Conformation and domain movement analysis of Human Matrix Metalloprotease-2: The role of associated Zn^{2+} and Ca^{2+} ions

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Introduction

Matrix Metalloproteinase-2 (MMP-2) is a 550 amino acid residue extracellular Zn^{2+} proteinase that recognizes and degrades type I and IV collagens.[1] MMP-2 expression is associated with several inflammatory, degenerative, and malignant diseases. MMP-2 has three domains catalytic (Cat), fibronectin (Fib), and hemopexin (Hpx) and five crystallographically assigned divalent cations (two Zn^{2+} and three Ca^{2+}). The Cat domain (Tyr¹¹⁰ through Tyr⁴⁴⁵) is a conserved matrix fold consisting of five β -sheets and three α -helices. The Fib domain (Glu²¹⁷ through Gln³⁹³) is inserted within the catalytic domain between the β 5-sheet and α 2-helix and contains three type II fibronectin fingers consisting of two antiparallel β -sheets connected by a short α -helix. The Hpx domain (Leu⁴⁶¹-Cys⁶⁶⁰) is a four bladed propeller fold that is partially oriented towards the Cat domain. The Cat and Hpx domains are connected by a proline rich flexible link (Lnk) region (Gly⁴⁴⁶-Thr⁴⁶⁰). In the present study we examine the domain movements within MMP-2 and evaluate the structural stability of the bound divalent ions using 1.0 μs NPT MD simulations.

Simulations were performed using GROMACS 5.1.2[2] with the CHARMM36m[3] force field, modified TIP3Pm water model, and a divalent metal ions model.[4] The system was solvated in a truncated dodecahedron with 45105 water molecules neutralized with Na^+ and Cl^- ions with a final NaCl concentration of 150 mM. Protein-ion distances were measured, ion hydration evaluated, the sampled conformational space analyzed with dihedral Principle Component Analysis (dPCA)[5] and Dynamic Cross-Correlation Matrix (DCCM) analysis[6]. Energy of binding of the Zn^{2+} and Ca^{2+} ions to the protein was calculated using the MM-PBSA method.[7]

Results and Discussion

A $C\alpha$ -trace overlay of the centroids representing the lowest energy conformations for each cluster (Fig. 1, Left) demonstrates that the most conformationally stable regions are the Cat and Hpx domains. The RMSDs of Cat and Hpx are 0.50 ± 0.03 nm and 0.34 ± 0.04 nm, respectively, while that of Fib is 3.69 ± 0.03 nm. The Fib domain is not disordered and the most significant flexibility occurs within the loops connecting the three type II fibronectin fingers. The four bladed anti-parallel β -sheet configuration the Hpx domain lends to its considerable conformational stability. The $C\alpha$ -trace overlay of the first five principle components of the domain cross correlation analysis are shown in Fig. 1 (Right). The most significant domain movements are accounted for by the first two principle components which represent 86.2% of the correlated and anti-correlated motions. The most significant contributions are an opening and closing of the center-of-mass distance between the Fib and Hpx domains mediated by the flexible Lnk domain

Energetically most favorable interactions occur between Zn^{2+} ion 1, Ca^{2+} ion 1 and Ca^{2+} ion 3 and the protein (Table 1). Zn^{2+} ion 1 is bound by three His residues arising from the α 1-helix and the Ω -loop and is associated with a single hydrating water molecule. Ca^{2+} ion 1 is bound within a pocket created by the distal portion of the S-loop and the interim loop connecting the Cat and Fib domains. Ca^{2+} ion 3 is closely associated with the Asp side chains surrounding the central cavity of the Hpx domain. Zn^{2+} ion 2 does not maintain its crystallographically defined binding site position but does stay in close proximity to the β -COO- group of Asp¹⁸⁰. Ca^{2+} ion 2 freely dissociates from its binding site and diffuses through the hydration box during the simulation.

Figure 1: Overlay of 10 representative lowest energy conformations identified from *k*-means clustering of the first two components of the dihedral principal components (Left). Red arrows representing β -sheet, blue α -helix, green tubes turn, aqua tubes coil, pink Zn^{2+} , and Yellow Ca^{2+} . $\text{C}\alpha$ -trace overlay of the first five principal components obtained from the DCCM analysis (Right). Correlated domain movements are indicated in blue and anti-correlated are in red.

Table 1: Binding energies between MMP-2 and the associated divalent cations as determined by the MMPBSA methods with its associated components.

Ion(s)	ΔE_{vdw}	ΔE_{elec}	ΔG_{polar}	$\Delta G_{\text{non-polar}}$	$\Delta E_{\text{binding}}$
All Ions	95.04 ± 2.92	-9264.45 ± 194.34	3142.58 ± 81.6	-3.95 ± 0.13	-6020.73 ± 116.75
Zn^{2+} Ion 1	25.41 ± 0.77	-2427.66 ± 72.19	1291.3 ± 42.75	-0.88 ± 0.04	-1114.16 ± 27.12
Zn^{2+} Ion 2	13.79 ± 0.96	-799.35 ± 63.45	188.73 ± 35.14	-0.47 ± 0.04	-598.61 ± 29.09
Ca^{2+} Ion 1	38.15 ± 1.90	-1796.42 ± 89.21	605.84 ± 30.55	-1.14 ± 0.07	-1154.73 ± 56.65
Ca^{2+} Ion 2	6.52 ± 0.58	-367.21 ± 36.96	73.71 ± 9.94	-0.35 ± 0.05	-289.24 ± 27.33
Ca^{2+} Ion 3	14.94 ± 0.57	-1480.59 ± 42.61	320.38 ± 11.31	-1.32 ± 0.05	-1147.96 ± 30.78

Only minor structural fluctuations occur within the Cat and Hpx domains. Flexibility within the Fib domain is accounted for by the loops connecting the three type II fibronectin fingers. Flexibility within this region may play an important role in collagen binding and unravelling necessary for substrate binding to the Cat domain. Both dPCA and DCCM analysis identify movement and change in orientation of the Hpx domain in relation to the Cat and Fib domains as mediated by the flexible Lnk region. This Hpx-to-Cat and Fib domain movement comprises the vast majority of correlated and anti-correlated motions within the protein and may allow for the enzyme to slide along the collagen triple helix to the next substrate binding site. The catalytic Zn^{2+} ion 1 has the greatest degree of conformational stability staying closely associated with the sidechains of His⁴⁰³, His⁴⁰⁷, and His⁴¹³ and an associated water molecule. Zn^{2+} ion 2, Ca^{2+} ion 1, and Ca^{2+} ion 3 are conformationally less stable but do stay within close proximity to the crystallographically identified binding sites.

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