Characterization of transient intermediates of a calmodulin- peptide complex

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Introduction

In the present work we have measured the dissociation kinetics of a Ca^{2+}-saturated calmodulin-peptide complex. The peptide studied is the Ca^{2+}-calmodulin dependent protein kinase IV calmodulin target-site. The effects of Ca^{2+} removal with a chelator were monitored using a SR-SAXS stopped-flow measurement. The present result allows the characterization of transient intermediates for the dissociation of $4Ca^{2+}$-calmodulin ($4Ca^{2+}$-CaM)-peptide complex. The result obtained is compared with other studies on the dissociation of calmodulin-peptide complexes. We confirm that the rate of the slowest step is determined by the contribution of a kinetic relaxation mechanism involving the intermediate species $2Ca^{2+}$-CaM-peptide, with two Ca^{2+} ions bound in the C-terminal domain, which has been previously suggested [1].

Materials and Methods

A 19-residue peptide having the sequence (RRKLKAAVKAVVASSRLGS; CaMKIVp) and bovine brain CaM were used. Stopped-flow experiments were performed using an instrument for SAXS with a stopped-flow apparatus (Unisoku Co ltd).

Results and Discussion

The changes in SR-SAXS profiles on mixing of CaMKIVp with $4Ca^{2+}$-CaM (at 25°C) were almost complete within the instrument deadtime, indicating a bimolecular association was complete within a few msec. We therefore studied the dissociation process of $4Ca^{2+}$-CaM-peptide complex (C^+). The results in Fig. 1 indicate 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 result[2]. Furthermore, the dissociation pathway is characterized by biphasic kinetics as shown in Fig. 2. 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. The intermediate species (I^+) with two Ca^{2+} ions in the C-domain is observed within the first 50-100 msec of the dissociation pathway. A subsequent activated state (A*) is observed at about 250 msecof the dissociation pathway. Final event is a conformational change in CaM-peptide complex (A^+).

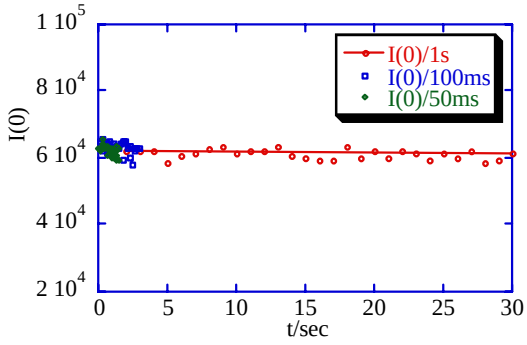

Fig. 1 Time cource of the forward scattering amplitude I(0) for the dissociation of $4Ca^{2+}$-CaM-peptide complex.

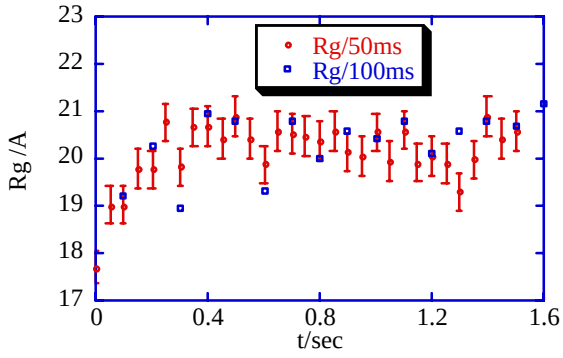

Fig. 2 Time cource of the radius of gyration of gyration Rg for the dissociation of $4Ca^{2+}$-CaM-peptide complex.

References

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