MOLECULAR MODELING OF INOSITOL 1,4,5-TRISPHOSPHATE RECEPTOR (IP3R) DYNAMICS AFTER IP3 BINDING

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Calcium concentration is strictly regulated in all cells. Homotetrameric inositol 1,4,5-trisphosphate receptors (IP3Rs) in the endoplasmic reticulum are ubiquitous intracellular Ca^{2+} channels and are among key molecules responsible for this regulation [1]. IP3 binding to the IP3-binding core (IBC) near the N terminus initiates conformational changes that lead to opening of a pore. The mechanisms underlying are unresolved. The opening of this channel requires binding of two intracellular messengers, which are inositol 1,4,5-trisphosphate (IP3) and Ca^{2+}. To promote the Ca^{2+} -channel gating and release from the endoplasmic reticulum, IP3 binds to the amino-terminal region of IP3R. At present, the structural understanding of the processes occurring upon IP3-binding and specifically of channel gating or the interpretation of mutagenesis data are based on six apo- and holo- 3D- structures of the inositol 1,4,5-trisphosphate receptor N-terminal parts: IP3-binding core (IBC, residues 224-604, PDB-code 1N4K, resolution 2.2 Å) [2], the suppressor domain (SD, residues 1-223, PDB-code 1XZZ, resolution 1.8 Å) [3], amino-terminal region (NT, residues 1-604, PDB-codes 3UJ0, 3UJ4, 3T8S, resolutions 3.0 Å, 3.6 Å 3.8 Å, respectively) [4, 5]. NT-structures 3UJ0, 3UJ4, 3T8S are presently the most complete atomic-resolution structures of IP3 receptor. However, they are obtained at low resolution, and furthermore, 3UJ0 and 3UJ4 are Cys-less forms. The backbone structures 3UJ0, 3UJ4 [4], on the one hand, and 3T8S apo and holo [5], on the other hand, are globally consistent to one another suggesting that they correspond to same structures. Both in [4] and [5] the apo- and holo- structures were used to predict IP3-evoked conformational changes. In accordance with these works, side chains of nine residues became organized around IP3 and the domain orientation angle between IBC-β and IBC-α was reduced (by, ~8-10°) after IP3 binding. This IP3-evoked domain-closure causes the distance across the entrance to the InsP3-binding pocket to decrease while retaining the extensive α-interface relatively static, yet moving the SD residues away from IBC-β at the β-interface. With the SD glued to IBC-α by the α-interface, IP3 binding causes the SD to twist (by ~9°) and move closer to the top of the IBC. This causes an amplified translational movement of the conserved HS loop in the SD. However, the kinetic feasibil-

ity of such conformational change after IP3 binding was not addressed. Furthermore, the holo- structure of IP3R in 3UJ0 contains cis peptide bond between Asp231 and Asp-232, while in ligand-free form such cis-form is absent. Since the inter-conversion between *cis* and *trans* isomers is a slow reaction which requires the overcoming an activation barrier of about 20 kcal/mol for this change [6], we have hypothesized that holo structures of the IP3R in 3UJ0 and 3T8S are not IP3-bound structures *in vivo*. To help address this issue we performed the modeling of conversion of apo structure to holo one by molecular modeling using two-stage simulation protocol. Because 3T8S apo structure contains cis peptide bond within the linker between SD and IBCβ which is absent in 3UJ4 while the latter is Cys-less, the 3T8S apo structure with the linker between SD and IBCβ taken from 3UJ4 (3TBS_3UJ4) was applied as a starting apo one. When obtaining this structure, Rosetta program [7] was used to close loops followed by minimization with TINKER [8]. At first stage of simulations IP3 was located at the position near IBCα identical to that in 1N4K. Then at first stage of simulations, LBFGS minimization using the software TINKER [8] has been carried out with only dihedral angles of loops between regular structures allowed to be changed. At the second stage the changes of all dihedrals were allowed. The force-field AMBER-99 has been used. The starting and resulting structures are shown in Fig.1.

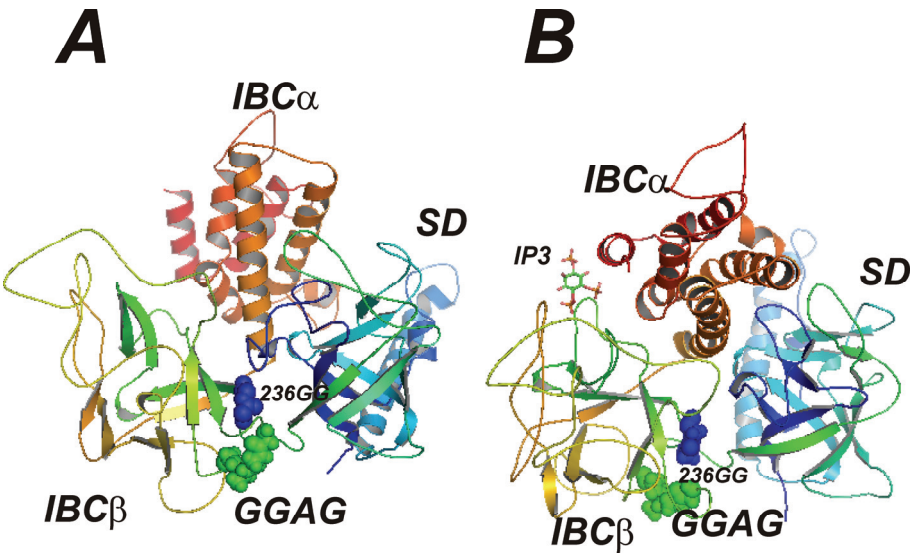

Figure 1 – Structure of the NT region of InsP3R1 without (A) and with IP3 bound (B) obtained by simulations

The analysis of final structure showed that: (i) the final structure of simulations differs significantly from that of holo X-ray structures 3UJ0 and 3T8S

and (ii) the greatest changes in dihedral angles took place within the loop containing residues 288-301 (Table I). Interestingly, the hinge group Gly294-Gly-Ala-Gly297 is conserved among different forms of IP3R (I-III) as well as between different species [2].

Table I – The most significant changes in IP3R backbone dihedral angles after IP3 binding

Residues	φ_{start}	ψ_{start}	φ_{final}	ψ_{final}
Gly294	49	-165	-79	-164
Gly295	141	179	121	-165
Ala296	-62	134	-75	-56
Gly297	-80	129	70	35

References

1. Foskett J. K., White C., Cheung K. H., Mak D. O. Inositol trisphosphate receptor Ca^{2+} release channels // Physiol. Rev. – 2007. – Vol. 87. – P. 593–658.
2. Bosanac, I. et al. Structure of the inositol 1,4,5-trisphosphate receptor binding core in complex with its ligand // Nature. – 2002. – Vol. 420. – P. 696 – 700.
3. Bosanac, I. et al. Crystal structure of the ligand binding suppressor domain of type 1 inositol 1,4,5-trisphosphate receptor // Mol. Cell. – 2005. – Vol. 17. – P. 193–203.
4. Seo M. D., Velamakanni S., Ishiyama N., Stathopulos P.B., et al. Structural and functional conservation of key domains in InsP3 and ryanodine receptors // Nature. -2012. – Vol. 483. –P. 108-112.
5. Lin, C. C., Baek K., Lu Z. Apo and InsP3-bound crystal structures of the ligand binding domain of an InsP3 receptor // Nature Struct. Mol. Biol. – 2011. – Vol.18. – P. 1172–1174.
6. Scherer G., Kramer M., Schutkowski M., Reimer U., Fischer G. Barriers to rotation of secondary amide peptide bonds // J. Am. Chem. Soc. – 1998. – Vol. 120. – P. 5568-5574.
7. Mandell, D. J., Coutsias, E. A., and Kortemme, T. Subangstrom accuracy in protein loop reconstruction by roboticsinspired conformational sampling // Nature Methods. – 2009. – Vol. 6. –P. 551–552.
8. http://dasher.wustl.edu/tinker/