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Tertiary structure of calmodulin predicted by bioinformatics: Experimental verification by SAXS, 2

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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 further 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 and B_{if} for various CaMs with $0Ca^{2+}$ or $4Ca^{2+}$

CaMs	R_0/Å 0Ca/4Ca	$B_{if}/10^{13}$ cm^5g^{-1}
Recombinant	21.5/21.9	6.8/2.7
D2K/E6K/E7K/E45K/E47K	20.9/22.2	0.1/-1.5
E47K/D50K/E54K/R74D/K75D	21.0/22.1	6.5/-3.1
R74D/K75D	21.6/21.0	9.8/2.4
D78K/D80K/E82K/E83K	20.8/21.6	5.4/-2.2
E14K/D78K/D80K/E82K/E83K/E84K/E87K	19.0/22.4	-3.4/-14.0

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

Results and Discussion

In all samples of mCaMs studied here, there is no evidence of any upward curvature at low s^2 values in the Guinier plots, which indicates that the data are free from the aggregation of samples. Using the SAXS data with above equations, the two parameters R_0 and B_{if} for various CaM with $0Ca^{2+}$ or $4Ca^{2+}$ were evaluated and compiled in Table 1.

The R_0 and B_{if} values of mCaMs except E47K/D50K/E54K/R74D/K75D with and without Ca^{2+} are different from the corresponding values of rCaMs, indicating that the mutation influendes the size of mCaMs as well as the interaction among mCaMs. However, the R_0 and B_{if} values for E47K/D50K/E54K/R74D/K75D almost don't appear to change those for rCaM, indicating that the electrostatic interaction between the acidic and basic amino acid residues is compensate each other because of the contiguous residues in the space.

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/E45K/E47K, D78K/D80K/E/82K/E83K, R74D/K75D and E14K/D78K/D80K/E82K/E83K/E84K/E87K appear to be consistent with this expectation, although the change in the parameters of B_{if} is very complex and cannot appear to be easily interpreted.

A decrease in the net charge associated with the binding of Ca^{2+} to mCaM can be expected to reduce the hydration shell and lead to a smaller Stokes radius. The result for R74D/K75D appears to be consistent with this expectation but those for other mutants are opposite against this expectation. A conformational change upon binding of Ca^{2+} might lead to the increase in the size of mCaM. Further SAXS study is needed to confirm this point.

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

References

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