The role of calcium ions for the stability of Sonic Hedgehog protein

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Abstract

Sonic Hedgehog proteins (Shh) are secreted signaling molecules that mediate essential tissue-patterning events during embryonic development and function in tissue homeostasis and regeneration throughout life [1]. The first crystal structure of Shh was resolved in 1995 and revealed the presence of a tetrahedrally coordinated zinc ion. Later on, in 2008 a complex of the Shh-Cdo revealed a previously unappreciated binuclear calcium binding site. Since that time, it has been difficult to predict the role of the metals; especially the role of calcium ions has not been clarified yet.

Therefore, in this study, molecular dynamics simulations of murine Shh (PDB codes: 1VHH, 3D1M, 3N1R, 2WFX) were carried out to explore the role of the calcium ions on the stability and the dynamic behavior of Hedgehog protein. We used the new set of Lennard-Jones parameters [2] to model Ca^{2+} protein interaction implemented in the GROMOS96 force field. All simulations were performed with the GROMACS 4.0 package. Each protein was simulated three times employing different seeds (considering three replicas per protein). The total simulation time was 10ns. In addition, the electrostatic potential surrounding the proteins was calculated with the APBS software [3] and also the effect of the absence of the zinc ion on the stability of Shh was tested.

The results show that the presence of calcium ions has a large effect on stability. When the calciums are bound, the protein is more compact and the loop which contains the ligands coordinating the ions is well order yielding a more stable secondary structure. Besides, the calcium binding site avoids the electrostatic repulsion between Shh and receptors. The presence of zinc ion also contributes to the stability of Shh (Fig 1).

We can conclude that the zinc binding site plays a catalytic role even though if the absence of the ion affects the stability of the protein. In contrast, the calcium site increases the stability of Shh and at the same time has a modulatory role. A comparison with experimental data [4,5] and the results reported by [6] support our findings. The present results can contribute to understand better the Hedgehog signaling pathway and could serve as a theoretical base for experimental studies.

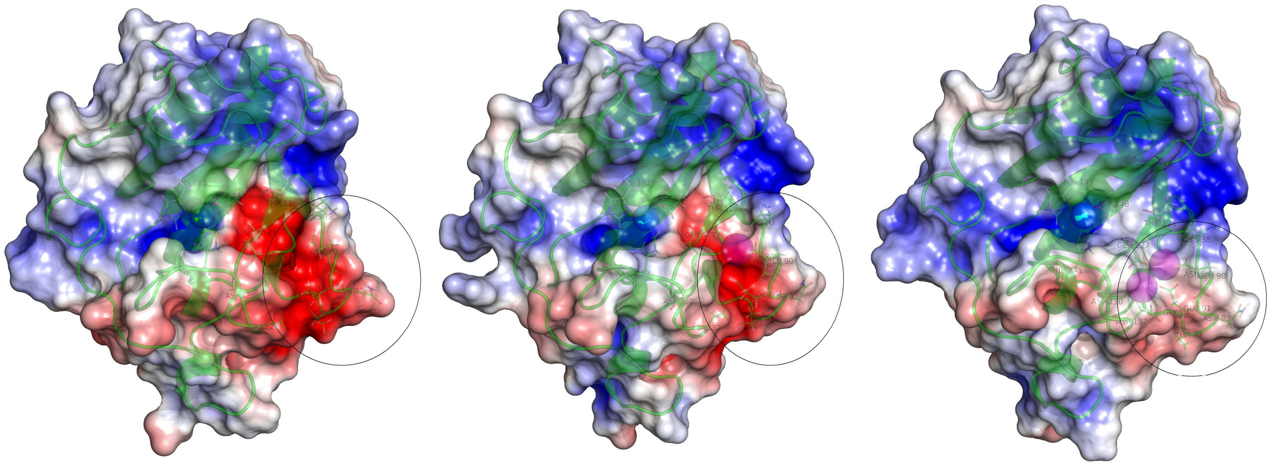

Figure 1. Electrostatic potential of murine Sonic Hedgehog proteins. From left to right: 1VHH, 3N1R and 3D1M. The pointed area represents the loop where the calcium ions are bound. Calcium and zinc ions are depicted as magenta and cyan spheres, respectively. Electrostatic potential of protein surfaces were scaled to the range of -5 (red) and 5 kT/e (blue) according to the APBS software.

References:

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