Characterization of transient intermediates of a calmodulin-peptide complex 2

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Introduction

We have previously reported that the EDTA-induced dissociation processes of Ca^{2+} ions from a complex of Ca^{2+}-saturated calmodulin ($4Ca^{2+}$/CaM) with Ca^{2+}/CaM-dependent protein kinase IV peptide (CaMKIVp) is characterized by biphasic kinetics[1], suggesting that the first event is the loss of two Ca^{2+} ions from the N-terminal lobe, followed the loss of two Ca^{2+} ions from the C-terminal lobe [2].

In the present work we have measured the dissociation kinetics of a complex of $2Ca^{2+}$ /CaM with CaMKIVp. The result obtained is compared with that calculated from an equimolar mixture of $4Ca^{2+}$/CaM/CaMKIVp and $0Ca^{2+}$/CaM/CaMKIVp. We confirm again that the rate of the slowest step is determined by the contribution of a kinetic relaxation mechanism involving the intermediate species, which have been previously suggested [2].

Materials and Methods

A 19-residue peptide having the sequence (CaMKIVp: RRKLKAAVKAVVASSRLGS) and recombinant CaM were used. Stopped-flow experiments were performed using an instrument for SAXS with a stopped-flow apparatus (Unisoku Co.Ltd) at BL10C of PF.

Results and Discussion

The result in Fig. 1 indicates that the molecular weight of the CaM-peptide complex does not change during the dissociation process, suggesting that the peptide binds to CaM even in the absence of Ca^{2+}, which is supported by a recent report [3]. Furthermore, the dissociation pathway is characterized by monophasic kinetics as shown in Fig. 2, in which the result corresponds to the loss of two Ca^{2+} ions from the C-terminal lobe. The experimental value of Rg at t=0 is 19.7A, while the calculated value under the condition in which $4Ca^{2+}$/CaM/CaMKIVp (Rg=17.6A) and $0Ca^{2+}$/CaM/CaMKIVp (Rg=20.3A) equally exist, is 19.1A. The significant difference indicates the existence of the intermediate species and supports the contribution of a kinetic relaxation mechanism involving them. From the Rg value, it is suggested that the conformation of $2Ca^{2+}$/CaM/CaMKIVp is a dumbbell-like structure similar to $4Ca^{2+}$/CaM/CaMKIV.

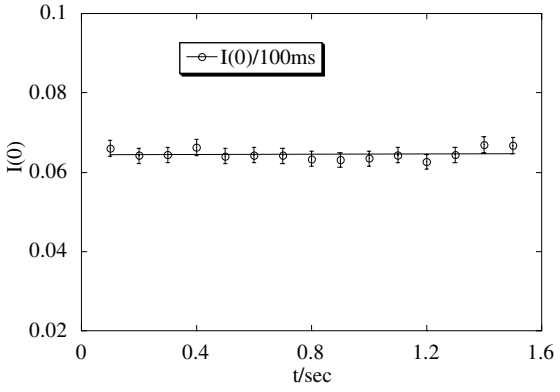

Fig. 1 Time course of the forward scattering amplitude I(0) for the dissociation of $2Ca^{2+}$/CaM/CaMKIVp complex.

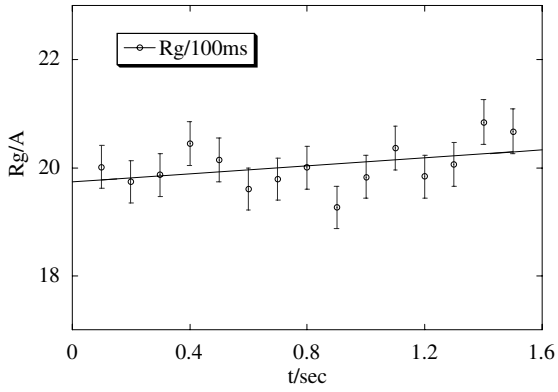

Fig. 2 Time course of the radius of gyration Rg for the dissociation of $2Ca^{2+}$/CaM/CaMKIVp complex.

References

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