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Tertiary structure of calmodulin predicted by bioinformatics: Experimental verification by SAXS, 3

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Introduction

Structure of calmodulin (CaM) consists of two globular terminal domains linked by a central linker. The each two domains are composed of two EF-hands, and CaM does not include the other moiety besides the four EF-hands. In spite of the simple structure, CaM is a high-performance bio-molecule, and one of the principal factors of intra-cellar signal transduction systems. Therefore, to elucidate structural–functional relationship of proteins, CaM is supposed to be an ideal subject of the study.

CaM takes a unique extended conformation referred to as the dumbbell shape, and the structural formation mechanism has not been elucidated so far. 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, and to understand principles of protein structure.

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. The methods of Guinier-Fournet were used for data analysis [3].

$I(s,c)=I(0,c)\exp[-(4\pi^2/3)R_g(c)^2s^2]$,
$Kc/I(0,c)=1/M+2A_2c+...$,
$R_g(c)^2=R_0^2-B_{it}c+...$

Table 1: Values of R_0 for various CaMs without Ca^{2+}

CaMs	R_0/Å
Recombinant	21.5
E47K/D50K/E54K/R74D/K75D	20.8
R74D/K75D	22.4
E47K/D50K/E54K	21.6

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

Results and Discussion

During the previous SAXS studies (10C/2004G392), it was suggested that E47 and D50 of CaM draw and fasten the two repelling domains. Details observations of CaM molecule (Fig.1), a negatively charged cluster composed of E47, D50, and E54 is located near from a positively charged cluster composed of R74 and K75. Two globular domains of CaM have large negative charges respectively. Nevertheless, CaM without Ca^{2+} takes a closed conformation. Electrostatic interaction between these two positively and negatively charged clusters was supposed to function as the clasp.

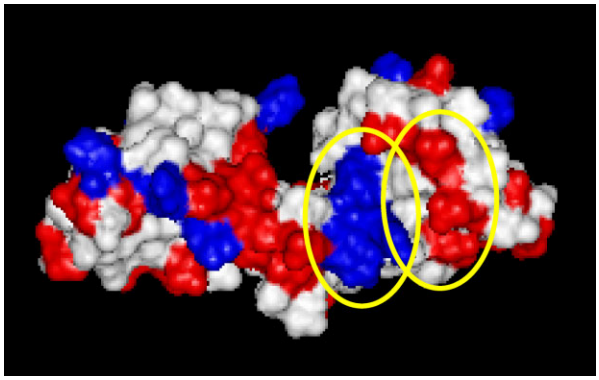

Figure 1: Molecular structure of CaM without Ca^{2+}. Red and blue show negatively and positively charged residues, respectively. Two yellow circles indicate the charged clusters mentioned in this report.

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 parameters R_0 for various CaM without Ca^{2+} were evaluated and compiled in Table 1.

The R_0 values of mCaMs are not so different from the corresponding values of rCaM, indicating that the mutation influences the conformation not so seriously as to induce collapse of the structures.

As shown in Table 1, inversions of charges of each cluster (R74D/K75D or E47K/D50K/E54K) induced expansion of distance of two globular domains of CaM, and the simultaneous inversion (R74D/K75D /E47K/D50K/E54K) resulted in the recovery. This result indicates that the electrostatic interaction between these two positively and negatively charged clusters function as the clasp of the two globular domains of CaM. In the presence of Ca^{2+}, some driving force may operate as to overcome the electrostatic interaction.

References

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