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Can Ca^{2+}-regulated photoproteins perform different functions than bioluminescence?

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Ca^{2+}-regulated photoproteins are single-chain polypeptide proteins (~22 kDa) that are responsible for the bioluminescence of marine coelenterates. The photoprotein represents an enzyme-substrate complex consisting of apoprotein with tightly but noncovalently bound preoxygenated coelenterazine derivative, 2-hydroperoxycoelenterazine. Blue light emission results from decarboxylation reaction of 2-hydroperoxycoelenterazine initiated by Ca^{2+} binding to a protein. All Ca^{2+}-regulated photoproteins contain three canonical EF-hand Ca^{2+}-binding sites, each consisting of 12 contiguous residues. It allows assigning these proteins to a family of EF-hand Ca^{2+}-binding proteins. Despite this fact, the photoproteins demonstrate no significant amino acid sequence homology with other proteins from this large family.

The ^{15}N-HSQC-NMR measurements carried out for obelin from *Obelia longissima* bound with different ligands offer five distinct conformational states of the photoproteins: (i) apophotoprotein; (ii) photoprotein bound with 2-hydroperoxycoelenterazine; (iii) Ca^{2+}-discharged photoprotein, i.e. protein bound with the reaction product, coelenteramide, and Ca^{2+}; (iv) Ca^{2+}-discharged photoprotein without Ca^{2+}; and (v) apophotoprotein bound with Ca^{2+}. To date, the crystal structures of four conformations (states ii-v) have been determined. The structures of these ligand-dependent conformations reveal a similar globular scaffold with some distinctions caused by the bound ligand type. Despite the numerous attempts, the crystals of apophotoprotein were not obtained. The cause may be the absence of an ordered three-dimensional structure that is supported by NMR measurements [1] and denaturation studies [2]. Thus, apophotoprotein displays the features characteristic of intrinsically disordered proteins.

Presently, many disordered Ca^{2+}-binding proteins have been discovered [3]. They assemble functionally active tertiary structure in response to calcium binding. Based on comparison of different conformational states of photoproteins with Ca^{2+}-binding proteins, we here hypothesize that photoproteins may perform another function in host organisms alongside with bioluminescence. In total, five types of Ca^{2+}-binding proteins were found in Protein Data Base which spatial structures reveal a high similarity with those of photoproteins. These are neuronal calcium-signaling protein calexcitin from the squid *Loligo pealei* (PDB 2CCM), juvenile hormone diol kinase from the silk worm *Bombix mori* (PDB 6KTH, 7PJD), okadaic acid binding protein (OABP) from the marine sponge *Halichondria okadai* (PDB 4WRI), three sarcoplasmic Ca^{2+}-binding proteins (SCBPs) from different species (PDB 2SAS, 2SCP, 3AKB) and Ca^{2+}-dependent coelenterazine-binding protein (CBP) from *Renilla muelleri* (PDB 2HQ8, 2HPS). All these proteins have similar globular structures and contain four EF-hand motifs but only

three loops with canonical amino acid sequences ensuring Ca^{2+} binding. There is only one exception—okadaic acid binding protein does not contain loops capable of binding Ca^{2+} owing to replacement of the key residues that provide an oxygen atom for coordination of Ca^{2+}. The function of these proteins is diverse. For instance, SCBPs serve as calcium buffers while OABP binds okadaic acid, a marine polyether cytotoxin. JHDK is involved in metabolism of insects; this enzyme catalyzes phosphorylation of juvenile hormone using ATP or GTP but in contrast to photoproteins, calcium ions inhibit its activity. Neuronal calcium-signaling protein calexcitin regulates different types of potassium channels and takes part in controlling the release of calcium ions from the endoplasmic reticulum by binding to the ryanodine receptor. After phosphorylation calexcitin can be translocated to the cell membrane where the one affects the membrane excitability. In addition, the calexcitin injection into photoreceptors of gastropods was capable to reproduce the electrophysiological effects of learning. In the last decade different groups showed that photoprotein aequorin may also administer the cognitive function [4]. Despite a very low sequence homology (18 %), Ca^{2+}-loaded conformational state of apo-aequorin and calexcitin reveal a very high structural similarity of their main chains (RMSD 3.6 Å). Moreover, the sequence analysis of aequorin and other photoproteins using NetPhos 3.1 software predicts the presence of different sites of phosphorylation with high probability, i.e., similar to calexcitin apo-aequorin can be also phosphorylated by protein kinase C. Earlier it was demonstrated that Aequorea jellyfish cannot synthesize coelenterazine; the substrate comes through diet [5]. At the same time, aequorin is expressed by jellyfish. Thus, in the absence of coelenterazine the aequorin should be in the apo-conformational state bound with either Ca^{2+} or Mg^{2+} or Ca^{2+}/Mg^{2+} depending on the affinity of its Ca^{2+}-binding sites and consequently can perform some other function, like, for example, calexcitin. Although the above mentioned experimental and theoretical findings imply other functions of photoproteins alongside with bioluminescence, only revealing the molecular mechanism of regulation of cognitive processes e.g. by aequorin, can definitely confirm it.

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