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Interactions among calmodulin, calcium and TFP studied by solution x-ray scattering

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Introduction

Calmodulin (CaM) is a ubiquitous Ca^{2+}-binding protein of 148 residues that regulates a variety of physiological processes in a Ca^{2+}-dependent manner. The regulation is achieved through the interaction of Ca^{2+}-bound CaM with a large number of target enzymes. The CaM molecule in both Ca^{2+}-bound and Ca^{2+}-free states adopts an 'elongated' structure in which the two globular domains are connected by a highly flexible linker, while the structures of Ca^{2+}-bound CaM complexed with a peptide from target enzymes adopt a compact globular shape caused by the bending of the domain linker. These structural studies suggest that the flexibility of the domain linker plays an important role in target recognition.

A very recent SAXS study provided evidence that, in solution, the structure of Ca^{2+}-bound CaM changes from an elongated shape to a compact globular shape upon the binding of two W-7 molecules [1] and four TFP molecules [2]. Here we studied structural change of CaM in the presence of TFP with molar ratios of 1:5 as a function of Ca^{2+}.

Here we performed small-angle x-ray scattering study to study how the binding of both Ca^{2+} and TFP to CaM influences the structural change of the CaM molecule.

Materials and Methods

The recombinant rat CaM was expressed in *Escherichia coli* and purified to homogeneity as previously described. For SAXS experiments, the recombinant was dissolved in Tris buffer (50 mM Tris-HCl, pH7.6) containing 120 mM NaCl. The protein concentration was determined by quantitative amino acid analysis. The TFP powder was dissolved in water containing 1% (v/v) dimethyl sulfoxide (DMSO), and added to the CaM solution. In every stage, the concentration of DMSO was kept at 1.0 %. Four series of CaM were prepared both in the presence of TFP with molar ratios of 1:5 and in the presence of Ca^{2+} with molar ratios of 0:0, 1:1, 1:2 and 1:5. Individual series were solutions at five protein concentrations of 4.0 to 18.0 mg/ml.

The measurements were performed using synchrotron orbital radiation with an instrument for SAXS installed at BL-10C of Photon Factory, Tsukuba. An X-ray wavelength of 1.488 Å was selected. The samples were contained in a quartz cell with a volume of 80 µl, and the temperature was maintained at 25±0.1ºC by circulating water through the sample holder. The reciprocal parameter, Q, equal to $4\pi\sin\theta/\lambda$, was calibrated by the observation of peaks from dried chicken collagen, where 2θ is the scattering angle and λ is the X-ray wavelength. Scattering data were collected for 250 or 300s at individual protein concentrations.

Results and Discussion

Guinier plots give the radius of gyration, *Rg*. The *Rg* values for CaM at zero concentration were estimated in the presence of TFP with molar ratios of 1:5 and in the presence of Ca^{2+} with molar ratios of 0:0, 1:1, 1:2 and 1:5, as shown in Table 1.

Table 1: The radius of gyration (*Rg*) for CaM in the presence of TFP with molar ratios of 1:5

Ca^{2+}/CaM			
0	1	2	5
2.05 nm	1.90 nm	1.84 mm	1.76 nm

The *Rg* with Ca^{2+}/CaM=0 shows that the *Rg* value for the Ca^{2+}-free CaM with TFP (CaM:TFP=1:5) is about 1 Å smaller than that (2.15 nm) of Ca^{2+}-free CaM alone [2]. The $p(r)$ function of the Ca^{2+}-free CaM with TFP shows a peak near 22 Å, which is consistent with that in the Ca^{2+}-saturated CaM-TFP complex. However, the shoulder near 40 Å seen in Ca^{2+}-saturated CaM alone grows very weak or disappears in the Ca^{2+}-free CaM with TFP. Consequently, the p (r) function of Ca^{2+}-free CaM with TFP is relatively symmetrical. This suggests that the Ca^{2+}-free CaM with TFP exist as an ellipsoid in solution [2].

The *Rg* with Ca^{2+}/CaM=5 shows that CaM changes from an elongated shape to a compact globular shape upon the binding of TFP molecules [2].

Interestingly, the present results indicate that the *Rg* values show no linearity with increasing molar ratio of Ca^{2+}/CaM. We think that this may be due to a cooperativity.

References

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