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Interaction of calmodulin with the cytoplasmic domain of a transmembrane gp41 : A novel binding mode of calmodulin molecular recognition

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Introduction

The HIV-1 envelope gp160 comprises a surface gp120 and a transmembrane gp41. Both are required for viral entry in to the cell. The gp41 is organized into three major regions: an N-terminal ectodomain, a transmembrane segment, and a C-terninal cytoplasmic tail. Two calmodulin (CaM)-binding sites have been identified near the cytoplasmic tail of gp41. Because the CaM-binding sites structurally and functionally resemble natural cytolytic peptides, they are designated the lentivirus lytic peptides, LLP1 and LLP2.

Previous studies [1] reported that the potential to form a highly positive charged amphipathic helix is essential for both the membrane-perturbative and CaM-binding activities of LLP1 and that CaM-binding function is well reserved despite the sequence variation observed in nature. However, it is still unknown about what mode of molecular recognition can be applied to the present CaM-target recognition.

Experimental

The recombinant rat CaM was expressed and purified as described previously [2]. Seven LLP1s were chosen from ENV_HV1A2 (aa 836-855; A2), HV1B1 (aa 837-856; B1), and HV1H2 (aa 837-856: H2).

SR-SAXS has been employed to analyze the interaction of Ca^{2+}/CaM with a LLP1. A Ca^{2+}/CaM/RS20 complex was used as a standard sample.

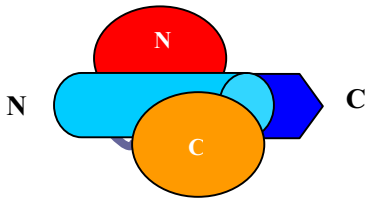
Results

All ratios of the molecular weight of a Ca^{2+}/CaM/LLP1complex to the calculated value are almost in the neighborhood of 1.0, indicating that Ca^{2+}/CaM binds each peptide at the molar ratio of 1:1. The value of A_2 for the Ca^{2+}/CaM/A2Δ3 (Δ represents a truncation of amino acid residues) complex is almost zero and that of B_{if} is negative, while these values for Ca^{2+}/CaM/A2Δ10 complex are positive, suggesting that the truncation at the C-terminus increases the solubility of the complex, if the sequence is truncated at a basic amino acid residue. The R_0 values for the Ca^{2+}/CaM/LLP1complexes take an intermediate value between 21.6 A for a dumbbell-shaped structure and 18.0 A for a compact globular structure, suggesting that these complexes adopt either a mixture of a dumbbell-shaped structure and a globular structure or a novel structure.

The data points in the Kratky plots are almost superimposed to those for the Ca^{2+}/CaM/RS20 complex, whose complex adopts a compact globular shape [3].

Discussion

The results for the SAXS measurements presented here show that Ca^{2+}/CaM binds each LLP1 at a molar ratio of 1:1, each complex adopts almost the same globular structure as that for the Ca^{2+}/CaM/RS20 complex, suggesting that each LLP1 adopts an alpha-helical structure in the complex. As the complex with HV1A2Δ3 adopts almost the same globular structure as that with HV1A2Δ10, the CaM-binding site is contained in the latter sequence. However, no CaM-binding mode in this sequence is retrieved using the CaM target database [4]. Moreover, Ca^{2+}/CaM binds a peptide with the opposite sequence and adopts almost the same globular structure as that for the Ca^{2+}/CaM/RS20 complex. At the two opposite outlets of the hydrophobic channel composed by two domains of CaM are clusters of acidic residues that are asymmetric in size [5]. The LLP1s that interacts with this channel possess complementary polarity created by a cluster of basic residues in the amino acid sequences. The basic cluster of RS20 is located at the N-terminal side of the CaM-binding region, while that of the LLP1s is located at the C-terminal side, as shown in Fig.1. Taken together, the results indicate that the location of a basic cluster within the CaM-binding region plays an important role in determining the direction of its binding with respect to CaM domains [5]. Furthermore, the binding mode of Ca^{2+}/CaM molecular recognition is well preserved despite of the sequence variation of three species of gp41 as shown in Fig. 1, suggesting that this region of the glycoprotein is important to viral replication.



HV1A2Δ10:DRVIEVAQRAYRAILHIHRR
HV1B1Δ10:DRVIEVVQGAYRAIRHIPRR
HV1H2Δ10:DRVIEVVQGACRAIRHIPRR

Fig. 1. Schematic model of Ca^{2+}/CaM/LLP1 complex: N and C-terminal domains of Ca^{2+}/CaM are shown in red and orange, respectively. N and C-terminal halves of the target peptide are shown in cyan and blue, respectively.

References

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