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Reconstruction of molecular shapes of the leukocyte-specific EF-hand protein, p65/L-plastin by the DAMMIN program

Hiroto SHINOMIYA*[1], Masaji SHINJO[2], Liu FENGZHI[1], Yoshihiro ASANO[1], Hiroshi KIHARA[2]
[1]Department of Immunology and Host Defenses, Ehime University School of Medicine, Ehime 791-0295, Japan
[2]Department of Physics, Kansai Medical University, Hirakata 573-1136, Japan

Introduction

Leukocytes play a crucial role in the first line of host defenses. In order for these cells to perform their functions, it is important that they can be recruited into infected tissues and be activated at the sites. In this respect, we have previously identified a 65-kDa protein (p65/L-plastin) that was phosphorylated in leukocytes by bacterial stimulation, finding that it had a series of two EF-hand Ca^{2+}-, a calmodulin-, and two β-actin-binding domains [1]. Interestingly, p65/L-plastin was demonstrated to contribute to the regulation of integrin-mediated leukocyte adhesion and activation [2]. We employed an X-ray scattering method to investigate their conformation and association in solution.

Material and Methods

Recombinant p65/L-plastin was prepared as described [3, 4]. All x-ray scattering experiments were done at BL-15A with the use of CCD detector.

Results

X-ray scattering experiments were performed with p65/L-plastin and mixture of p65/L-plastin with Ca^{2+}.

We calculate P(r) function, as shown in Fig 1.

They are similar in shape and have single peak respectively. So p65/L-plastin does not take a dumbbell-type shape like calmodulin, another famous EF hand protein. Ca^{2+} does not induce aggregation as is the case of grancalcin.

The peak position of p65/Lplastin with Ca^{2+} is slightly larger than p65/Lplastin, which suggests Ca^{2+} induces conformational change of the protein. It is also confirmed the change of radius of gyration in guinier analysis (data not shown).

DAMMIN program [5] was used to reconstruct molecular structure. Fig. 2 shows their shapes. Conformation changed in the presence of Ca^{2+}. The conformational change should have strong relevance to the p65/L-plastin's actin-bundle activity since the activity is greatly reduced in the presence of high concentration of Ca^{2+} [6].

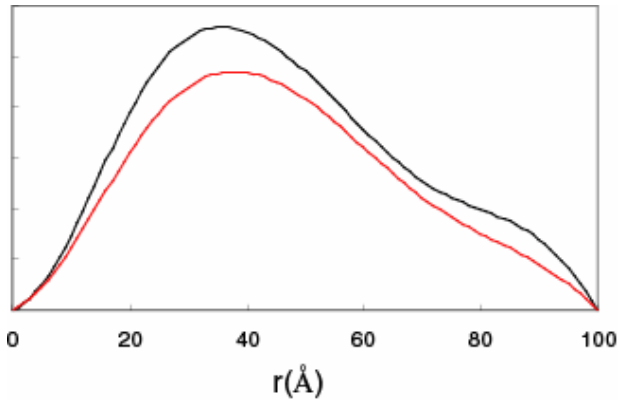
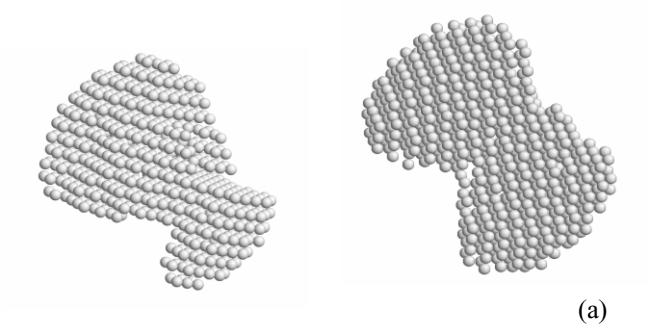


Fig. 1. P(r) function of p65/L-plastin (black) and p65/L-plastin + Ca^{2+}(red)



(a)

(b)

Fig .2. Calculated structure by DAMMIN
(a) p65/L-plastin (b) p65/L-plastin with Ca^{2+}

References

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*hiroto@m.ehime-u.ac.jp