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A biophysical and structural approach to investigate calcium sensor properties of plant calmodulin-like proteins

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Calcium is an essential second messenger in plants that regulates various signaling pathways through stimulus-specific Ca^{2+} signatures, which are decoded and converted into a wide variety of biochemical changes by Ca^{2+} sensors. Besides evolutionarily conserved calmodulin (CaM), plants exclusively possess a group of calmodulin-like proteins (CMLs), which play central roles in the coordination of plant responses to different external stimuli. Nevertheless, only few of these proteins have been thoroughly characterized and demonstrated to function as Ca^{2+} sensors. Our research is focused on the investigation of the metal-binding, physicochemical and structural properties of various plant CMLs using complementary biophysical and structural approaches to correlate their properties with the biological activity. We have recently characterized CML36 from *Arabidopsis thaliana*, demonstrating that *in vitro* the protein shows feature consistent with Ca^{2+} sensor function. ITC analysis revealed that CML36 possesses two high affinity Ca^{2+}/Mg^{2+} mixed sites and two low affinity Ca^{2+}-specific sites. Binding of Ca^{2+} to CML36 increases its α-helical content and triggers a conformational change that exposes hydrophobic surfaces necessary for target recognition. Ca^{2+} and Mg^{2+} ions also stabilized the tertiary structure of CML36. Cations binding to the Ca^{2+}/Mg^{2+} mixed sites appear to guide a large structural transition from a loosely packed molten globule apo-state to a well-defined, stable holo-structure. Through *in vitro* binding experiments, we showed that CML36 directly interacts with the N-terminal domain of *Arabidopsis* Ca^{2+}-ATPase isoform 8 (ACA8), a type IIB Ca^{2+} pump localized at the plasma membrane (PM). Moreover, we demonstrated that this interaction promotes ACA8 Ca^{2+}-dependent hydrolytic activity *in vitro*.

Biography

Alessandra Astegno is interested in various aspects of Protein Chemistry, including folding, Evolution and structure-function relationship of proteins and Macromolecular assemblies. She obtained a PhD in Applied Biotechnologies from University of Verona in 2010. She is currently working as an Assistant Professor in Biochemistry at the Department of Biotechnology of the University of Verona. She has a solid background in recombinant protein expression and purification, functional and structural characterization of metallo-proteins as well as PLP-dependent enzymes. Recently, her work focused on the study of calcium signaling in higher plants through biophysical, biochemical and structural characterization of calcium sensor proteins, such as calmodulin and calmodulin like proteins of *Arabidopsis thaliana*.

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