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Conformational analysis of the leukocyte-specific EF-hand protein, grancalcin in solution by X-ray scattering

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Introduction

Grancalcin, a leukocyte-specific protein, is member of a new protein family named penta-EF-hand (PEF), which contains five repetitive EF-hand motifs [1]. This family proteins are characterized by unique biological significance. So, grancalcin should also play a vital role in leukocyte functions. We employed an X-ray scattering method to investigate their conformation and association in solution.

Material and Methods

Recombinant grancalcin (28-kDa) was prepared as described [1,2]. All x-ray scattering experiments were done at BL-15A with the use of CCD detector.

All data show aggregation and we use two-component analysis in Guinier plot as below.

$$I(h) = I_1 \exp(-\frac{R_{g1}^2 h^2}{3}) + I_2 \exp(-\frac{R_{g2}^2 h^2}{3}) \qquad \text{(a)}$$

I is intensity, Rg is radius of gyration and h is scattering vector.

. In case of mixed solution of monomer and oligomers, the smaller Rg gives the Rg of the monomer, while the larger one gives the average Rg of oligomers, if they are well separated (unpublished data). Then we can estimate monomer component.

Results

X-ray scattering experiments were performed with grancalcin, grancalcin + 1M urea, and grancalcin + Ca^{2+}.

We calculate P(r) function from scattering intensity. They are shown in Fig 1.In grancalcin with 1M urea, monomer is the main component, but grancalcin shows aggregation and this property is strongly emphasized in the presence of Ca^{2+}.

Grancalcin shows some peaks at P(r) function. The first peak is coincident with monomer component and the second peak comes from distance between two centers of molecules as dimmer. If molecule is sphere it correspond to diameter. But this is in contradiction with crystallographic data as dimer [3] that looks like one globule and give only one peak at P(r) function. Molecular structure in solution was calculated by DAMMIN program [4]. Oligomers part was subtracted from scattering intensity by equation (a), then calculation was done about monomer component. Fig. 2 shows calculated image and crystal data. They are similar except for bottom part.

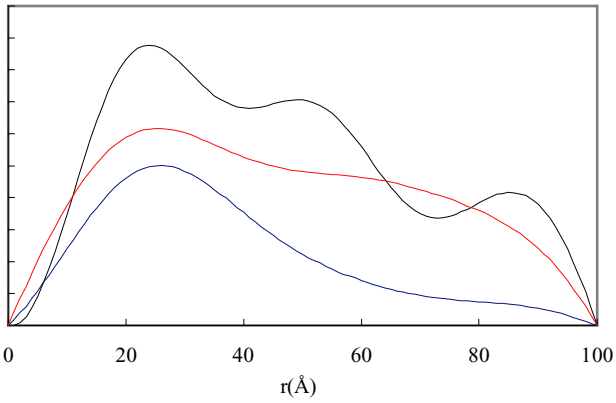
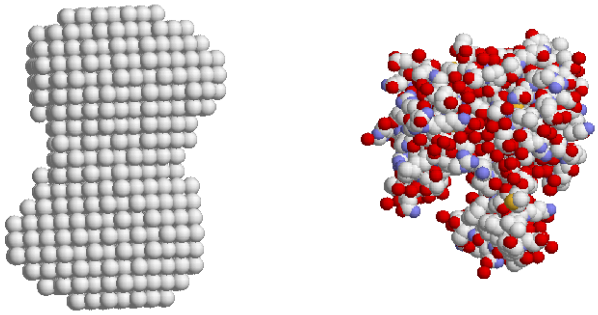


Fig.1.P(r) function. Black:grancalcin, Red:grancalcin with Ca^{2+}, Blue: grancalcin with 1M urea



(a) (b)

Fig.2. (a) calculated structure by DAMMIN
(b) crystal structure: PDB ID: 1k95

References

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