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Solution structure of plant calmodulin in the absence and presence of targets: Implications on its structure and function

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Introduction

Calmodulin (CaM) is a ubiquitous multifunctional Ca^{2+} signal receptor in eukaryotes that participates in most of the important signaling pathways in cells. It plays a key role in transducing Ca^{2+} signals to different physiological effects. In order to elucidate the functional mechanism of CaM, structural and biological studies have been carried out. The three-dimensional structure of potato CaM (PCM6) has been recently reported [1]. This structure is different from animal CaMs in the central helix region. In the present work, SAXS has been applied to characterize the solution structure of PCM6 in the absence and presence of a target.

Materials and Methods

PCM6 was prepared as described previously [2]. The PCM6-binding domains (PCM6BD) of *arabidopsis* DWARF1 (DWF1) and mutants were prepared as described previously [3]. Table 1 summarizes the primary sequences of three synthetic peptides. Trifluoperazine (TFP) was used as a CaM antagonist as described previously [4]. Two methods of data analysis were used. The first method was that of Guinier-Fournet.

$I(s,c)=I(0,c)\exp[-(4\pi^2/3)R_g(c)^2s^2]$,

$Kc/I(0,c)=1/M+2A_2c+\ldots$,

$R_g(c)^2= R_0^2-B_{if}c+\ldots$

The second method was that of Kratky. The details are described in a previous paper [3].

Table 1: Primary sequences of PCM6BDs of DWF1

Peptide name	Primary sequence
DWF1 WT	RKKYRAIGTFMSVYYKSKKGR
DWF1 F528D	RKKYRAIGTDMSVYYKSKKGR
DWF1 V531D	RKKYRAIGTFMSDYYKSKKGR

Acidic and basic residues are shown in red and blue, respectively.

Results and Discussion

Solution structure of PCM6 in the absence of target

The R_g value of apoPCM6 was 19.8Å. When Ca^{2+} was added, it increased to 20.3Å. As compared with the corresponding change of animal CaM, the change is one half, suggesting that the Ca^{2+}-induced shape change for PCM6 is smaller. The infinite dilution value was 20.9Å for Ca^{2+}-saturated PCM6, while that was 21.9Å for Ca^{2+}-saturated animal CaM. The PCM6 adopts a dumbbell shape smaller than animal CaM. The results suggest that the two lobes of PCM6 are more approaching than those of animal CaM.

Solution structure of PCM6/DWF1

The *M* values for three complexes of PCM6/DWF1 indicate that Ca^{2+}-saturated PCM6 binds each peptide with the molar ratio of 1:1. The R_0 values were 18.8A for PCM6/DWF1WT, 19.3A for PCM6/DWF1V531D, and 20.7A for PCM6/DWF1F528D, respectively. Each complex adopts a relaxed globular shape, because the corresponding anchoring residues are not observed at the appropriate positions. The results suggest that Phe at 528 of DWF1 plays more important role than Val at 531 in the formation of the compact complex. However, any binding motif was not retrieved in the DWF1 sequence, suggesting a novel recognition motif.

Solution structure of PCM6/TFP

The R_g value of Ca^{2+}-saturated PCM6 without TFP was 21.0Å at the protein concentration of 9.0 mg/ml. When TFP was added, the values decreased drastically: they were 16.9Å for the 1:1 complex, 17.0Å for the 1:2 complex, 17.2Å for 1:3 complex, and 17.2Åfor the 1:4 complex. The infinite dilution value for the 1:1 complex was 18.0Å and thus the complex adopts a compact globular shape. The results are equivalent with those for the canonical complex of animal CaM and the CaM-binding domain of smooth muscle myosin light chain kinase (RS20). However, the compact globular shape in the animal CaM required the binding of four TFP molecules per one CaM molecule in solution [4]. The present results suggest that the molecular recognition to TFP molecule is significantly different between animal CaM and PCM6. The approaching between the two lobes of PCM6 might explain the difference. A detailed modeling study is necessary to confirm this point. Research is in progress along this line.

References

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