Biological Science

10C/2005G296

# Interaction of Alzheimer's disease proteins with calmodulin: Experimental evidence by SAXS

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## Introduction

The calcium hypothesis of Alzheimer's disease (AD) invokes the disruption of calcium signaling as the underlying cause of neuronal dysfunction and ultimately apoptosis. Calmodulin (CaM), as a primary calcium signal transducer, responds to cytosolic calcium fluxes by binding to and regulating the activity of target CaM-binding proteins (CaMBPs). $Ca^{2+}$-dependent CaMBPs are classified by 1-10, 1-14, and 1-16 motifs, while $Ca^{2+}$-independent CaMBPs are characterized an IQ or IQ-like motif [1]. A recent study has suggested that many of proteins intimately linked to AD may be CAMBPs, opening new avenues for research on this devastating disease [2]. The present SAXS study has been performed to reveal the role of CaM in AD.

## Materials and Methods

Recombinant CaM was prepared as described previously [3]. The synthetic peptides were a series of putative peptides based on a search for CaMBPs [4]. Table 1 summarizes the primary sequences of these peptides. 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 [5].

Table 1: Primary sequences of AD peptides and SAXS results for $Ca^{2+}$/CaM/AD peptide complexes

| Peptide name | Sequence[1] | $M_{exp}/M_{cal}$[2] | Shape[3] /$R_0$/ Å |
|---|---|---|---|
| APH1a | LQE*V*FRA YYKL*L*KK | 1.00 | I+/19.3 |
| APP | RRRLA*L*ENY*I*TALQ*A*VPPRPRH | 1.04 | I-/20.5 |
| PEN2 | KLNLCRKY*Y*LGG*F*AFLP*F* | 1.14 | G/18.1 |
| PSN1 | RG*V*KLG*L*GDFI*F*YSVL*V*GK | ---- | I+/20.1 |
| BACE2 | *F*YVI*F*DRAQKRVG*F* | ---- | D/20.5 |
| NIC | D*L*MEKLKGRTSRIAGL*A*VSLTK | 0.92 | I-/19.6 |
| BACE1 | RRGS*F*VEMVDNLRGKSGQG*Y*YVE | 1.12 | D+/21.9 |
| Tau | SSG*A*KEMKLKGADGKTKI*A*T | 1.00 | D-/21.5 |
| α-Syu | KT*V*EGTAGS*I*AA*A*TGF*V*KK | 1.00 | I+/21.7 |

1: The italic letter represents the putative anchoring residue.

2: $M_{cal}$ is calculated from $M$ of $Ca^{2+}$/CaM and AD peptide.

3: I+, I, I-: three different intermediate shapes; G: globular shape; D+, D, D-: three different dumbbell-like shapes.

*: The solution of CaM with PSN1 or BACE2 was cloudy and each supernatant was measured.

## Results and Discussion

The present results indicate that, in the presence of $Ca^{2+}$, CaM binds each AD peptide with the molar ratio of 1:1. The $R_0$ values of the complexes changes from 18.1 Å, a typical value for a compact globular shape, to 21.9Å, a typical value for a dumbbell shape. From the $R_0$ value of CaM/PEN2, the complex adopts a compact globular shape similar to the CaM/RS20 complex. However, the $R_0$ values for other complexes are larger than this value.

The SAXS data also indicate that the complexes adopt various shapes depending on the AD peptide. The shapes were not classified by the classic motifs, indicating that the shape of the complex is very specific. In order to characterize the various shapes, the shapes of the dumbbell, globular, and intermediate shapes were further subdivided as shown in Table 1. The result suggests that the shape of each complex is determined by the sequence of the AD peptide. As the present CaM target database is based on the classic continuous CaMBDs, which is 60-80% accurate in revealing CaMBPs, some CaMBDs of AD proteins might contain a novel type.

In the absence of $Ca^{2+}$, the solution of CaM and APH1a was cloudy. When $Ca^{2+}$ was added, the solution became clear. The result suggests that the hydrophobic residues in APH1a interact with the hydrophobic patch in each lobe of CaM exposed upon binding of $Ca^{2+}$.

In α-Syu, any CaMBD was not retrieved using the present CaM target database. However, the present result indicates that CaM binds this peptide. In actual, the opposite motif (rev1-5-8-14) exists in the sequence as shown in Table 1. A revised version of the CaM target database is necessary to retrieve the molecular recognition motif in the opposite orientation.

Taken together, the results provide that CaM binds various AD proteins via the CaMBDs and regulates their functions in a $Ca^{2+}$-dependent manner.

## References

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