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단 보

A Study on the Three Dimensional Structure of Bowman-Birk Type Proteinase Isoinhibitor-CII Using Computer Aided Molecular Modeling

Yoongho Lim*, Mina Oh, Jiyoung Kim, and Su-il Kim[1]

Department of Agricultural Chemistry, Kon-Kuk University, Seoul 133-701,
[1]Department of Agricultural Chemistry, Seoul National University. Suwon 441-744, Korea
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Introduction

Proteinase inhibitors are found in various plants. Among those, especially, they are found in leguminous seeds.[1,2] Bowman-Birk Proteinase Inhibitor (BBPI) is a kind of serine proteinase inhibitors, and found in leguminous seeds. It is a single polypeptide which is consisted of 71 amino acid residues, including 7 disulfide bonds. That is, sulfur amino acids account for the ratio of 20%. The molecular weight of BBPI is 6-9 kDa.[3] Its crystallographic structure has been known. BBPI has two independent active sites which inhibit trypsin and chymotrypsin simultaneously. Several BBPI-like isoinhibitors were found. Those are double-headed inhibitors, and have 7 disulfide bonds too, and are named A, B, C-II, D-II, E-I. Among those, the primary sequences of CII and DII have been known.[4] C-II and BBPI show the homology % of 66.2 in their residues. Because three dimensional crystallographic structure of BBPI has been known, three dimensional structure of C-II can be predicted by homology modeling. In this study, the crystallographic structure of BBPI was used for a template structure to determine the structure of C-II by Homology modeling. From the sequence homology, highly conserved residues can be identified, and the conformation of non-conserved residues can be determined by molecular dynamics.

Computational methods.

The three dimensional structure of BBPI obtained from Brookheaven Protein Data Bank (PDB) was used for a template structure. The arbitrary structure based on its primary sequence of C-II was built by Viewer module of InsightII, and the primary sequence was extracted using Homology module of InsightII. Next, the structurally conserved regions (SCRs) were simulated. The methods used for the automatic determination of SCRs are as follows[5];

① A Cα distance matrix is made for a comparison of three dimensional structures of two proteins.

② A probe from the distance matrix of the template is overlaid onto that of the target.

③ Until a minimum value of RMS(Root Mean Squares) difference of the corresponding matrix is found, the probe is moved down the diagonal of the matrix of the target repeatedly.

④ When the RMS difference of the off-diagonal blocks is less than the threshold of the target, the two pairs of the template and the target are considered to be similar structurally.

In order to align the sequence of CII with that of BBPI, a few gaps were inserted in BBPI. The non-SCRs and the side chain were built. After that, the structure of C-II was refined with Discover module of InsightII, where the consistent-valence forcefield (CVFF) as used and the calculation was carried out for 500ps. The analytic form of the energy expression used in CVFF is given in Eq. [1].[6]

$$E_{pot} = \Sigma D_b[1-\exp(-\alpha(b-b_0))] \ (1) + \Sigma H_\theta(\theta-\theta_0)^2 \ (2) + \Sigma H_\phi [1 + \mathrm{scos}(n\phi)] \ (3) + Ha_x x^2 \ (4) + \Sigma\Sigma F_{bb'}(b - b_0)(b' - b'_0) \ (5) + \Sigma\Sigma F_{\theta\theta'}(\theta - \theta_0)(\theta' - \theta'_0) \ (6) + \Sigma\Sigma F_{b\theta}(b - b_0)(\theta - \theta_0) \ (7) + \Sigma F_{\phi\theta\theta'}\cos\phi \ (\theta - \theta_0)(\theta' - \theta'_0) \ (8) + \Sigma\Sigma F_{xx'}xx' \ (9) + \Sigma\varepsilon[(r^*/r)^{12}-2(r^*/r)^6] \ (10) + \Sigma q_i q_j/\varepsilon r_{ij} \ (11)$$ ----------Eq. [1]

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*Corresponding author

Where the terms are expressed as follows;

1 : bond length
2 : bond angle
3 : torsion angle
4 : out-of-plane interaction
5-9 : couplings between deformations of internal coordinates
10-11 : nonbond interactions.

During the calculation, any other constraints except 7 disulfide bonds were not given.

Results and Discussions

C-II is consisted of 76 amino acid residues. A comparison of the primary sequence of BBPI and C-II is shown in Fig. 1. There are found several SCRs between two inhibitors, which are residues 1-10, 14-15, 19-22, 28-36, 48-49, 55-59, and 61-63. As shown in Fig. 2, the crystallographic structure of BBPI is separated in two similar regions which have 3 loops individually. Seven disulfide bonds are Cys8-Cys62, Cys9-Cys24, Cys12-Cys 58, Cys14-Cys22, Cys32-Cys39, Cys36-Cys5, and Cys41-Cys49. Double headed active sites are known Lys16-Ser 17 and Leu43-Ser44. Since Asp1-Asp10 of BBPI is in accord with that of C-II, 6 gaps are inserted in the beginning of BBPI. C-II contains seven disulfide bonds too, and their residue numbers are the same. Constrained energy minimization is applied to obtain the refined srtucture of C-II. An entire comparison of the carbon backbone gives the RMS value of 0.04A. Among four residues contained in the active sites, Ser17 and Ser44 of BBPI are the same as those of C-II. But Leu43 of BBPI is replaced by Arg43 of C-II, and Lys16 of BBPI, by Ala 16 of C-II. The methyl group of Ala16 of C-II is aligned to Cβ of Lys16 of BBPI. As a result, the first active site is overlapped well. Cβ, Cγ, and Cδ of Arg43 of C-II are overlaid on Cβ, Cγ, and Cδ of Leu43 of BBPI, respectively. Therefore, the second active site is overlapped too. In the case of Arg23 of BBPI and His23 of C-II, an interesting partial structure is observed as shown in Fig. 3. Cα, Cβ and Cγ of Arg23 in the template are overlaid onto those of His23 in the target well. Likewise, Tyr45 of BBPI and Met45 of C-II are overlapped well and its partial structure is shown in Fig. 4. Phe50 of BBPI and Arg50 of C-II, and Pro64 of BBPI and Ser64 of C-II show same results as shown in Figs. 5 and 6, respec-

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                (1)        (10)       (20)       (30)       (40)
BBPI :          DDESSKPCCDQCACTKSNPPQCRCSDMRLNSCHSACKSCI
CII  : SDHSSSDDESSKPCCDLCMCTASMPPQCHCADIRLNSCHSACDRCA

                (50)       (60)       (70)
BBPI : CALSYPAQCFCVDITDFCYEPCKPSEDDKEN
CII  : CTRSMPGQCRCLDTTDFCYKPCKSSDEDDD
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Fig. 1. A comparison of the primary sequence of BBPI and that of C-II.

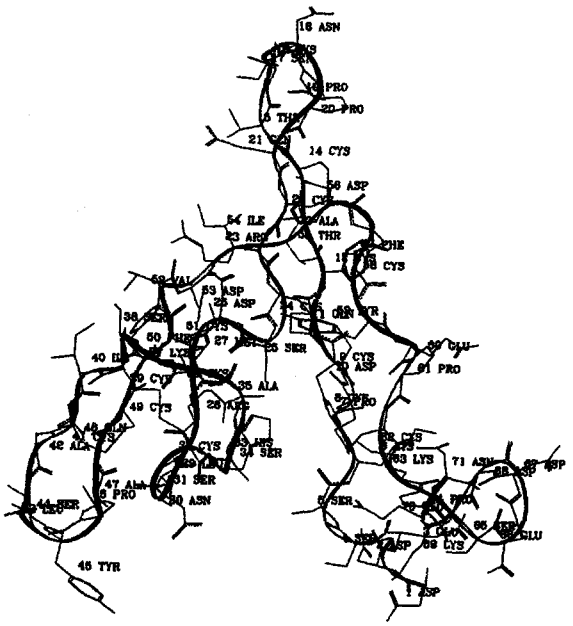

Fig. 2. Crystallographic structure of BBPI.

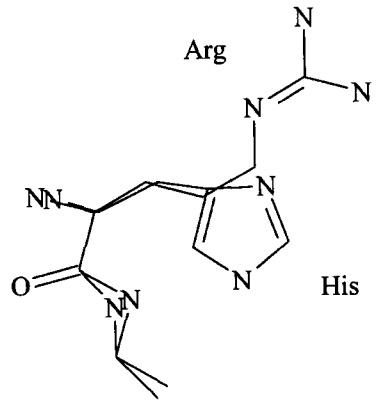

Fig. 3. A partial structure of overlapped Arg 23 of BBPI and His 23 of CII.

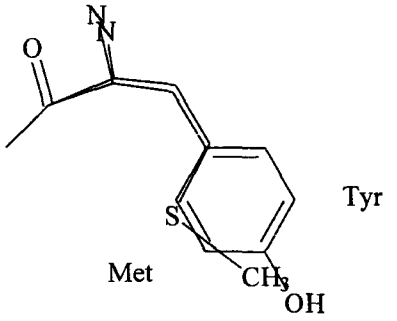

Fig. 4. A partial structure of overlapped Tyr 45 of BBPI and Met 45 of CII.

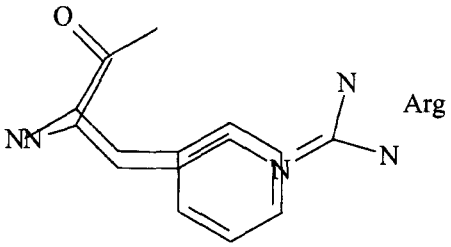

Fig. 5. A partial structure of overlapped Phe 50 of BBPI and Arg 50 of CII.

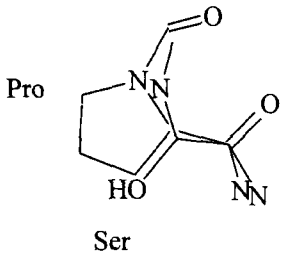

Fig. 6. A partial structure of overlapped Pro 64 of BBPI and Ser 64 of CII.

tively.

Since the method used in this experiment can give information of the regions of the target overlaid onto those of the template, the structure of the N-terminal of C-II which are not contained in BBPI, SDHSSS, can not be determined.

Consequently, entire refined structures of C-II and BBPI are overlapped very well. Because the structures of two active sites are conserved, the activity of C-II is expected to be almost same as that of BBPI. The refined structure of C-II compared with BBPI is shown in Fig. 7.

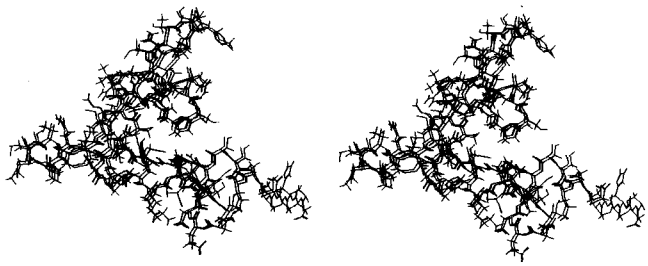

Fig. 7. A comparison of the refined structure of C-II and BBPI.

Acknowledgements

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컴퓨터분자설계를 이용한 BBPI-CII의 3차 구조 연구

임융호* · 오민아 · 김지영 · 김수일[1](건국대학교 농화학과, [1]서울대학교 농화학과)

찾는말 : BBPI, homology, CII

* 연락저자