Assessing the effect of calcium and magnesium ions in the structural stability of the protein kinase A through molecular dynamics simulations

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Introduction

cAMP-dependent protein kinase, also called protein kinase A (PKA), is one of the most well studied protein kinases, and because of the high conservation of the protein kinase family, it serves as a model for all protein kinases[1]. Mg^{2+}, as the most abundant divalent metal ion in the cell, is believed to be the favored coordinating ion for kinases, however it has been proven experimentally than other divalent metals such as Ca^{2+} can also promote the phosphoryl transfer but at much lower rates. Based on these experimental results, the main goal of this research was to determine how the retention of the products occurs and to identify which interactions in the presence of Ca^{2+} overstabilize the final state of the catalysis in PKA.

In order to get a better understanding of these events, PKA in its product state was evaluated through molecular dynamics simulations using two previously crystallized systems, one using the ion Mg^{2+} as a cofactor, and other using the Ca^{2+} ion[2,3]. We observed, that Ca^{2+} reduce the mobility of PKA and of the phosphorylated substrate; thereby corroborating experimental observations about a "trapping effect" and consequently an inhibitory effect produced by Ca2+. This information is expected to be valuable for the understanding of the catalytic mechanisms in protein kinases which could lead to the design of more potent inhibitors as well as to understand a possible regulation mechanism exerted by Ca2+ on kinases.

Experimental Data

"Ca^{+2} increases complex stabilization in the product state."

PKA:PKS	k_a (1/Ms)	k_d (1/s)	K_D (nM)
Mg^{2+}ADP	1.7×10^6	6.5×10^{-2}	38.2
Ca^{2+}ADP	2.3×10^6	4.7×10^{-3}	2.0

Table1. SPR analysis of product dissociation after phosphoryl transfer[1].

X-Ray Crystallography Facts

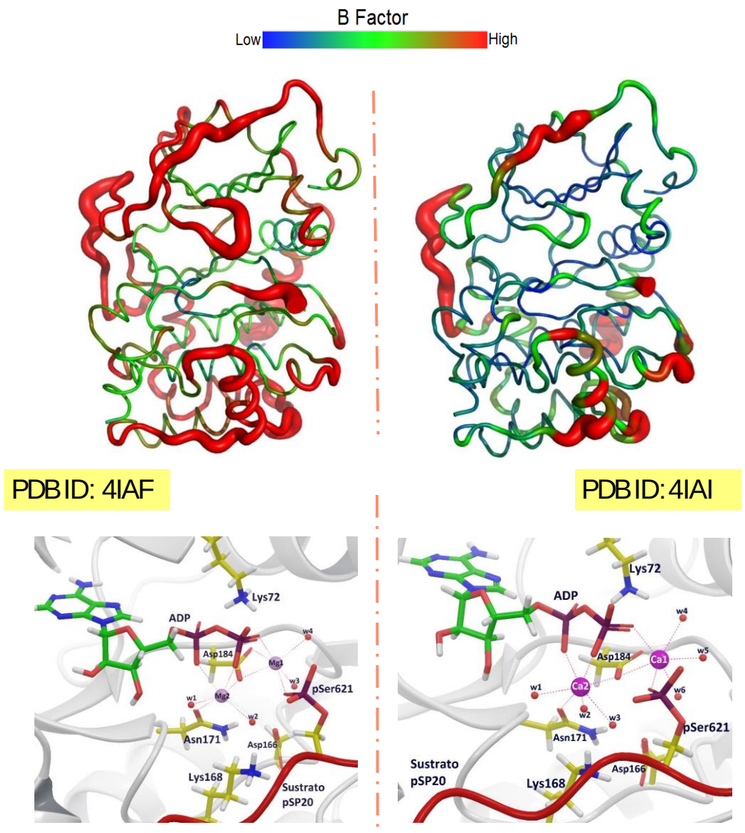

Fig. 1 β-Factor visualization of the ternary complexes showing regions with higher flexibility in red and lower in blue. Coordination spheres are shown for each ion in the active site.

Materials & Methods

Crystal structures with PDB codes 4IAF and 4IAI were used as starting points. Protein preparation was performed with the *Protein Preparation Wizard* tool implemented in Maestro[4]. Molecular dynamics simulations were carried out with the software Amber 14[5] and the force field ff99SB[6]. The systems were firstly energy minimized and equilibrated by short MD simulations in the NVT and NPT ensembles. Temperature and pressure were kept fixed at 300 K and 1 atm, respectively. Production runs were performed for 100 ns in the NPT ensemble which were used for subsequent analysis. All analysis were carried out with the tool *cpptraj* of Amber 14 and VMD plugins. VMD was used for the display of the molecular dynamics simulations.

Results

Distances atoms key

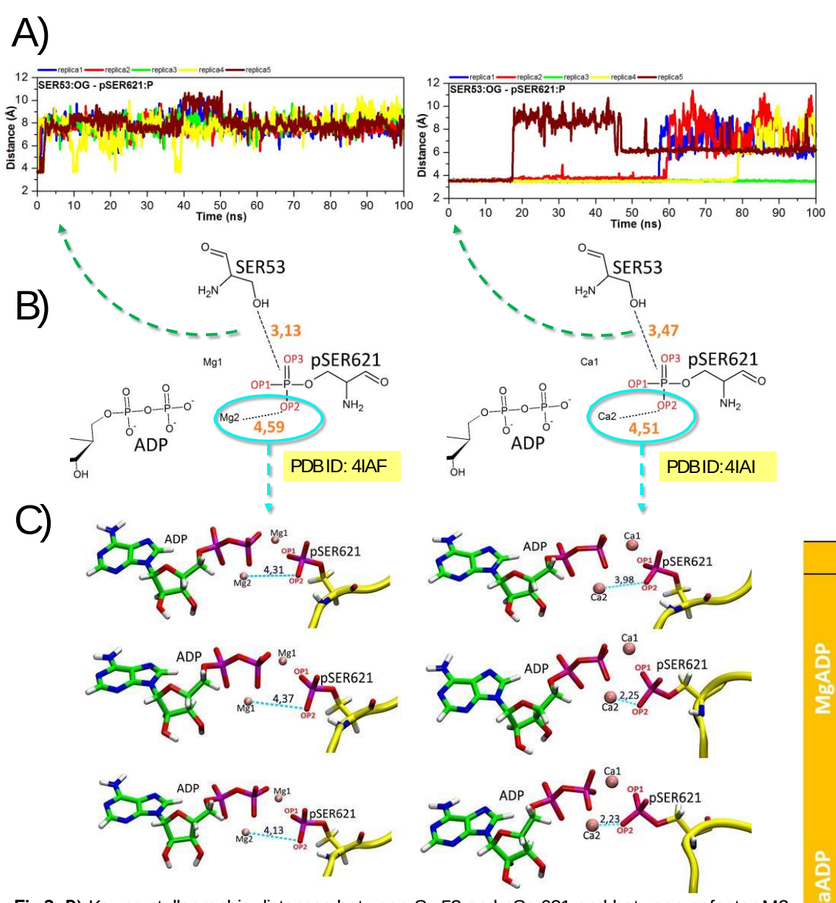

Fig 2. B) Key crystallographic distances between Ser53 and pSer621 and between cofactor M2 and the oxygen atom OP2 of the phosphate group of pSER621 in both crystallographic complexes (PDB IDs: 4IAF y 4IAI). **A)** The time dependence of the distance between the side chain oxygen atom of Ser53 located in glycine-rich loop and phosphorous atom of pSP20 (running averages are shown). **C)** Comparison of the "trapping effect" produced by the cofactor Ca2 in the CaADP system on the phosphate group of the pSer621 residue in the substrate pSP20. For the comparison, replication 2 of both systems has been used. All distances in Angstrom.

Hydrogen bond analysis

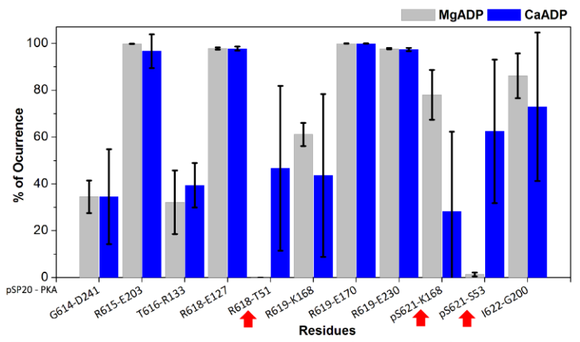

Fig 3. Percentage of occurrence of hydrogen bonds, between residues located at the substrate binding interface pSP20 and PKA. The hydrogen bonding interaction between R618-T51, pS621-K168 and pS621-S53, stands out with one of the most varied variations between MgADP and CaADP.

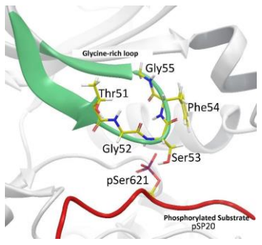

Fig 4. Glycine-rich loop described as an active site sensor involved in the release of products

Structural fluctuation analysis

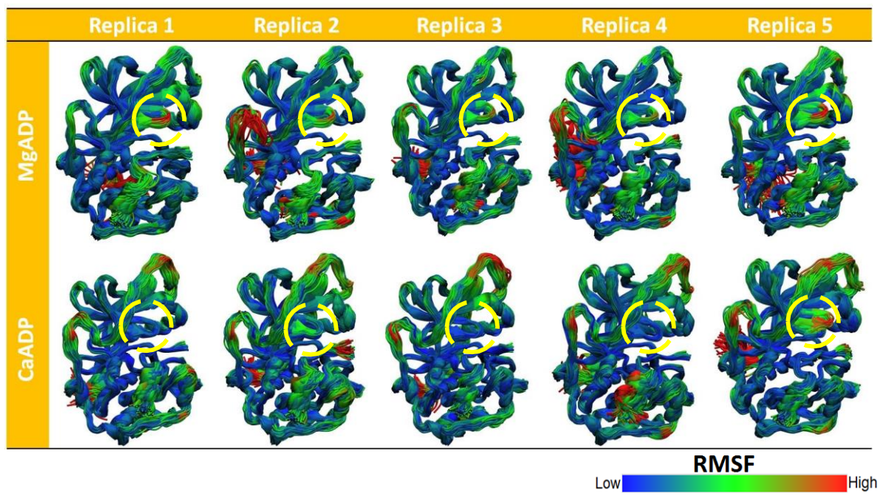

Fig 5. Results of MDlovoFit in the replications of the MgADP and CaADP systems. Structural alignment of 100 frames equidistant from the first 50 ns of simulation and colored according to the RMSF.

MM-PBSA analysis

MMPBSA (kcal/mol)				
Replicas	MgADP		CaADP	
	Average	Dev Std	Average	Dev Std
Replica 1	-119,335	7,5	-116,622	7,6
Replica 2	-119,504	6,8	-90,5651	14,6
Replica 3	-117,096	7,0	-123,0744	6,3
Replica 4	-113,248	6,5	-123,592	6,7
Replica 5	-115,806	6,4	-110,705	7,5

Table 2. Comparative table of the results obtained by the MM-PBSA energy calculation for the MgADP and CaADP systems, and their respective replicas. All values are in kcal/mol.

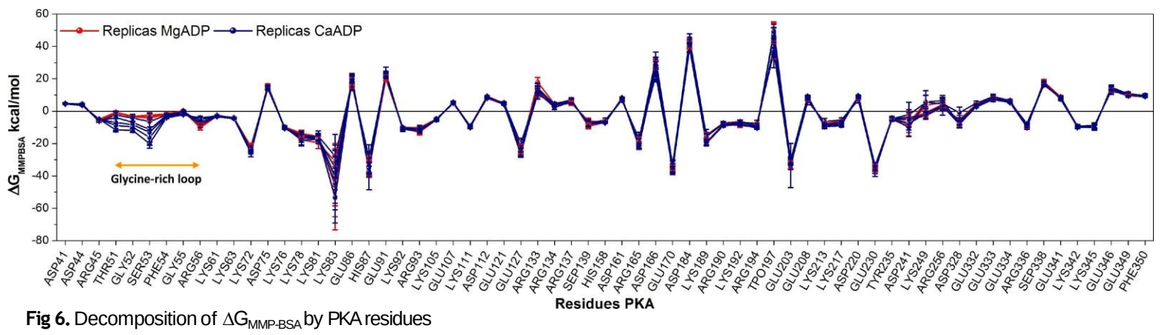

Fig 6. Decomposition of $\Delta G_{MMP\text{-}BSA}$ by PKA residues

Conclusion

- The release of the products in the catalytic cycle of PKA is the event that shows the greatest change in Ca^{2+} and the most probable reason for the low catalytic activity reported previously.
- The loop rich in glycine is the structural motive that has the greatest impact on the release of products. In MgADP it opens rapidly leaving the active site exposed by contrast CaADP more slowly opens trapping a region of pSP20 and stabilizing the coordination spheres of both ions.
- Significant evidence has been provided on how metal ions such as Ca^{2+} and Mg^{2+} can achieve opposite effects on the protein kinase activity in their final state of reaction and that they are related to the low dissociation of the catalysis products in the CaADP system.
- It is necessary to further study on the role that both ions would have in the function of protein kinases, and how their chemical properties would be adapted for a given function.

References

[1] Bastidas, A. *et al.*, *Biochemistry* 54, 2-10 (2014) [2] Knape, M. J. *et al.*, *ACS Chem Biol* 10, 2303-2315 (2015) [3] Gerlits, O. *et al.*, *Biochemistry* 53, 3179-3186 (2014) [4] Schrodinger, Maestro versión 10.2 (2015) [5] Case, D. A. *et al.*, *Univ. California* (2014) [6] Lindorff-Larsen, K. *et al.*, *Proteins* 78, 1950-1958 (2010)

Acknowledgments

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