known as synthetic inhibitor. So the first step to develop synthetically in laboratory we must know about structural components of the MMPs. Here we explored computationally the structural component of the MMP-2 by freely available web facilities as database, software etc.

**KEYWORDS:** MMPs, MMP-2, Prostate cancer, Structural components, *In-silico*.

## INTRODUCTION

The matrix metalloproteinases (MMPs) are larger family of enzymes has been a pharmaceutical target for >25 years.[1-2] MMPs comprise a large family of zinc dependent endoproteinases, collectively capable of degrading all extracellular matrix (ECM) components and also regarded as matrixins. They are found in all organisms belong to the metzincin superfamily of metalloproteinases.[3] About 28 different MMPs have been

identified[4], 24 of which are present in humans.[5] Based on their substrate specificity, MMPs have been divided into distinct subclasses: collagenases (MMP-1, MMP-8, MMP-13, and MMP-18), gelatinases (MMP-2, MMP-9), stromelysins (MMP-3, MMP-10 and MMP-11) and matrilysins (MMP-7, MMP-26) and other MMPs.[6] Tissue inhibitors of matrix metalloproteinases (TIMPs) are specific endogenous tissue inhibitors for MMPs. The proteolytic activities of MMPs influence essential cellular processes like cell proliferation, migration and adhesion, as well as many fundamental physiological events involving tissue remodeling, such as angiogenesis, bone development, wound healing, and uterine and mammary involution.[1] Gene transcription and the synthesis of pro-MMPs are different levels of MMP regulation, beside thes activation of proenzymes and the inhibition of MMPs by TIMPs are important regulatory processes. MMPs are secreted in a latent form as pro-MMPs, which require activation. The balance between MMPs and TIMPs regulates tissue remodeling under normal conditions. On other hand up-regulation of MMPs is leads to different types of cancer and several other diseases.[7]

MMP-2 (gelatinase-A) is expressed by many cell types including fibroblasts, keratinocytes, endothelial cells, chondrocytes and monocytes. It degrade a broad spectrum of ECM molecules such as collagen types I, IV, V, VII, X, IX, elastin, fibronectin, aggrecan, vitronectin, laminin. Various studies have shown role metastasis and invasion of cancerous cells.[8]

MMPs consist of a propeptide domain (80 amino acids), a catalytic domain (170 amino acids), a linker peptide also called as the “hinge region” (length varies) and a hemopexin domain (200 amino acids).[9-10] Activities of MMPs are regulated by endogenous inhibitors, such as tissue inhibitors of metalloproteinase (TIMPs), α2-macroglobin, heparin and the reversion-inducing cysteine-rich protein with kazal motifs (RECK). Beside four TIMPs in humans (TIMP-1, -2, -3 and -4) with 22–29 kDa there are several natural and synthetic inhibitors can also be used for the MMP regulation by main maintaining the MMP and MMP inhibitor imbalance.

To inhibit the upregulatory activity of the MMP-2, as therapeutic option MMP-2 inhibitor must be there as natural or synthetic inhibitors. Before exploring the MMP-2 inhibitors natural or synthetic development, the good understanding of the nature of protein structure and the conformational characteristics of MMP-2 must be explored. Here we will study the different structural component of the MMP-2 computationally. This computational study is

also known as the *in-silico* study or bioinformatics study with saving money as well as the time.

## MATERIALS AND METHODS

This insilico study was carried out with use of all required facilities at Department of Pharmacology & Therapeutics, King George's Medical University, Lucknow.

### Computer and Internet Connection

The study was completely *in-silico* off-course with the use of computer and here there is no need to introduce the computer because now it becomes necessity for today's life. Whenever and wherever we talk about the computer we can't ignore the internet i.e. world wide web (WWW), because without internet there disconnection from the world. Hence we used the computer for the structure analysis by different programs and internet for gathering the information to complete the study.

### Databases

All the information regarding MMP-2 was explored from the different freely available biological databases. The information may be in the form of information/structure/domain of the MMP-2. These different freely available online databases were PDB[11], MMPdatabase[12], NCBI[13], EXPASY[14], UNIPROT[15], INFORMATICS[16], Wikipedia[4] etc.

### Software

To analyze the different structural components we used the freely available online program Swiss-PdbViewer.[17] It is an application that provides a user friendly interface allowing to analyze several proteins at the same time. The proteins can be superimposed in order to deduce structural alignments and compare their active sites or any other relevant parts. Amino acid mutations, H-bonds, angles and distances between atoms are easy to obtain thanks to the intuitive graphic and menu interface.

## RESULTS AND DISCUSSION

The full length structure of MMP-2 downloaded from the PDB database with the PDB ID 1ck7 with Chain: A; Length: 631 amino acids; Theoretical weight: 71 KDa; Source organism: Homo *sapiens*; Expression system: *Trichoplusia ni*; Gene names: CLG4A, MMP2 and with the FASTA Sequence.

>pdb|1ck7|A

APSPIIKFPGDVAPKTDKELAVQYLNTFYGCPKESCNLFVLKDTLKKMQKFFGLPQT
GDLDQNTIETMRKPRCGNPDVANYNFFPRKPKWDKNQITYRIIGYTPDLDPETVDDA
FARAFQVWSDVTPLRFSRIHDGEADIMINFGRWEHGDGYPFDGKDGLLAHAFAPGT
GVGGDSHFDDDELWTLGEGQVVRVKYGNADGEYCKFPFLFNGKEYNSCTDTGRSD
GFLWCSTTYNFEKDGKYGFCPHEALFTMGGNAEGQPCKFPFRFQGTSYDSCTTEGRT
DGYRWCGTTEDYDRDKKYGFCPETAMSTVGGNSEGAPCVFPFTFLGNKYESCTSAG
RSDGKMWCATTANYDDDRKWGFCPDQGYSLFLVAAHAFGHAMGLEHSQDPGALM
APIYTYTKNFRLSQDDIKGIQELYGASPDIDLGTGPTPTLGPVTPEICKQDIVFDGIAQI
RGEIFFFKDRFIWRTVTPRDKPMGPLLVATFWPELPEKIDAVYEAPQEEKAVFFAGNE
YWIYSASTLERGYPKPLTSLGLPPDVQRVDAAFNWSKNKKTYIFAGDKFWRYNEVK
KKMDPGFPKLIADAWNAIPDNLDAVVDLQGGGHSYFFKGAYYLKLENQSLKSVKFG
SIKSDWLGC.

**Domain Structure**

The structure of MMP-2 is composed of by the Pre: signal sequence; Pro: propeptide with a free zinc-ligating thiol (SH) group; Zn: zinc-binding site; II: collagen-binding fibronectin type II inserts; H: hinge region; The hemopexin/vitronectin-like domain contains four repeats with the first and last linked by a disulfide bond (Fig. 1).

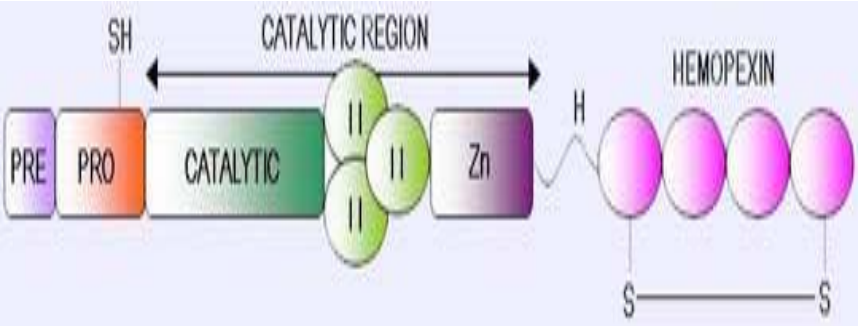


**Fig. 1: Domain structure of the MMP-2**

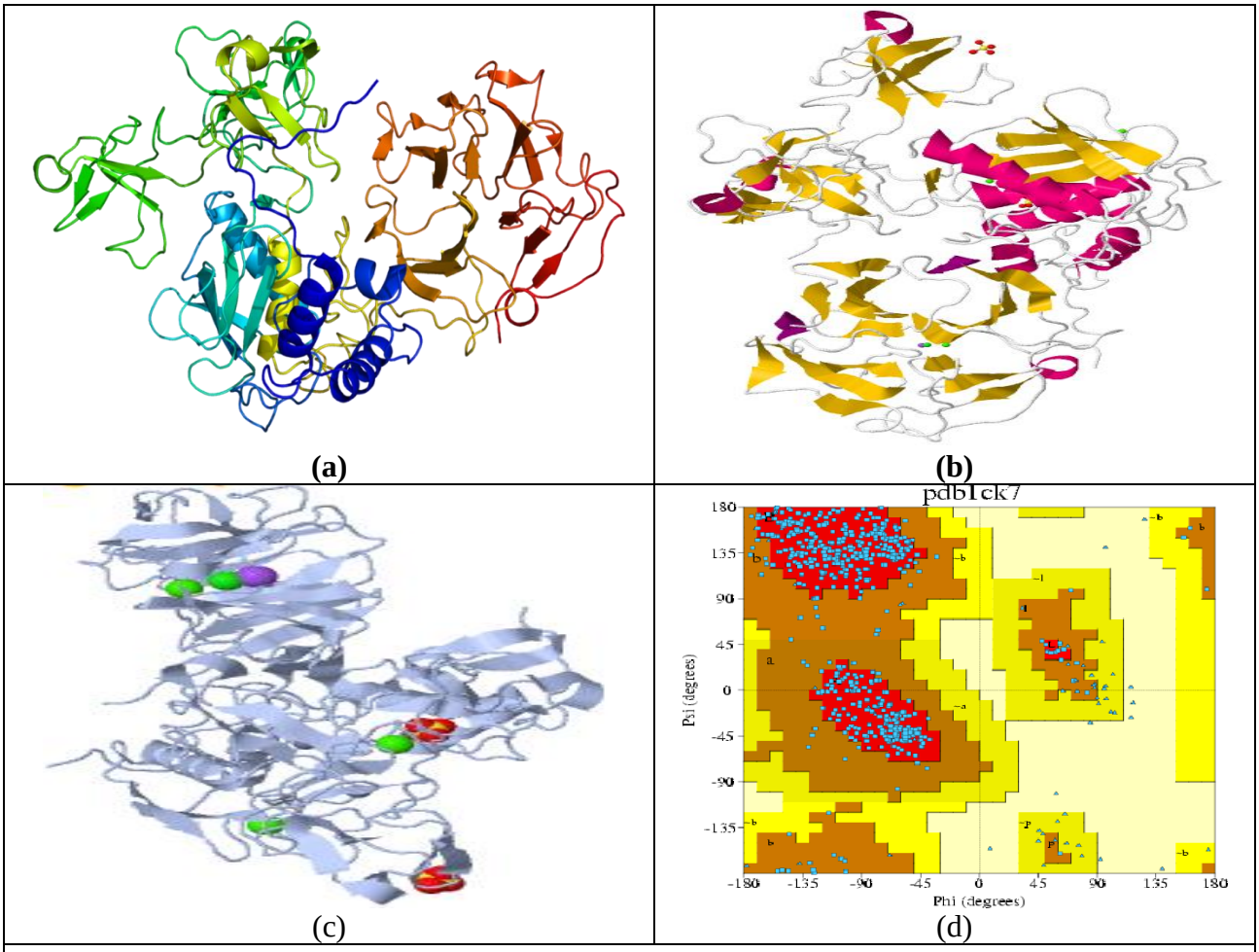


**Fig. 2: MMP-2 Structures (a) Full length 3-D structure, (b) 3-D structure with different ligands, (c) 3-D structure with different ligands with space fill model**

**Helices and Sheets**

There are 11 helix structures (Helix 1-Helix 11) found in the MMP-2 Structure of PDB ID 1CK7. All the helices are of chain A and right-Handed alpha besides helix 9 and helix 10. On the other hand there are 34 sheets are found in PDB ID 1CK7 within chain A and first strand sense (Fig. 2a,b,c).

**Ligands**

There are total 5 types of ligands are present in the MMP-2 structure including Two Zinc ion (1 atom), three calcium Ion (1 atom), one chloride ion (1 atom), one sodium ion(1 atom) and two sulfate ions (5 atoms) in chain A (Tab. 1).

**Table 1: Showing different legands present in the MMP-2 structure**

| Ligand Name | Ligand Notation | No of Ligand Molecule | No. of Atoms | Chain ID |
|---|---|---|---|---|
| Zinc Ion | Zn | 2 | 1 | A |
| Calcium Ion | Ca | 3 | 1 | A |
| Chloride Ion | Cl | 1 | 1 | A |
| Sodium Ion | Na | 1 | 1 | A |
| Sulfate Ion | $SO_4$ | 2 | 5 | A |

**Ramachandran Plot**

A Ramachandran plot is a way to visualize backbone dihedral angles ψ against φ of amino acid residues in protein structure. 91.9% (565/615) of all residues were in favored (98%) regions. 98.0% (603/615) of all residues were in allowed (>99.8%) regions. There were 12 outliers (phi, psi): 32 SER (174.0, 99.3) 91 GLN (-39.0, -28.0) 165 GLY (49.1, -172.6) 176 TRP (-40.0, -76.6) 186 GLY (8.0, -155.8) 448 SER (156.0, 150.4) 481 ILE (-115.5, -120.8) 490 ASP (-62.7, -96.8) 572 PHE (161.2, 159.6) 601 GLY (33.1, 80.7) 627 GLY (-58.5, 85.1) 659 GLY (-60.4, 83.8) (Fig. 2(d).

## CONCLUSIONS

MMP-2 plays an important role in cellular pathways activation leading to carcinogenesis, promotion, and metastasis. Still we are waiting for such molecule to inhibit the activity of the MMP-2 in treatment of the different types of the cancers, while so many clinical studies going on as well as have been completed without beneficial results. Till we are aware about the structure and activities of the targeted molecule to be inhibited in the treatment of the disease, we cannot find the specific inhibitors. So we have tried to explore all the structural component of MMP-2, which can be very helpful for the new drug molecule development firstly *in-silico* and then in laboratory following the clinical trials as well as a novel drug discovery against the prostate cancer treatment.

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