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Crystal structure of hevein at 2.8 Å resolution

A. Rodríguez-Romero, K.G Ravichandran* and M. Soriano-García

Departamento de Química, Universidad Autónoma Metropolitana Iztapalapa, Iztapalapa, D F 09340, México and *Instituto de Química, Universidad Nacional Autónoma de Mexico, Circuito Exterior, Ciudad Universitaria, Coyoacan D F 04510, México*

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The three-dimensional structure of hevein, a low molecular weight protein isolated from the latex of *Hevea brasiliensis*, has been determined by X-ray diffraction at 2 8 Å resolution The protein crystallizes in space group $P2_12_12_1$, with lattice constants a=21 78, b=31 86, c=51 12 Å The structure was solved by molecular replacement methods using the domain C of wheat germ agglutinin (WGA) as search model The positions and individual isotropic temperature factors of the 324 atoms have been refined by the Hendrickson and Konnert restrained refinement procedure While tight restraints have been maintained on the bonded distances and angles, the R-factor has dropped to 24 1% and an averaged B value of 9 5 Å^2, using 78% (802) of the total possible number of reflections in the resolution range 5–2 8 Å The tertiary structure is very similar to that of domain C of WGA from residues 3–31

Hevein, Crystal structure, Agglutinin, *Hevea brasiliensis*

1 INTRODUCTION

Hevein is a small, four-disulfide protein present in the bottom fraction of ultracentrifuged rubber-tree (*Hevea brasiliensis*) latex, whose physiological role is still unknown The amino acid sequence in its 43-residue polypeptide chain has been reported [1] In terms of primary structure, hevein shows 56% sequence identity to the C domain of wheat germ agglutinin (WGA) [2], for which an accurately refined crystal structure is available [3]. Recent circular dichroism studies support the idea that the polypeptide conformations of WGA and hevein are closely related [4], in addition, on the basis of these studies it has been suggested that some common spectral characteristics of the two proteins are a reflection of a similar arrangement of disulfide bonds Therefore, hevein and each of the four domains of WGA are expected to have a similar structure This paper describes the crystallization, structure determination and refinement of hevein at 2.8 Å, and compares its structure with that of WGA. Furthermore, the hemagglutinating activity of hevein was also investigated

2. MATERIALS AND METHODS

2 1 *Crystallization*

The protein was purified from the bottom fraction of *H brasiliensis* latex (clone GV-31) as previously described [5] Crystals of hevein were grown using the hanging drop method as reported before [6] Samples (12 μl) containing 10 5 mg/ml protein were mixed with 12 μl of 60% 2-methyl-2,4-pentanediol (MPD), 0 01 M $CaCl_2$ buffered with 40 mM Tris, pH 8 06 Each droplet was equilibrated against 500 μl of 60% MPD, 0 15 M NaCl in the same buffer Crystals (0 6 × 0 1 × 0 08 mm) appeared within 2–3 weeks at 6°C The crystals are orthorhombic, space group $P2_12_12_1$, a=21 78, b=31 86, c=51 12 Å, one molecule per asymmetric unit with a protein fraction of 64%

2 2 *Data collection and processing*

For intensity data collection a crystal was mounted and sealed in 0 7 mm glass capillary with a plug of well solution The crystal was placed on the goniostat of a Nicolet P3/F four-circle diffractometer, using a graphite monochromator with CuKα radiation at 2000 W (40 mA) power, and optically centered Crystals of hevein are orthorhombic, $P2_12_12_1$, only 1/8 of the diffraction data is unique An octant of intensity data was measured at 2 8 Å resolution (1022 reflections observed of 1033, 98%) by using a Wyckoff step-scan procedure and techniques described elsewhere [7] An empirical absorption correction was applied to the intensity data based on the measurement of transmission curves near χ=90° [8] Decay correction was not applied and twice the absolute value of the average of the negative intensities (averaged-background-corrected intensities) was used as the observable limit for data reduction

2 3 *Structure determination and refinement*

The structure of hevein was solved by molecular replacement rotation–translation method [9] using the structure of the domain C of WGA as the search model The atomic coordinates of WGA, refined at 1 8 Å resolution [2], are available from the Brookhaven Protein Data Bank [10] The main chain and 24 conserved side chain positions of WGA (domain C) were employed in the calculations (252 atoms, 78%) (Fig 1) The rotation search was carried out using the PROTEIN package of programs [11] The model was placed in a triclinic cell with orthogonal axes of length 48 Å Triclinic structure factors were calculated from 8 0 to 3 0 Å resolution and an overall temperature factor of 15 Å^2 A 3 0 Å model Patterson map was calculated and only the largest 1618 vectors within a radial shell of 3–12 Å were chosen for the rotation search The crystal Patterson synthesis was calculated using all reflections from 8 0 to 3 0 Å The product corre-

Present address Howard Hughes Medical Institute, Dallas TX 75235, USA

Correspondence address A Rodriguez-Romero, Instituto de Quimica, Universidad Nacional Autonoma de Mexico, Circuito Exterior, Ciudad Universitaria, Coyoacán 04510, México D F Fax (525) 548-5448

lation of the crystal and the model Patterson maps was calculated as a function of the orientation angles θ_1, θ_2, θ_3 (Eulerian angles) in steps of 5° in the angle range θ_1=0–180°, θ_2=0–180°, θ_3=0–180°, and subsequently in smaller steps of 1° and finally in 0 5° steps The highest correlation of each search appeared consistently at the same set of rotation angles The rotation angles of the best solution for hevein were 70 0°, 105 5°, 194 0° and coresponded to a peak height of 16 0 (4 7σ above background) The next highest peak in the same solution is 4 2σ A translation vector search was carried out using the program BRUTE [12] for the three highest rotation solutions, using data from 5 0–3 0 Å, 6 0–3 0 Å, 5 0–2 8 Å and 6 0–2 8 Å resolution The best solutions had values ranging between 6σ and 8σ, corresponding to correlation coefficients of 0 30–0 36 As a single solution was not obvious from the translation sea • results, the possible correctness of a number of highest solutions, from each rotation solution, was determined by examination of the resulting Cα-structure packing using an Evans and Sutherland PS 390 interactive graphics system with FRODO [13] software Three translation possibilities from the best solution and two of the second and third best solution were examined The interpenetration and short contacts in molecular packing of hevein molecules in the crystal easily eliminated all but one solution The first translation solution from 5 0 to 3 0 Å of the best rotation solution shows a tight and regular packing of molecules in the crystal The rotation angles and translation solution were then refined simultaneously in 0 3° and 0 1 Å increments The best solution of the rotation/translation refinements gave a correlation coefficient of 0 44 corresponding to Eulerian angles of 68 5° 107 2° 193 3° with a translation vector of 13 5 Å, 2 8 Å, 20 1 Å A ($2|F_o|-|F_c|$) map, based on these rotation/translation results, revealed most of the hevein structure (residues 1–31) The region of the structure from residues 32–43 proved to have a number of breaks in the density and a definite path for this region was not clear Consequently, it was not considered in the structure factor calculations at the start of the refinement

The structure of hevein was refined at 3 0 Å resolution using restrained least-squares methods with the program PROLSQ [14] and PROFFT [15] for 34 cycles The R factor dropped from 51 9% to 34 5% At this stage the $2F_o-F_c$ and F_o-F_c maps were inspected using FRODO, and it was possible to fit residues 32–37 into the electron density A further 40 cycles of least-squares minimization were performed with the use of an average overall thermal parameter and the R factor dropped to 30% Graphic refitting of the model structure at this stage revealed residues 38–43 An additional 35 cycles of refinement, using data from 5–2 8 Å and individual temperature factors for side and main chain atoms for each residue, resulted in the current R factor of 24 1% and an average B-value of 9 5 Å^2 The root-mean square (r m s) deviations of the structure from the ideality are 0 020 Å for covalent bond distances, 0 053 Å for the interbond angle distances, 0 015 Å for the planar groups and 2 4° for the peptide bond torsional angles

2 3 *Hemagglutination tests*

A 2% suspension of native erythrocytes of the different human bloodgroups AOB was used to determine the hemagglutination capacity of hevein, according to the serial dilution procedure in microtiter plates [16] Specificity was determined by mixing the protein solution with various carbohydrates (0 1 M), and then testing their agglutination activity as above

3. RESULTS AND DISCUSSION

3 1 *Structure determination*

The three-dimensional folding of the hevein polypeptide chain is shown in Fig 1A. The structure is folded into a series of loops all linked together by four disulfide bonds and similar to the domain C of WGA (Fig. 1B) A least-squares superposition of the structurally equivalent α-carbon atoms of hevein and domain C of WGA

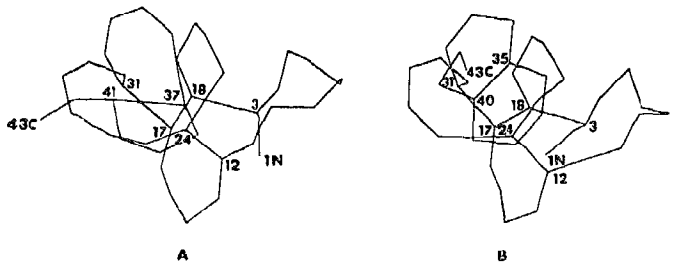

Fig 1 Schematic representation of the α-carbon backbone and disulfide bonds of hevein (A) and domain C of WGA (B) The disulfide bonds are represented by double lines

gives at this stage of refinement an r m s deviation of 1.2 Å However, the r m s difference in the tightly folded core region (residues 3–37) is only 0 78 Å

Three out of the four disulfide bonds (Cys^3–Cys^{18}, Cys^{12}–Cys^{24} and Cys^{17}–Cys^{31}) occupy analogous positions in the two structures The disulfide bridges (Cys^3–Cys^{18} and Cys^{12}–Cys^{24}, Cys^{17}–Cys^{31} and Cys^{37}–Cys^{41}) are nearly perpendicular to one another, with a distance between disulfide midpoints of 4 76 Å and 4 8 Å, respectively. As a result of these close disulfide contacts, the different loops tend to extend away from each other. A solvent accessibility calculation [17] indicates essentially zero accessibility for all disulfides of hevein, with the exception of Cys^{41} which showed 14 and 19% for side chain and total, respectively

The stereo drawing shown in Fig 2 depicts the complete hevein structure. Due to its small size and its lack of a hydrophobic core, the three aromatic residues of this molecule (Trp^{21}, Trp^{23}, Tyr^{30}) and Pro^{33} are all exposed to solvent Of the charged side-chains, all glutamic acids, lysines and two aspartic acids (Asp^{28}, Asp^{43}) are extended into solvent, while Arg^5 and Asp^{34} form intramolecular hydrogen bonds Two serine (Ser^{26}, Ser^{32}), a threonine (Thr^{27}) and a histidine (His^{15}) residues are relatively exposed to the solvent

3 2. *Hemagglutination tests*

It was found that hevein agglutinates human erythrocytes of blood group A In a preliminary attempt to identify which sugars inhibited this activity, we used *N*-acetyl-D-glucosamine, *N*-acetyl-D-galactosamine and galactose, observing that the main inhibition was produced with *N*-acetyl-D-glucosamine It is already known that WGA binds specifically to oligosaccharides such as sialic acid (*N*-acetylneuraminic acid) and chitin (*N*-acetyl-D-glucosamine) [18] and that there are two unique binding sites [3]. In these sites there are involved two tyrosyl side chains (Tyr^{73}, Tyr^{159}) which assume precise orientations for optimum hydrophobic contact with the *N*-acetyl methyl groups of the sugar ligand, also involved are some side-chains that form hydrogen bonds (Ser^{62}, Glu^{115}, Ser^{148} and Asp^{29}). The specific orientation of the three aromatic residues in hevein and also the presence of Asp^{28} and Asp^{34} in the same region,

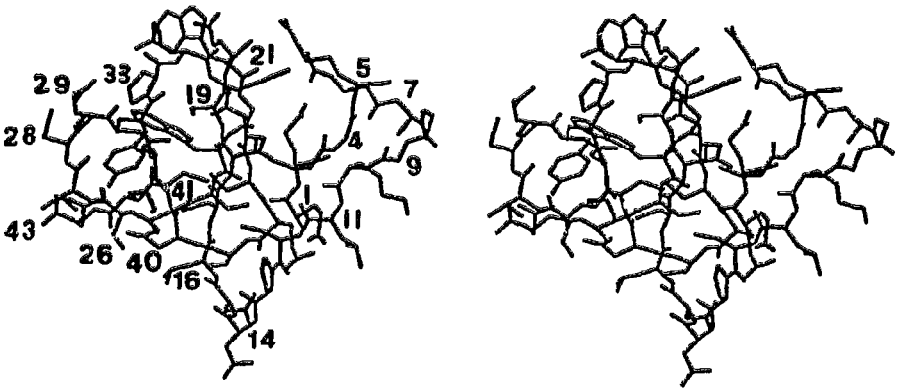

Fig 2 Stereoview of the complete tertiary structure of hevein Selected residues are numbered for guidance

seem to indicate that the sugar might bind at this site The fact that this small protein has the ability to bind sugars makes it a candidate for research in cancer diagnostic work and/or chemotherapy

A high resolution analsys of hevein is presently in progress, and that should enable one to make a detailed comparison between this protein and WGA

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