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# Tertiary structure of calmodulin predicted by bioinformatics: Experimental verification by SAXS

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## Introduction

Extended proteins such as calmodulin (CaM) and troponin C have two globular terminal domains linked by a central linker. The mechanisms that stabilize the tertiary structure of extended proteins appear to differ greatly from those of globular proteins. Identifying such differences in physical properties of amino acid sequences between extended proteins and globular proteins can provide clues useful for identification of extended proteins from complete genomes including orphan sequences. Along line of this thought, a recent study has indicated that electrostatic interaction is a dominant factor in stabilization of extended proteins and plays important roles in structural changes of extended proteins [1]. The present study has been performed to obtain the experimental verification by SAXS.

## Materials and Methods

The mutant and recombinant CaMs (denoted as mCaM and rCaM, respectively) were prepared as described previously [2]. Table 1 summarizes various CaMs used in this work. Two methods of data analysis were used. The first method was that of Guinier-Fournet.

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

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

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

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

Table 1: Values of $R_g$ for various CaMs

| CaMs | $R_0$/0Ca$^{2+}$ | $R_0$/4Ca$^{2+}$ |
|---|---|---|
| Recombinant | 21.5 | 21.9 |
| D2K/E6K/E7K | 21.1 | 21.4 |
| E45Q/E47Q | 23.4 | 21.9 |
| E45K/E47K | 22.8 | 22.0 |
| E7Q/E45K/E47K | 20.9 | - |
| D2K/E6K/E7K/E45K/E47K | 21.9 | 22.4 |
| D2K/E6K/E7K/E45K/D50K/E54K | 21.6 | 25.3 |
| E14K/D78K/D80K/E82K/E83K/E84K/E87K | 22.5 | 31.6 |
| E87K | 23.4 | 22.1 |
| E114K/D118K/E120K | 22.4 | 26.1 |

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

## Results and Discussion

In almost all samples of mCaMs studied here, there is an evidence of slight upward curvature at low $s^2$ values in the Guinier plots, which indicates that the data are not free from the aggregation of samples. Using the SAXS data with above equations, the three parameters $R_0$, $A_2$ and $B_{if}$ were evaluated and only the former was compiled in Table 1.

The $R_0$ values of D2K/E6K/E7K with and without Ca$^{2+}$ are slightly smaller than those of rCaM. The $R_0$ values of E45Q/E47Q and E45K/E47K without Ca$^{2+}$ are larger than that of rCaM, but those with Ca$^{2+}$ don't change. The $R_0$ values of D2K/E6K/E47K/E45K/E47K, D2K/E6K/E7K/E45K/D50K/E54K, E14K/D78K/D80K/E82K/E83K/E84K/E87K, E87K, and E114K/D118K/E120K without Ca$^{2+}$ are almost equal or larger than that of rCaM, but those with Ca$^{2+}$ are larger than that of rCaM.

A decrease in the net charge associated with the mutation might be expected to bind two domains of CaM and reduce the $R_0$ value compared with that of rCaM. The results for D2K/E6K/E7K and E7Q/E45K/E47K appear to be consistent with this expectation, but those for other mutants appear to be inconsistent. In latter case, a drastic change in the parameters of $A_2$ and $B_{if}$ was observed, suggesting an intermolecular association among mCaMs.

A decrease in the net charge associated with the binding of Ca$^{2+}$ to mCaM might be expected to reduce the hydration shell and lead to a smaller Stokes radius. The results for E45Q/E47Q, E45K/E47K, and E87K appear to be consistent with this expectation, but those for other mutants appear to be inconsistent. A conformational change upon binding of Ca$^{2+}$ might lead to an increase in the size of mCaM. However, the large increase in the size of mCaMs such as D2K/E6K/E7K/E45K/D50K/E54K, E14K/D78K/D80K/E82K/E83K/E84K/E87K, and E114K/D118K/E120K might be induced by an intermolecular association among mCaMs. Moreover, excess Ca$^{2+}$ in all samples might promote the intermolecular association.

The present work supports that electrostatic interaction is an important factor in stabilization of CaM.

## References

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