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Report:

Aim: We intend to determine the crystal structures of Ca^{2+} complexes of some model proteins in order to examine functional and structural aspects of the biological role of the essential metal ion Ca^{2+}.

Structural Evidence for Non-canonical Binding of Ca^{2+} to a Canonical EF-hand of a Conventional Myosin [1]

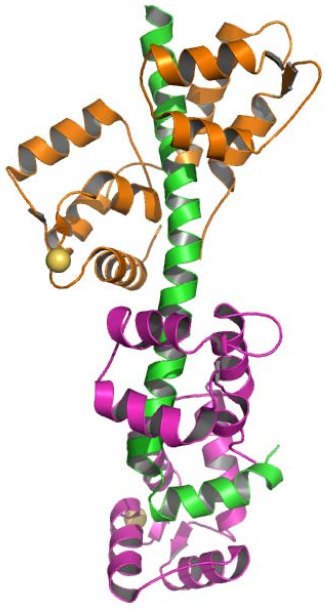
We have previously identified a single inhibitory Ca^{2+}-binding site in the first EF-hand of the essential light chain of *Physarum* conventional myosin. As a general rule, conformation of the EF-hand-containing domains in the calmodulin family is “closed” in the absence and “open” in the presence of bound cations; a notable exception is the unusual Ca^{2+}-bound closed domain in the essential light chain of the Ca^{2+}-activated scallop muscle myosin.

We solved the 1.8 Å resolution structure of the regulatory domain (RD) of Physarum myosin II in which Ca^{2+} is bound to a canonical EF-hand that is also in a closed state (Figure 1). The 12th position of the EF-hand loop, which normally provides a bidentate ligand for Ca^{2+} in the open state, is too far in the structure to participate in coordination of the ion. The structure includes a second Ca^{2+} that only mediates crystal contacts.

To reveal the mechanism behind the regulatory effect of Ca^{2+} we compared conformational flexibilities of the liganded and unliganded RD. Our working hypothesis, i.e. the modulatory

effect of Ca^{2+} on conformational flexibility of RD, is in line with the observed suppression of hydrogen-deuterium exchange rate in the Ca^{2+}-bound form, as well as with results of molecular dynamics calculations. Based on this evidence, we concluded that Ca^{2+}-induced change in structural dynamics of RD is a major factor in Ca^{2+}-mediated regulation of *Physarum* myosin II activity.

Figure 1 Overall structure of *Physarum* myosin regulatory domain. A ribbon representation of the three-chain regulatory domain complex is shown. Orange, essential light chain; magenta, regulatory light chain; green, heavy chain fragment (Ile-771–Gly-841). The inhibitory Ca^{2+} ion, bound to EF-hand I of ELC, and a crystal packing Ca^{2+} in EF-hand II of RLC (coordinated partially by a crystal symmetry-related ELC) are shown as yellow spheres.



Calmodulin-antagonist complexes [2]

Tests of crystals of calmodulin complexed with small molecules of vinblastine and arylalkilamine types. Two datasets collected, but possible twinning and loss of resolution caused bad statistics.

References:

1 Debreczeni JE, Farkas L, Harmat V, Hetenyi C, Hajdu I, Zavodszky P, Kohama K, Nyitray L. "Structural evidence for non-canonical binding of Ca2+ to a canonical EF-hand of a conventional myosin." JBC 280:41458-64. (2005)

2 Horvath I, Harmat V, Perczel A, Palfi V, Nyitray L, Nagy A, Hlavanda E, Naray-Szabo G, Ovadi J. "The structure of the complex of calmodulin with KAR-2: a novel mode of binding explains the unique pharmacology of the drug." JBC. 280:8266-74. (2005)