The control of bionanomachine employing calmodulin-based ionochromic nanodevice

Yassine Sabek [1], Zhang Ziun[2] Kondo Kazunori[2]
supervisor Shinsaku Maruta[1,2]

1) Soka University Graduate School of Science and Engineering, Department of Biosciences, Maruta Laboratory (Laboratory of Bio-nanotechnology)
2) Soka University, Faculty of Science and Engineering, Department of Science and Engineering for Sustainable Innovation

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1. Background and Aim of Study

Recent research reveals structural and functional similarities between kinesin and myosin motor domains, suggesting they are likely connected through divergent development from a similar core nucleotide-binding motif.. The topology of these proteins uses analogous chemical and physical mechanisms to sense nucleotide status in in the active site and send this information to protein partners.[1]

Chromic chemicals, including photochromic, thermochromic, ionochromic, electrochromic, and piezoceramic switches, are sensitive to color change or absorption[2]. This paper discusses chromic compounds, specifically calmodulin, a calcium-binding nanoswitch that targets M13 peptide when calmodulin binds to 4 Ca2+ ions, a conformational shift in protein structure, and its application in biochemistry.

Calmodulin (CaM) plays an important role in signaling and regulating numerous calcium-dependent processes in almost all eukaryotic cells. The structure of CaM has been particularly conserved throughout eukaryotic evolution. Many of the target proteins of CaM enzymes activity is known to be stimulated by Ca^{2+}-dependent association with CaM[3,4];

Small GTPase, a nucleotide-driven bionanomachine, plays a crucial role in cell proliferation and differentiation. Ras, a small G-protein, stimulates downstream pathways and is regulated by GTPase-activating proteins (GAPs) and guanine nucleotide exchange factors (GEFs). Calmodulin (CaM) is involved in calcium-dependent processes and is used as a regulatory device to control Ras function with calcium ion dependence. Two fusion proteins were designed and prepared for this purpose.Ca2+-dependent interaction with CaM activates enzymes like smMLCK, crucial for muscle contraction regulation. CaM, a 148 amino acid protein, has EF-hand motifs in two domains, with four EF-hands in CAM binding to four Ca2+. causes conformational changes within each EF hand[3,4]; this assumed conformation is significantly flexible, with exposed hydrophobic clefts available for target binding[7].

2. Materials and Methods

Protein design (CaM.Gb.M13)
expression and purification were expressed and purified as follows.

The fusion protein was synthesized by Funakoshi Coltd,
CLC sequence vie I r 8.0 and Eurofins genomic were respectively used to design. and order The CaM.Gb.M13 and DNA sequence those structures were modeled using Pymol software monitoring the crystal structures of CaM.

I constructed plasmid pET15b (Novagen, Madison, WI,USA), peptide sequence RWKKNFIAVSAANRFKKIS, with a C-terminal 6 His tag. E. coli BL21 (DE3) cells were transformed with the plasmid (pET15b: CaM.Gb.M13 for expression. (CaM.Gb.M13) were then purified starting with E. coli treatment, lysate by co-chelating chromatography follo I d by dialysis against 100 mM NaCl, 30 mM Tris–HCl (pH 7.5), 0.3 mM $MgCl_2$, 0.2 mM ATP, and 1 mM DTT. purified ant stored at -80 °C until use.

GTPase assay

to determine GTPase activity. H-Ras (2 μM) was preincubated for 5 minutes in the presence of 2 μM GAP coexisting and competing with our Gap-based inhibitor (CaM.Gb.M13) in a concentration of 5μM in the presence of Ca^{2+} ($CaCl_2$ 0.5mM) and in the absence of Ca^{2+} (EGTA 0.5mM) and GEF 2 μM in GTPase activity assay buffer (30 mM Tris-HCl pH 7.5, 60 mM NaCl, 2 mM $MgCl_2$). Then, 1 mM GTP was added to start the GTPase assay at 25 °C for 30 min before stopping with 10% trichloroacetic acid (TCA). BioMol Green Reagent was added to the supernatant and, after centrifugation at 15000 rpm for 5 min at 4 °C, incubated at 25 °C for 30 min to determine the amount of Pi produced by GTP hydrolysis.

3. RESULTS and DISCUSSION

3-1 Ionochromic regulation using CaM fused with inhibitory peptide and M13 peptide.

MADQLTEEQIAEFKEAFSLFDKDGDNTITTKELGTVMRSLGQNPTEAEL
QDMINEVDADGNGTIDFPEFLTMMARKMKDTDSEEEIREAFRVFDKDGN
GYISAAELRHVMTNLGEKLTDEEVDEMIREADIDGDGQVNYEEFVQMMT
AKLESGGSGGGSSALTIQLIQNHFVDEYRWKKNFIAVSAANRFKKIS

Fig. 1 DNA Encoding peptide sequence The modification is formed by four starting with the calmodulin (black) parts, the linker (grey), Gap binding peptide (pink), and the calmodulin target peptide M13 as represented in the figure

I designed the two CaM fused with inhibitors to show which conformation is more responsive in terms of controlling the H RAS cycle. The linker SG was enlarged, giving more flexibility to the calmodulin/M13 target peptide and allowing a larger binding angle. Subsequent GTPase results will provide further details.

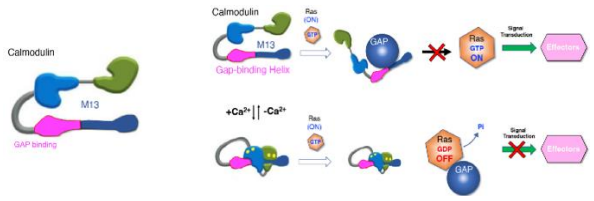

Fig. 2 Representing the CAM-Gb-M13 bionanodevice: showing the expected mechanism for RAS activity inhibition. The inhibitor structure is represented by the GAP binding part (pink) and the calmodulin attached with a linker (grey) to the Inhibitor and M13 CAM target protein (dark blue)

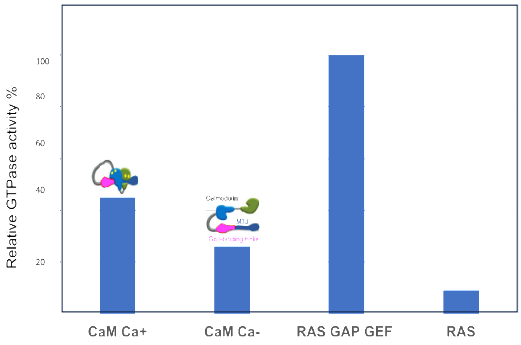

Fig. 3 GTP Hydrolysis inhibition of H-RAS in the presence and absence of CAM-Gb-M13, 5μM A) GTPase was inhibited in the condition of 2μM H-Ras with the presence of 2μM GAP and 2μM GEF by 5μM CAM-Gb-M13 in active form Ca- (absence of Ca^{2+}) and a little less inhibition in its inactive form Ca+ (presence of Ca^{2+}) in both cases in the presence of Ras and GEF at 25C absorption of 630 nm. H-RAS cycle in the presence of GAP and GEF shows an accelerated GTPas circle compared to Ras in the absence of the regulators, however, the incorporation of our CAM-Gb-M13 showed a considerate decrease in the RAS activity in the

The expected mechanism shows Ras cycle control by CAM-Gb-M13 Gap competition avoiding GTP to GDP exchange accelerated by Gap and allowing a potential H-Ras cycle control.

The results of the GTPase assay in the presence and absence of Ca^{2+} showed inhibition of more than 70% in the absence of Ca2+, and surprisingly, the Ras cycle was inhibited even in the presence of Ca^{2+} but slightly more than CAM -Gb-M13 in the absence of Ca2+, which prompted us to investigate more about the structure and mechanism.

To better understand the phenomenon, I proposed a Dose-dependency experiment to clarify the best dosage for optimal functionality

An effective H-RAS cycle control is obtained in the presence of GAP and GEF, the effective competition between our bionanodevise and H-RAS results in a Ca2+-dependent ionochromic inhibition with a promising application in the near future.

Conclusion

The bio nanodevice showed regular Ras cycle control compared with CAM.Gb.M13, which exhibited Ras control,

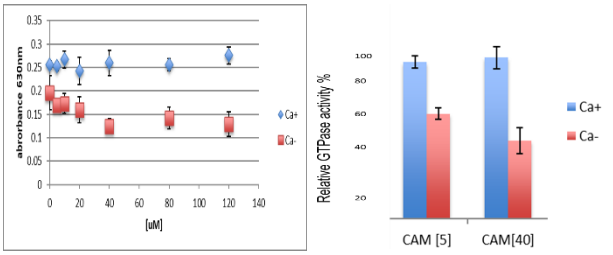

Fig. 4 Showing Effective dose and ionochromic control control are shown in this figure with the (red, absence of calcium) and (blue, presence of calcium) the red dots are representing inhibition compared to the blue dots , the bar Graph shows an active inhibition in the presence of GEF,GAP,H-RAS and Calmodulin based nanodevice in two different concentrations .

On the other part of this research and even if the GTPase assay shows an opposite behavior from the expected.

Controlling biologically and chemically the H-Ras cycle is an intriguing field of study that has the potential to further both scientific understanding and possible therapeutic approaches in disorders where H-Ras signaling is involved.

5. Future Plan

Chemical modification of GAP and GEF to control by calmodulin.

6. References

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