

Theoretical study on the activation mechanism of AMP-kinase by means of Molecular Dynamics Simulations

C. Estarellas,¹ G. Leal Moraes,² A. Castro,³ J. Lameira,² A. Bidon-Chanal,¹ F. J. Luque¹

¹Departament of Physical Chemistry, Faculty of Pharmacy, Campus de l'Alimentació de Torribera, Universitat de Barcelona, Av. Prat de la Riba, 171, 08921 Santa Coloma de Gramenet, Spain, ²Department of Chemistry, Universidade Federal do Pará, Belem, Brasil, ³Institute of Medicinal Chemistry, CSIC, Madrid, Spain
cestarellas@ub.edu

Abstract-Mammalian AMP-activated protein kinase (AMPK) is a Ser108/Thr132 heterotrimeric enzyme complex (one catalytic subunit α and two regulatory subunits β and γ) with a key role as sensor in the cellular energy homeostasis. This function confers AMPK a major role in numerous metabolic disorders, such as type 2 diabetes, obesity and cancer, and explains the progressive interest as a therapeutic target. AMPK is regulated by several mechanisms including indirect and direct activators, which show clear specificity by a particular subunit. We have carried out a series of molecular dynamic simulations of the apo and holo forms of AMPK to gain insight into the mechanism of AMPK activation.

I. INTRODUCTION

Mammalian AMP-activated protein kinase (AMPK) is a Ser108/Thr132 protein kinase with a key role as sensor in the cellular energy homeostasis [1]. This function confers AMPK a major role in numerous metabolic disorders, such as type 2 diabetes, obesity and cancer, and explains the progressive interest as a therapeutic target. AMPK is a heterotrimeric enzyme complex composed by a catalytic α -subunit and two regulatory subunits known as β and γ . It is regulated by several mechanisms, including indirect activators such as metformin, rosiglitazone and resveratrol, and direct activators, such as compound A-769662 [2] (Figure 1A). Some of these activators show a clear specificity for a particular AMPK subunit, which provide a possible way to design new compounds that could activate AMPK in specific tissues, minimizing the putative negative effects of AMPK activation at whole body level [3].

The X-ray structure of AMPK bound to the direct activator A-769662 has been recently reported (PDB entry: 4CFF) [2]. We have carried out a series of molecular dynamic simulations of the apo and holo forms of AMPK, with the phosphorylated Ser108 (pSer108), to gain insight into the mechanism of AMPK activation. The results indicate that the holo structure is less flexible than the apo form, most likely due to the strong interactions formed between pSer108 and Lys29/Lys31 upon binding of the activator. Furthermore, the presence of the activator leads to a significant alteration in the shape and size of the ATP-binding pocket. Moreover, this effect is linked to the dynamical fluctuations observed between the N-terminus of the catalytic α -subunit and the regulatory domain of the β -subunit. On the basis of these results, we speculate that these structural and dynamical features may provide the molecular basis for the synergy found experimentally between the binding of activator and phosphorylation of pSer108 in regulating the activity of AMPK.

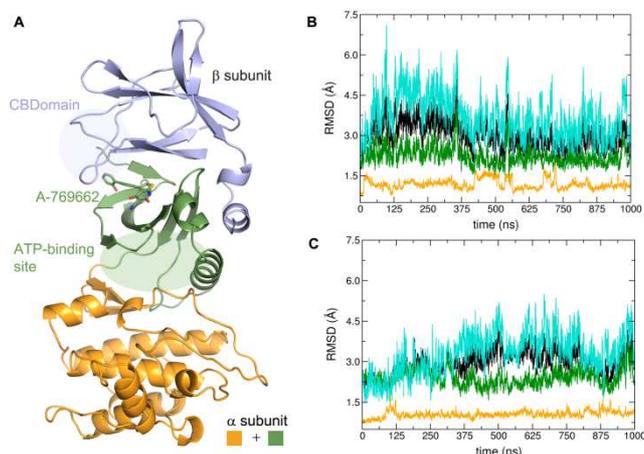


Fig. 1. A) 3D-structure of AMPK. B) RMSD of apo protein. C) RMSD of phosphorylated holo protein (complex with A769662 activator)

II. RESULTS

A. Conformational flexibility

MD simulations show that the complex between the phosphorylated Ser108 (pSer108) kinase domain of AMPK (pAMPK) complexed with the activator A-769662 presents an overall reduction in the conformational flexibility compared to the apo form, which is especially significant in the N-lobe region (Figure 1).

B. Essential dynamics

The apo form shows two regions with different dynamic behavior. One region formed by the N-terminal domain of the kinase and the Carbohydrate Binding Domain (CBM) located in the β -subunit, which show much higher flexibility than the rest of the structure. Remarkably, in the apo form the ATP-binding loop presents a reduced flexibility (marked with a red arrow in Figure 2, top). In contrast, the dynamical behavior of the holo form involves a correlated movement of the N-terminal domain of the kinase, the β -subunit and the flexible loop located in the upper part of the ATP-binding site (Figure 2, bottom).

This remarkable difference in the dynamics of the apo and holo forms might be related to the presence of the activator. Thus, we speculate that it would act like a glue, by releasing the water molecules present in the binding pocket and filling the space between the N-terminal domain of the α subunit and the CBM of the β subunit, and therefore, transferring the movement of these regions to the ATP-binding site.

It can be expected that the increased flexibility of the upper loop in the ATP-binding site should favor the population of

conformations suitable for accommodating ATP, which in turn should lead to an increase in the activity of AMPK.

relevant for keeping the activator tightly bound to the pocket at the N-terminus domain.

CONCLUSIONS

On the basis of these findings, it can be speculated that binding of A769662 and Ser108 phosphorylation act synergistically to enhance the binding of ATP.

At present, we hypothesize that this effect implies a sizable rearrangement of the binding pocket residues that would shape the ATP-binding site, specifically leading to a conformational change in the mouth of the binding pocket that would alter the balance between open and closed states. In turn, this effect should favour the binding of ATP, and the concomitant increase in enzymatic activity.

Future studies will extend these simulations to the complexes with bound ATP in order to ascertain the impact on the ATP arrangement in the binding site and the association/dissociation events.

ACKNOWLEDGMENT

We thank Spanish Ministerio de Innovación y Ciencia (SAF2014-57094-R), Generalitat de Catalunya (2014SGR1189), ICREA Acadèmia and Xarxa de Recerca en Química Teòrica i Computacional (XRQTC) for financial support, and the Barcelona Supercomputation Center for computational resources. C.E. thanks to Spanish Ministerio de Economía y Competitividad for the postdoctoral fellowship (FPD-2013-15572).

REFERENCES

- [1] D. Carling, F.V. Mayer, M.J. Sanders, S.J. Gamblin. "AMP-activated protein kinase: nature's energy sensor," *Nat. Chem. Biol.* 2011, 7, 512–518.
- [2] B. Xiao, M.J. Sanders, D. Carmena, N.J. Bright, L.F. Haire, E. Underwood, B.R. Patel, R.B. Heath, P.A. Walker, S. Hallen, F. Giordanetto, S.R. Martin, D. Carling, S.J. Gamblin. "Structural basis of AMPK regulation by small molecule activators," *Nat. Commun.*, 2013, 4, 3017.
- [3] J.W. Scott, N. Ling, S.M. Issa, T.A. Dite, M.T. O'Brien, Z.P. Chen, S. Galic, C.G. Langendorf, G.R. Steinberg, B.E. Kemp, J.S. Oakhill. "Small Molecule Drug A-769662 and AMP Synergistically Activate Naive AMPK Independent of Upstream Kinase Signaling," *Chem. Biol.* 2014, 21, 619–627.

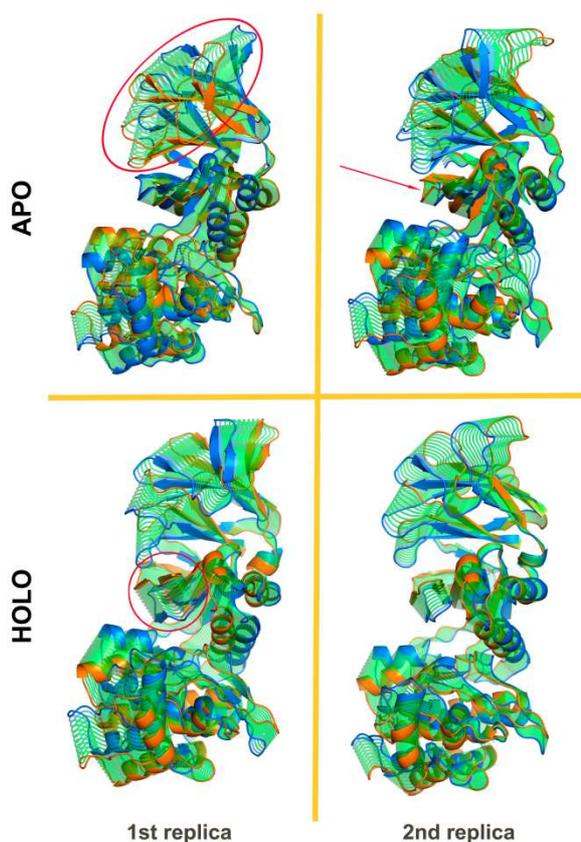


Fig. 2. Representation of the first essential mode, which contributes between 20-30% for apo and holo forms.

C. Distances

A key molecular determinant of this structural alteration is the residue Arg83, which is highly flexible in the *apo* form, being able to form salt bridge interactions with either Asp88 or Asp136. The *apo* form shows frequent exchange between those salt bridge interactions along the MD simulation in comparison to the *holo* form, where the presence of the activator shifts the interaction pattern toward the contact with Asp88.

The activator is tightly bound in the binding pocket. Most of the contacts comprise van der Waals interactions with hydrophobic residues, but the binding is also assisted by the formation of hydrogen bonds between the amide NH unit and the carboxylate group of the Asp88 and between the hydroxy unit of the phenyl ring with the carbonylic oxygen of Gly19. In addition, the binding of A769662 is further assisted by the contact between the phosphate group of pSer108 and Lys29. However the conformational flexibility of the phosphate group allows the formation of complementary interactions with either the hydroxyl group attached to the fused bicycle of the activator or with Lys31. Overall, this interaction seems