Crystal Structures of Hydrophobin HFBII in the Presence of Detergent Implicate the Formation of Fibrils and Monolayer Films

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Hydrophobins are small, amphiphilic proteins secreted by filamentous fungi. Their functionality arises from a patch of hydrophobic residues on the protein surface. Spontaneous self-assembly of hydrophobins leads to the formation of an amphiphilic layer that remarkably reduces the surface tension of water. We have determined by x-ray diffraction two new crystal structures of Trichoderma reesei hydrophobin HFBII in the presence of a detergent. The monoclinic crystal structure (2.2 Å resolution, R = 22, Rfree = 28) is composed of layers of hydrophobin molecules where the hydrophobic surface areas of the molecules are aligned within the layer. Viewed perpendicular to the aligned hydrophobic surface areas, the molecules in the layer pack together to form six-membered rings, thus leaving small pores in the layer. Similar packing has been observed in the atomic force microscopy images of the self-assembled layers of class II hydrophobin, indicating that the crystal structure resembles that of natural hydrophobin film. The orthorhombic crystal structure (1.0 Å resolution, R = 13, Rfree = 15) is composed of fiber-like arrays of protein molecules. Rodlet structures have been observed on amphiphilic layers formed by class I hydrophobins; fibrils of class II hydrophobins appear by vigorous shaking. We propose that the structure of the fibrils and/or rodlets is similar to that observed in the crystal structure.

Filamentous fungi secrete small proteins, hydrophobins, that have a unique ability to spontaneously form amphiphilic layers on hydrophobic-hydrophilic interfaces (1). Hydrophobins are distributed in two classes according to the pattern of hydrophobic and hydrophilic amino acid residues in the protein sequence and the dissociation resistance of the assembled amphiphilic layers (2). The pioneering work (3) with the class I hydrophobin SC3 from Schizophyllum commune revealed the dual role served by this protein in the growth of the fungal hypha: hydrophobins reduce the surface tension of water and provide a protective coating on the hyphal surface, once exposed to air.

Hydrophobins are an attractive target of study due to their vast application potential provided by their unique characteristics (4). Examples include anti-fouling applications, coatings that increase biocompatibility in medical instruments, coatings in drug delivery, surface patterning, use in products of personal care as emulsifiers, use in immobilization techniques, and as very potent foam-forming agents (5, 6). In addition, protein purification of hydrophobin fusion proteins in aqueous two-phase systems has been demonstrated (7).

So that we understand the mechanism of the function of hydrophobins, the structural information of these proteins is crucial. The first hydrophobin structure was determined by x-ray diffraction in 2004 (PDB codes 1R2M, 2B97) (8, 9), describing a class II hydrophobin HFBII from Trichoderma reesei. Now, the structure of a class I hydrophobin EAS from Neurospora crassa has been determined by the NMR method (PDB code 2FMC) (10). Class I and II hydrophobins, on the basis of determined structures, seem to share a common fold with a small β-barrel forming the core structure and being reinforced by four disulfide bridges formed by the eight conserved cysteine residues that are characteristic of all hydrophobin sequences.

The disulfide bridges form in the same, non-consecutive order (Cys-1-Cys-6, Cys-2-Cys-5, Cys-3-Cys-4, Cys-7-Cys-8) in both class I and II structures and align perfectly when the structures are superimposed. The main differences between class I and II hydrophobins are that the class I structure does not contain an α-helix and does contain two large disordered regions.

The functional site of class II hydrophobins has been assigned to the hydrophobic area in the molecular surface, composed of hydrophobic side chains of 13 amino acid residues (8). The hydrophobic patch covers ~18% of the total surface area, and contributing residues are mainly located between the third and the fourth and the seventh and the eighth cysteines in the protein sequence (supplemental data). Unfortunately, this is also the area in which the disordered loops occur in the class I EAS structure (Met-22-Ser-42 and Val-65-Phe-72), and therefore it is difficult to interpret whether the functional site is the same in both classes. However, the disordered regions include many hydrophobic residues, indicating a similar function. In addition, the sequences of class I hydrophobins contain more residues in these areas in comparison to class II hydrophobins.

The sequence comparison also reveals that the segment between the fourth and the fifth cysteines in class I hydropho-
bins is shorter than in class II. This is the location of the α-helix in class II structures and indicates that the lack of the α-helix is a common feature for class I hydrophobins. The place occupied by the α-helix in the class II structure is taken up by two short pieces of β-strands, one of which originates from the N terminus, in class I structure.

Based on the x-ray structure of HFBII and the x-ray structures of native and variant forms of HFBI (PDB codes 2FZ6, 2GVVM) (11), which is also a class II hydrophobin from T. reesei, we have previously proposed that oligomerization in solution is an important property of hydrophobins. Hydrophobins seem to oligomerize via the hydrophobic surface areas in order to conceal the hydrophobic areas from the solvent. This also explains why hydrophobins are readily soluble in water. The solution behavior of HFBI and HFBII has been studied with the use of Förster resonance energy transfer and size exclusion chromatography (12) with the conclusion that the preferred oligomerization state is a tetramer, while monomers and dimers are also observed. Crystallographic results show dimeric and tetrameric structures and the tetrameric structure indicates that the tetramer is composed of two dimers (11). Similar results related to oligomerization have been reported for class I hydrophobin SC3 from S. commune (13), for which the primary oligomerization state was determined to be a dimer. However, the experiments with SC3 were conducted at lower protein concentrations in comparison to the experiments with HFBI and HFBII, while the oligomerization process is also thought to be a concentration-dependent phenomenon.

The natural function of hydrophobins is to form amphiphilic layers on the hydrophobic-hydrophilic interfaces. How these layers are formed from the building blocks that originate from the solution is the key problem we wish to examine here. Another matter that has received a vast amount of attention is the formation of a rodlet structure on the hydrophilic surface of the assembled layers of class I, but not class II, hydrophobins. These rodlets have been identified as amyloidal, because they are stained by the amyloid-specific dyes Congo Red and thioflavin and the appearance of the rodlet resembles that of amyloid fibrils (14). Even though the rodlet formation is specific to class I hydrophobins only, fibrils are also formed by class II hydrophobins (15). In this report, we describe a fiber structure, possibly similar to fibrils and/or rodlets, observed in the new, orthorhombic crystal form of HFBII.

**MATERIALS AND METHODS**

**Crystalization**—The protein was produced and purified as described previously (16). The protein was crystallized at room temperature using the hanging drop vapor diffusion method. The crystallization solution contained 0.1 M lithium sulfate, 20% polyethylene glycol (MW 2000), and 0.1 M Tris buffer at pH 8.5. The pH of the crystallization solution was 8.48 and 6.91 for the crystals of space groups P2₁ and P2₁2₁2₁, respectively. The crystallization drops contained 4 μl of crystallization solution, 1 μl of 300 mM detergent (heptyl-β-D-thioglycoside), and 5 μl of hydrophobin, dissolved in pure water at a concentration of 8 mg/ml. Streak seeding was used to produce the monoclinic crystals.

| Crystal form | Monoclinic | Orthorhombic |
|--------------|------------|--------------|
| a (Å)        | 61.08      | 25.3         |
| b (Å)        | 66.41      | 57.1         |
| c (Å)        | 79.77      | 72.1         |
| β (°)        | 99.23      | 90.0         |
| Space group  | P2₁        | P2₁2₁2₁      |
| Source       | 4F4-E5. ESRF | X12, EM4      |
| Wavelength (Å)| 0.931      | 0.900        |
| Resolution range (Å)| 20–2.2 (2.5–2.2) | 20–1.0 (1.2–1.0) |
| No. of observations | 145265      | 355555       |
| No. of unique reflections | 61949 (19671) | 107245 (44537) |
| Completeness (%) | 98.6 (98.4) | 98.3 (96.9) |
| R-factor (%) | 11.9 (40.2) | 12.0 (30.9)  |
| I/σ(I)       | 7.70 (3.10) | 8.82 (3.89)  |
| R (°)        | 22.4       | 12.5         |
| Rsym (%)     | 28.0       | 15.2         |
| Rmsd bond length (Å) | 0.995      | 0.014        |
| Rmsd bond angle (°) | 1.363      | 2.289        |
| No. protein atoms | 4033       | 963          |
| No. of water molecules | 521        | 149          |
| No. of other atoms | 57         | 24           |
| Average B-factor (Å²) | 33.3       | 10.2         |

**RESULTS**

**The Monoclinic Crystal Form of HFBII**—The structure of the monoclinic crystal form has been deposited with the PDB under code 2PL6. The structure included eight protein molecules, polypeptide chains assigned from A to H, each consisting of 71 amino acid residues, three detergent molecules (heptyl-β-D-thioglycoside), and 521 molecules of water. Six residues...
The structures of individual hydrophobin molecules were very similar to those that have been previously described (8). However, a structural change was identified in some of the molecules at the area of Ala-55-Ala-61 (Ala-60-Ala-66 in the HFBII sequence). Here, the position of the main chain in the middle of this area differs by 7–11 Å from that typically observed. Molecules B, D, F, and G of the monoclinic HFBII structure harbor this structural change. In all of the molecules A-H, the corresponding area was slightly disordered and the temperature factors were higher than elsewhere in the structure.

Fig. 1 shows the area of Ala-55-Ala-61 superimposed for all molecules (A-H). Molecules B, F, and G in the HFBII structure are in a similar, extended conformation. Molecules A, C, E, and H are in a similar conformation to each other; this conformation is that previously observed for HFBII at an ultra-high resolution. However, molecule D has the area Ala-55-Ala-61 in a conformation that is between the closed and the extended conformation. Most likely, judging by the high temperature factors in this area for all of the molecules, several conformations are present in each molecule, but only the predominant one is presented in the model. The conformational change in molecules B, F, and G also resulted in a slight change in the secondary structure of the protein; the fourth β-sheet was shortened from 8 residues (59–66) to 3 (64–66), which decreased the β-sheet content of the structure from 37 to 31%.

FIGURE 1. The overall structure of T. reesei HFBII. a, molecules B, F, and G (P2₁) are in an extended conformation (orange). Molecules A, C, E, and H (P₂₁) are in regular, closed conformation (blue). Molecules A and B of the orthorhombic structure (P₂₁2₁2₁) also represent this conformation (blue). Molecule D (P₂₁) is in an intermediate conformation (magenta). b, enlargement of the area of the conformational change.

(ile-22 in molecule C, Thr-28 in molecules D and H, and Gln-65 in molecules A, E, and F) showed density indicative of being ordered into two positions; two conformations for these residues were included into the model.

The functional site of hydrophobins, a hydrophobic patch consisting of the side chains of residues Leu-7, Val-18, Leu-19, Leu-21, Ile-22, Val-24, Val-54, Ala-55, Val-57, Ala-58, Ala-61, Leu-62, and Leu-63, was located on the protein surface as previously described (8). The three detergent molecules that originated from the crystallization solution were coordinated between two hydrophobin molecules in the region of the hydrophobic patches. The electron density produced by the detergent molecules (Fig. 2a) was ambiguous and the tempera-
Another detergent molecule, was seen elsewhere but also close coordinated in the vicinity of the hydrophobic patches. Some weak yet clearly visible, and therefore the detergent molecule as asymmetric units (Fig. 2)

between the hydrophobic surface areas of two neighboring hydrophobic surface. The detergent molecules were packed one layer all pointed in the same direction, thus creating a larger anti-parallel fashion and the hydrophobic surface areas within when the helical parts of the molecules packed together in an crystal packing resulted in the formation of a layer structure

unmodeled.

FIGURE 3. The structure of HFBII in space group P2₁2₁2₁, and the fiber formation. a, surface representation of the dimer in the asymmetric unit (hydrophobic areas in red, detergent in yellow), b, the packing of translation-related dimers leads to the formation of a continuous hydrophobic surface. c, the packing of rotation-related dimers produces the hydrophilic fiber. d, representative electron density of the detergent molecule (red) and the protein (blue).

structure factors remained high, despite the evident presence of the detergents. This is likely due to the detergent molecules being slightly disordered because of their structural flexibility. At fairly low resolution, it was also difficult to distinguish between clusters of water and detergent molecules. Indeed, some residual density remained in clearly hydrophobic locations, indicating that partially occupied detergent sites may have been left unmodeled.

The eight molecules of the asymmetric unit were packed in two rows and arranged in the shape of a horseshoe (Fig. 2b). The crystal packing resulted in the formation of a layer structure when the helical parts of the molecules packed together in an anti-parallel fashion and the hydrophobic surface areas within one layer all pointed in the same direction, thus creating a larger hydrophobic surface. The detergent molecules were packed between the hydrophobic surface areas of two neighboring asymmetric units (Fig. 2c). When the layer structure is viewed in a perpendicular direction, a very symmetrical packing of six-membered rings, formed by hydrophobin molecules, can be observed (Fig. 2d). The diameter of these rings is ~62 Å. The ring is slightly tilted; in other words, it winds up in a manner typical of helical structures.

The Orthorhombic Crystal Form of HFBII—The structure of the orthorhombic crystal form has been deposited at the PDB under code 2PL7. The structure contained two molecules of hydrophobin HFBII, designated A and B, one detergent molecule, a sulfate ion, and 149 molecules of water. No conformational changes were observed in this structure. The C termini were found to be either degraded or so flexible that some of the residues could not be seen in the electron density maps. The three C-terminal residues were not fitted to the model due to lack of electron density; thus, the sequences end with Ala-67.

The electron density for the heptyl-β-D-thioglycoside was weak yet clearly visible, and therefore the detergent molecule was modeled with 50% occupancy (Fig. 3d). The detergent was coordinated in the vicinity of the hydrophobic patches. Some residual density, quite likely caused by partial presence of another detergent molecule, was seen elsewhere but also close to the hydrophobic surface areas. However, this density was too ambiguous for a detergent molecule to fit it.

The two molecules of the asymmetric unit formed a dimer in such a way that contact between the molecules was made through the N-terminal loops, the first β-hairpin loop, and the fourth β-strand. This contact resulted in the hydrophobic surface areas being located close together on one side of a dimer, creating a shared hydrophobic surface (Fig. 3a). More of these dimers packed together sideways, in the same rotation angle and only by translation of one unit along the x-axes. In this way, an elongated structure was created, with a shared, continuous hydrophobic surface (Fig. 3b). On top of this surface there was, in very close contact, a symmetrical row of molecules, with a symmetry matrix x + 1/2, −y + 1/2, −z relating the molecules to x, y, z and the row being continuous with the translation of one unit along the x-axes (Fig. 3c). The interaction between these two elongated chains was through the areas of the hydrophobic surface patch. The packing through the hydrophobic patch proceeded in a zigzag pattern: molecule A was in contact with molecule B of an adjacent dimer through the hydrophobic surface and molecule B was in contact with molecule A of another adjacent dimer. Thus, this kind of packing led to a fiber-like structure, with most of the hydrophobic surface areas buried inside the fiber. The entire crystal was composed of these fibers packed close together. However, the interactions between adjacent fibers were considerably weaker than within a fiber. Only a small portion of the hydrophobic patches was left exposed to the solvent after the formation of the fiber structure and close-packing through the hydrophobic surface areas. This hydrophobic pocket hosted the carbon tail of the detergent molecule.

DISCUSSION

The Conformational Change—Some structural flexibility was found in the primitive monoclinic structure of HFBII at the area of Ala-55-Ala-61 (Fig. 1). A similar conformational change was previously observed for HFBI (11). This type of change has only been observed when class II hydrophobins have been crystallized in the presence of a detergent and also only on half of the
molecules in the asymmetric unit. The precise function of the conformational change is not evident from the crystal structures. It could be hypothesized that the movement is driven by an attempt to adjust the position of the hydrophobic patch in relation to the hydrophobic substrate, as with the previously observed plasticity in the molecular structure of hydrophobins (11).

The NMR structure of the class I hydrophobin EAS from *N. crassa* (10) contains two unstructured loop regions, Met-22-Ser-42 and Val-65-Phe-72. The latter, smaller area may partially coincide with the area of the conformational change observed in class II hydrophobin structures, because both of these areas lay between the last 2 cysteine residues in the sequence. The disordered regions in the EAS structure concern 29 residues (35%) while 7 residues (10%) are involved in the conformational change in HFBI and HFBII structures. It remains slightly unclear whether the loop areas of the EAS are truly disordered or just undefined by NMR, because earlier NMR results (23) indicated that the entire protein is largely unstructured. In addition, it is not possible to model the unstructured areas using the crystals structures of class II hydrophobins, because the loops are significantly larger in size in class I hydrophobins.

**Oligomer Formation and the Mechanism of Function**—Hydrophobins are typically readily soluble in water. We have previously proposed that oligomerization is the mechanism of how hydrophobins remain soluble despite the amphiphilicity of the molecules, and we have described the energetic principles as the driving force of the self-assembly (8, 11). In the previous crystal structures of hydrophobins the governing interactions between the protein molecules have occurred via the hydrophobic surface areas. For the first time, in the orthorhombic crystal structure presented in this report a type of tight interaction, likely similar to a lateral interaction in the amphiphilic layer, is observed. The two molecules in the asymmetric unit form a closely packed dimer that does not bury the hydrophobic surface areas but aligns them on one side of the dimer (Fig. 4, b and e). When this amphiphilic dimer is superimposed on the tetramer with hydrophobic interaction (Fig. 4, d and e), its position is close to that of two non-dimeric molecules (just slightly tilted), which originate from two different constituent dimers. We suppose that the monomeric HFBII (Fig. 4, a and e) oligomerizes in solution first to dimers held together by hydrophobic interaction (Fig. 4, c and e) and then further forms tetramers by combining two dimers. The tetramer may break down to a dimer similar to that in the orthorhombic crystal structure. This dimer has the hydrophobic surface patches of both molecules aligned on one side of the dimer, ready to interact with the hydrophobic surface. The amphiphilic dimer is then incorporated to the assembly on the hydrophobic-hydrophilic interface or (in the presence of an excess amount of hydrophobin) to the fibril structure. A schematic representation of the complete mechanism of function is shown in Fig. 5.

**Interfacial Structures**—Both class I and II hydrophobins form amphiphilic layer structures, with the distinction that class I layers are more resistant to dissociation and the class I layers show a rodlet-like surface pattern. For class II hydrophobins, the surface of the layer appears to be smooth, but closer exam-
Fibril and Monolayer Film of Hydrophobin HFBII

![Diagram of hydrophobin structures](image)

**FIGURE 5. The mechanism of the function for hydrophobins, schematic representation.** Monomers multimerize to dimers, two of which form a tetramer. The tetramer may split into two new dimers with hydrophobic surface areas aligned. These amphiphilic dimers precede the formation of amphiphilic monolayer on hydrophobic-hydrophilic interface. At high concentration, excess hydrophobin forms fibril structures.

**Fibril Formation—**The basic structural unit in the orthorhombic structure is an amphiphilic dimer. These dimers are packed in a fiber-like array throughout the crystal. These fibers are mostly hydrophobic on the surface and their diameter is ~55 Å. We propose that this fiber-like array (Fig. 3c) is, in fact, the structure of fibrils observed for class II hydrophobins. The fibrils are formed upon vigorous shaking and appear to be a way to efficiently conceal the hydrophobic surface areas when an excess amount of hydrophobin is present. It is not known how the fibrils formed by class II hydrophobins are related to the rodlets formed by class I hydrophobins, if they are in any way. Rodlets have been observed on the hydrophobic side of the self-assembled films of class I hydrophobins (24), and it has recently been reported for class I hydrophobin SC3 that during the self-assembly a featureless film is formed first and that after overnight incubation a 10-nm film with rodlet patterning forms (25). In relation to the thickness of hydrophobin films, it is also noteworthy that SC3 is heavily glycosylated (31), whereas HFBI (32), HFBII (33), and EAS (23) are not. Glycosylations add to the thickness of the layer and affect the surface pattern on the hydrophilic side.

Alignment of hydrophobic surface areas necessitates favorable lateral interactions in the hydrophobin assembly. In the orthorhombic crystal structures, residues Pro-4, Phe-8, Asp-20, and Gln-65 interact between the two molecules of the asymmetric unit, whereas Thr-16, Asn-17, and Asp-20 tie together the two dimers in the side-by-side manner of the fiber formation. In the primitive monoclinic structure, residues Pro-4, Phe-8, Thr-16, Asp-20, and Gln-65 participate in contacts between the molecules of the asymmetric unit. Residues Pro-29, Thr-30, and Pro-56 mediate the packing of adjacent ring-like structures in the primitive monoclinic structure of HFBII, whereas Asp-59 and Gln-60 seem to strengthen the interaction when the hydrophobic patches pack close together in the orthorhombic fiber-like structure. All the residues involved in the lateral interactions are well conserved in class II hydrophobin sequences and have no other apparent reason for conservation other than the involvement in lateral interactions.

Whether the assembled amphiphilic layer is a mono- or multilayer of hydrophobins is not clear. For films of hydrophobin HFBI on a water droplet, the thickness of the dried surface layer has been determined to be 1.1–3.0 nm (28), indicating a monolayer. Also, judging by the detergent-mediated layer structure in the primitive monoclinic crystal form, it would seem that multilayers would require additional hydrophobic moieties, i.e. the detergent, in order to form. For class I hydrophobin SC3, the formation of both 10- and 3-nm-thick layers has been reported (29, 30), indicating multi- and monolayer, respectively. The formation of the layer is said to be concentration-dependent, and it has been found for SC3 that a featureless film is formed first and that after overnight incubation the 10-nm film with rodlet patterning forms (25). In relation to the thickness of hydrophobin films, it is also noteworthy that SC3 is heavily glycosylated (31), whereas HFBI (32), HFBII (33), and EAS (23) are not. Glycosylations add to the thickness of the layer and affect the surface pattern on the hydrophilic side.

The two crystal structures described in this report have given clear indications with regard to major issues in hydrophobin.
research. These structures have allowed us to build an energetically reasonable pathway, starting from a soluble molecule and ending with an amphiphilic layer. The crystal structures also indicated how the layers and fibril structures may be constructed of their building blocks, the hydrophobin molecules. However, it still needs to be clarified how the loop areas in class I hydrophobins are structured before we can make full comparisons of the two hydrophobin classes.

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REFERENCES

1. Wessels, J., de Vries, O., Åsgersdóttir, S., and Schuren, F. (1991) Plant Cell 3, 793–799
2. Wessels, J. (1994) Annu. Rev. Phytopathol. 32, 413–437
3. Wösthen, H., van Wetter, M., Lugones, L., van der Mei, H., Buscher, H., and Wessels, J. (1999) Curr. Biol. 9, 85–88
4. Scholtmeijer, K., Wessels, J., and Wösthen, H. (2001) Appl. Microbiol. Biotechnol. 56, 1–8
5. Hektor, H., and Scholtmeijer, K. (2005) Curr. Opin. Biotechnol. 16, 1–6
6. Linder, M., Szilvay, G., Nakari-Setälä, T., and Penttilä, M. (2005) FEMS Microbiol. Rev. 29, 877–896
7. Linder, M., Qiao, M., Laumen, F., Selber, K., Hyttiä, T., Nakari-Setälä, T., and Penttilä, M. (2004) Biochemistry 43, 11873–11882
8. Hakamäki, J., Paananen, A., Askolin, S., Nakari-Setälä, T., Parkkiniemi, T., Penttilä, M., Linder, M., and Rouvinen, J. (2004) J. Biol. Chem. 279, 534–539
9. Hakamäki, J., Linder, M., Popov, A., Schmidt, A., and Rouvinen, J. (2006) Acta Crystallogr. Sect. D Biol. Crystallogr. 62, 356–367
10. Kwan, A., Winefield, R., Sunde, M., Matthews, J., Haverkamp, R., Templeton, M., and Mackay, J. (2006) Proc. Natl. Acad. Sci. U. S. A. 103, 3621–3626
11. Hakamäki, J., Szilvay, G., Kaljumäe, H., Maksimainen, M., Linder, M., and Rouvinen, J. (2006) Protein Sci. 15, 2119–2124
12. Szilvay, G., Nakari-Setälä, T., and Linder, M. (2006) Biochemistry 45, 8590–8598
13. Wang, X., Graveland-Biker, J., de Kruif, C., and Robillard, G. (2004) Protein Sci. 13, 810–821
14. Wösthen, H., and de Vocht, M. (2000) Biochim. Biophys. Acta Rev. Biomembr. 1469, 79–86
15. Torkkeli, M., Serimaa, R., Ikkala, O., and Linder, M. (2002) Biophys. J. 83, 2240–2247
16. Hakamäki, J., Parkkiniemi, T., Hakulinen, N., Linder, M., and Rouvinen, J. (2004) Acta Crystallogr. Sect. D Biol. Crystallogr. 60, 163–165
17. Kabsch, W. (1993) J. Appl. Crystallogr. 26, 795–800
18. McCoy, A., Grosse-Kunstleve, R., Storoni, L., and Read, R. (2005) Acta Crystallogr. Sect. D Biol. Crystallogr. 61, 458–464
19. Collaborative Computational Project, Number 4 (1994) Acta Crystallogr. Sect. D Biol. Crystallogr. 50, 760–763
20. Brünger, A., Adams, P., Clore, G., DeLano, W., Gros, P., Grosse-Kunstleve, R., Jiang, J., Kuszewski, J., Milles, N., Pannu, N., Read, R., Rice, L., Simonson, T., and Warren, G. (1998) Acta Crystallogr. Sect. D Biol. Crystallogr. 54, 905–921
21. Sheldrick, G., and Schneider, T. (1997) Methods Enzymol. 277, 319–343
22. Jones, T., Zhou, J., Cowan, S., and Kjeldgaard, M. (1991) Acta Crystallogr. Sect. A Found. Crystallogr. 47, 110–119
23. Mackay, J., Matthews, J., Winefield, R., Mackay, L., Haverkamp, R., and Templeton, M. (2001) Structure 9, 83–91
24. Ma, H., Snook, L., Tian, C., Kaminskyy, S., and Dahms, T. (2006) Mycol. Res. 110, 879–886
25. de Vocht, M., Reviaikine, I., Ulrich, W., Bergsma-Schutter, W., Wösthen, H., Vogel, H., Briisson, A., Wessels, J., and Robillard, G. (2002) Protein Sci. 11, 1199–1205
26. Paananen, A., Vuorimaa, E., Torkkeli, M., Penttilä, M., Kauranen, M., Ikkala, O., Lemmetyinen, H., Serimaa, R., and Linder, M. (2003) Biochemistry 42, 5253–5258
27. Szilvay, G., Paananen, A., Laurikainen, K., Vuorimaa, E., Lemmetyinen, H., Peltonen, J., and Linder, M. (2007) Biochemistry 46, 2345–2354
28. Serimaa, R., Torkkeli, M., Paananen, A., Linder, M., Kisko, K., Knapila, M., Ikkala, O., Vuorimaa, E., Lemmetyinen, H., and Seeck, O. (2003) J. Appl. Crystallogr. 36, 499–502
29. Wang, X., Shi, F., Wösthen, H., Hektor, H., Poolman, B., and Robillard, G. (2005) Biochem. J. 388, 3434–3443
30. Wösthen, H., de Vries, O., and Wessels, J. (1993) Plant Cell 5, 1567–1574
31. Wösthen, H., de Vries, O., van der Mei, H., Busscher, H., and Wessels, J. (1994) J. Bacteriol. 176, 7085–7086
32. Nakari-Setälä, T., Aro, N., Kalkkinen, N., Alatalo, E., and Penttilä, M. (1996) Eur. J. Biochem. 235, 248–255
33. Nakari-Setälä, T., Aro, N., Ilnén, M., Muñoz, G., Kalkkinen, N., and Penttilä, M. (1997) Eur. J. Biochem. 248, 415–423