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 Ca²⁺-saturated calmodulin-peptide complex. The peptide studied is the Ca²⁺-calmodulin dependent protein kinase IV calmodulin target-site. The effects of Ca²⁺ 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 4Ca²⁺-calmodulin (4Ca²⁺-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 2Ca²⁺-CaM-peptide, with two Ca²⁺ 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 4Ca²⁺-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 4Ca²⁺-CaM-peptide complex (C¹). 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 Ca²⁺, 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 Ca²⁺ ions from the N-terminal lobe, followed the loss of two Ca²⁺ ions from the C-terminal lobe. The intermediate species (I) with two Ca²⁺ 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 msec of the dissociation pathway. Final event is a conformational change in CaM-peptide complex (A⁺).

Fig. 1 Time course of the forward scattering amplitude I(0) for the dissociation of 4Ca²⁺-CaM-peptide complex.

Fig. 2 Time course of the radius of gyration, Rg for the dissociation of 4Ca²⁺-CaM-peptide complex.

References