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X-ray solution scattering study on the leukocyte-specific EF-hand proteins, p65/L-plastin and grancalcin

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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 have also identified grancalcin, a possible binding partner of p65/L-plastin in leukocytes, finding that it was a member of a new protein family named penta-EF-hand (PEF), which contains five repetitive EF-hand motifs [3]. Since the precise molecular nature of these two proteins remains to be elucidated, we employed an X-ray scattering method to investigate their conformation and association in solution.

Material and Methods

Recombinant p65/L-plastin and recombinant grancalcin (28-kDa) were prepared as described [3, 4]. Control proteins with similar molecular masses, bovine serum albumin (67-kDa) and bovine carbonic anhydrase (29-kDa), were purchased. All x-ray scattering experiments were done at BL-15A1 with the use of CCD detector.

Results

X-ray scattering experiments were performed with grancalcin, grancalcin + 1M urea, p65/L-plastin and mixture of grancalcin with p65/L-plastin. All measurements show more or less aggregates. Guinier anlalyses were done with the model of double exponential . Radius of gyration (Rg) of smaller components were 21.2 +/- 0.6A, 21.1 +/- 0.1A, 27.5 +/- 0.5 A, for grancalcin, grancalcin + 1M urea, and P65/L-plastin, respectively. In Fig. 1, P(r) functions are plotted. In case of grancalcin, P(r) function shows double peak with the peak position at 24A and 50A. In the presence of 1 M urea, P(r) shows a single peak with the peak position at 24 A. This suggests that the second peak is corresponding to the grancalcin dimer. In contrast, P(r) function of p65/L-plastin shows a single peak at 35A.

It is interesting to know if grancalcin and p65/L-plastin directly forms complex or not. P(r) function of the mixture of the two proteins show double peak, which might be in agreement with the complex formation. However, when P(r) from grancalcin is subtracted, the second peak disappeared and it is qualitatively similar with the P(r) of p65/L-plastin. These data suggest that the complex of grancalcin with p65/L-plastin is not formed.

References

[1] H. Shinomiya *et al*., J. Immunol. 154, 3471 (1995)
[2] S.L. Jones *et al*., Proc. Natl. Acad. Sci. 95 9331 (1998)
[3] F. Liu *et al*., Biosci. Biotechnol. Bichem. 68, 894 (2004)
[4] H. Shinomiya *et al*., Biosci. Biotechnol. Bichem. 67, 1368 (2003)

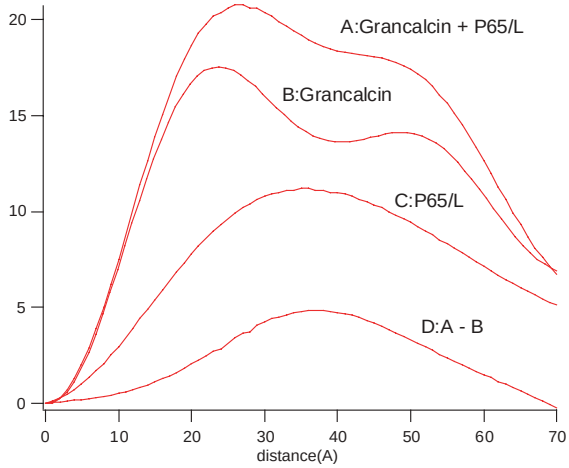

Figure 1. P(r) function of grancalcin, p65/L-plastin and the mixture of grancalcin + p65/L-plastin. Graph D means the subtraction of grancalcin from the mixture of the two proteins.

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