Apocalmodulin binds to the Ca^{2+}/calmodulin-dependent protein kinase IV calmodulin target-site

Yoshinobu IZUMI*[1], Shigeo KUWAMOTO[1], Yuji JINBO[1], Hidenori YOSHINO[2]
[1]Graduate School of Science and Engineering, Yamagata University, Yonezawa 992-8510, Japan
[2]Department of Chemistry, Sapporo Medical University, Sapporo, 060-8566, Japan

Introduction

The aim of the present study is to investigate the complex state in apocalmodulin (apoCaM) induced by the binding of a peptide with Ca^{2+}-dependent calmodulin (CaM) binding motifs by small-angle X-ray scattering (SAXS). We used 19-residue peptide encompassing the Ca^{2+}/CaM-dependent protein kinase IV (human) CaM target-site as a natural target binding domain of CaM. This peptide has been classified as the 1-5-8-14 motif. The present results provide the first detailed SAXS evidence for the formtion of apoCaM/peptide complex [1].

Materials and Methods

A 19-residue peptide having the sequence (RRKLKAAVKAVVASSRLGS) and bovine brain CaM were used. Two methods of data analysis were used. The first method was that of Guinier.

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

$Kc/I(0,c)=1/M+2A_2C+\ldots$

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

Using these equations, we estimated the four parameters M, A_2, R_0 and B_{if}. The details are described in a previous paper [2].

The second method was that of Kratky.

Results and Discussion

In all of the samples studied here, there is no evidence of any upward curvature at low s 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 four parameters M, A_2, R_0 and B_{if} were evaluated and compiled Table 1.

Table 1: Molecular weight (M) , second virial coefficient (A_2), radius of gyration at infinite dilution (R_0), parameter of interparticle intereference (B_{if}) for CaM at pH 7.6

	$10^{-3}M$	10^4A_2	R_0/A	$10^{13}B_{if}$
Apo CaM	16.7	3.1	21.2	6.8
Apo CaM/TP	18.1	1.9	22.2	5.7
$4Ca^{2+}$/ CaM	16.7	2.1	21.5	4.7

The molecular weight M increased about 8.4% in the presence of 1 mol of the target peptide, whose increment almost cooresponds to the molecular weight of 1 mol of the target peptide, while the value of A_2 decreased about 30%. The increase in the molecular weight provides the direct evidence that a complex is formed between apoCaM and the target peptide even in the absence of Ca^{2+}. The formation of a complex with the peptide increases the radius of gyration of apoCaM by 1.0A in comparison with that in the absence of target peptide. Furthermore, the formation of the complex decreases the value of B_{if} by 16%.

The Kratky plot for apoCaM without the target peptide is characterized by the presence of a broad asymmetric maximum near $s=0.0153A^{-1}$, indicating that apoCam without peptide adopts a dumbbell-shaped structure. In the presence of target peptide, the changes in the Kratky plot consist of a slight change, i.e., the position of the maximum shifts to the low value of $s=0.0139A^{-1}$. The results indicate that the overall size of complex increases but apoCaM still preserves a dumbbell-shaped structure even in the presence of target peptide.

In order to specify the location of the peptide in the complex, we applyed a three-body model. The results suggest that the peptide does not interact with both lobes of apoCaM but one lobe of apoCaM. Furthermore, we assume that the peptide interacts solely with the C-terminal lobe of apoCaM. The results indicate that the semi-open conformation doesn't pre-exist in the C-terminal lobe of apoCaM but induced by the binding of the target peptide. The residues E114 and K115 of apoCaM would interact with the peptide prior to expose the hydrophobic residues like M109 and L112 on its surface. Because these electrostatic residues locate on the surface of the C-terminal lobe of apoCaM.

Thus, the complex formation is induced by the electrostatic interactions and subsequent van der Waals interactions between apoCaM and the peptide, which is responsible for the decrease in A_2 andB_{if}.

References

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* yizumi@dip.yz.yamagata-u.ac.jp