Muscle fructose-1,6-bisphosphatase (FBPase), which catalyzes the hydrolysis of fructose-1,6-bisphosphate (F1,6BP) to fructose-6-phosphate (F6P) and inorganic phosphate, regulates glucose homeostasis by controlling the gluconeogenic pathway. FBPase requires divalent cations, such as Mg\textsuperscript{2+}, Mn\textsuperscript{2+}, or Zn\textsuperscript{2+}, for its catalytic activity; however, calcium ions inhibit the muscle isoform of FBPase by interrupting the movement of the catalytic loop. It has been shown that residue E69 in this loop plays a key role in the sensitivity of muscle FBPase towards calcium ions. The study presented here is based on five crystal structures of wild-type human muscle FBPase and its E69Q mutant in complexes with the substrate and product of the enzymatic reaction, namely F1,6BP and F6P. The ligands are bound in the active site of the studied proteins in the same manner, and have excellent definition in the electron density maps. In all studied crystals, the homotetrameric enzyme assumes the same cruciform quaternary structure, with the α angle, which describes the orientation of the upper dimer with respect to the lower dimer, of ~85°. This unusual quaternary arrangement of the subunits, characteristic of the R-state of muscle FBPase, is also observed in solution by small-angle X-ray scattering (SAXS).

**Keywords**: fructose-1,6-bisphosphatase; glycolysis; energy metabolism; active site; gluconeogenesis; glyconeogenesis; T/R-state enzyme;

**Acknowledgements of Financial Support**: This work was supported in part by the Polish National Science Centre (NCN) grant No. 2013/09/B/NZI/01081. Research leading to these results has received funding from the European Community’s Seventh Framework Programme (FP7/2007–2013) under BioStruct-X (grant agreement N 283570).

**Abbreviations**: FBPase, fructose-1,6-bisphosphatase; F1,6BP, fructose-1,6-bisphosphate; WT, wild type

**INTRODUCTION**

Fructose-1,6-bisphosphatase (FBPase, EC 3.1.3.11), which catalyzes the hydrolysis of fructose-1,6-bisphosphate (F1,6BP) to fructose-6-phosphate (F6P) and inorganic phosphate, is the main regulatory enzyme of gluconeogenesis and glyconeogenesis and thus it controls such fundamental processes as energy metabolism and glucose homeostasis (Tejwani, 1983; Gidh-Jain et al., 1994). Vertebrate genomes contain two distinct FBPase genes, FBP1 and FBP2, encoding two isozymes. Liver FBPase (or FBP1), the product of the FBP1 gene, is mainly expressed in the gluconeogenic organs, where it functions as a regulator of glucose synthesis from non-carbohydrates (Al-Robayi & Eschrich, 1999). Unlike liver FBPase, the muscle isoform (or FBP2), encoded by FBP2, is expressed in all vertebrate cells, whether gluconeogenic (e.g., in muscle fibers) or not (such as neurons, which reportedly do not synthesize glycogen from carbohydrate precursors) (Löffler et al., 2001).

Despite high level of similarity of their primary structure, the two isozymes play entirely different biological functions and significantly differ in such kinetic properties as susceptibility to inhibition (Dzugaj, 2006). FBPase activity is regulated by such physiological inhibitors as AMP and NAD\textsuperscript{+}, which bind to an allosteric site, and fructose-2,6-bisphosphate (F2,6BP), which binds to the active site, as well as by calcium ions. The enzyme requires divalent cations such as Mg\textsuperscript{2+} or Zn\textsuperscript{2+} or Mn\textsuperscript{2+} for its activity (Gidh-Jain et al., 1994; Choe et al., 1998). Interestingly, calcium ions inhibit muscle FBPase, but not liver FBPase (Gizak et al., 2004; Zarzycki et al., 2007).

In line with its key role in glucose homeostasis, the activity of FBPase is under hormonal control (Bartrons et al., 1983; Pilks et al., 1995). Glucagon and insulin, respectively, stimulate and inhibit the synthesis of F2,6BP, which acts as a competitive inhibitor of FBPase. F2,6BP acts synergistically with AMP to regulate the enzyme activity (Van Schaftingen & Hers, 1981; Gidh-Jain et al., 1994; Choe et al., 1998). Muscle FBPase is about 100 times more susceptible to the action of the allosteric inhibitors AMP (Rakus et al., 2003) and NAD\textsuperscript{+} (Rakus et al., 2003) than the liver isoform, and about 1,000 times more sensitive to inhibition by Ca\textsuperscript{2+} (Gizak et al., 2004, 2008; Zarzycki et al., 2007). It was also shown that calcium ions inhibit muscle FBPase competitively in respect to Mg\textsuperscript{2+} and disrupt the Z-line-based FBPase-aldolase complex in striated muscles, blocking glycogen re-synthesis during high-intensity exercise (Gizak et al., 2013). While the different sensitivity of muscle and liver FBPases to Ca\textsuperscript{2+} was shown to be dependent on the single E69Q point mutation (Zarzycki et al., 2007; Rakus et al., 2013), the structural differences leading to the unique responses to allosteric inhibitors and different ability to interact with various binding partners are not well understood. Recent studies of Wiśniewski and others (Wiśniewski et al., 2017) revealed that muscle FBPase exists in equilibrium between tetramers, dimers and monomers and that only the tetrameric form of FBPase is retained in the cell nucleus, whereas only the dimeric form...
associates with mitochondria and protects them against stress stimuli, such as elevated calcium and H2O2 levels.

Our knowledge about the structure of FBPase is predominantly based on studies of the enzyme isolated from porcine liver, while information about the muscle isoform is scarce and limited to only a few structural studies (Zarzycki et al., 2011; Shi et al., 2013, Barciszewski et al., 2016). Although Wśniiewski and collaborators demonstrated that in the absence of AMP muscle FBPase exists as a mixture of various oligomers (Wśniiewski et al., 2017), in all published crystal structures, the FBPases are homotetramers comprised of the upper (subunits C1•C2) and lower (C3•C4) dimers, which rotate with respect to each other during the catalytic cycle. Our earlier studies revealed that the structure of the inactive (T-state), AMP-saturated muscle FBPase is practically identical to that of the T-state liver isozyme (Zarzycki et al., 2011; Barciszewski et al., 2016; Ruf et al., 2016), with the two dimers forming a small angle \( \beta \). Based on the previously reported structures (known at that time for the liver isoform only) of the active R-state, we suggested that the explanation of the markedly different kinetic properties of the liver and muscle isoforms must reside in different quaternary arrangements of the active R-states of the isozymes. The crystal structure of the R-state of the muscle isozyme revealed (Barciszewski et al., 2016) that its cross-like form is indeed diametrically different from the flat form of the R-state of the liver isozyme. The cruciform R-state of muscle FBPase is stabilized by a hydrophobic motif, termed the “leucine lock”, which plays an important role in the R-to-T transition, and in particular blocks the residue (D187) responsible for parking the catalytic loop in the disengaged (inactive) conformation when the enzyme is in the T-state (Barciszewski et al., 2016). The leucine lock is not present in the liver isozyme. All these observations have led us to several important questions: is the perpendicular R-state also present in solution? What is the R-state structure of the E69Q mutant? Are there any differences in substrate/product binding between the wild-type and E69Q muscle FBPase?

To address these questions, here we resolved and present the first crystal structures of both the wild-type and E69Q muscle FBPase in its active R-state in complexes with F1,6BP and F6P, which are, respectively, the substrate and the product of the enzyme. The results show that the arrangement of the substrate/product binding site in the two proteins is identical and that the sensitivity to calcium ions is not related to direct disruption of the F1,6BP/F6P binding site. Our SAXS experiments confirm that in solution muscle FBPase forms the cross-like quaternary arrangement known from crystallographic studies, thus supporting the conclusion about physiologically different R-states of the two isoforms.

**MATERIALS AND METHODS**

**Protein expression, purification and crystallization**

Mutagenesis, expression and purification of wild type (WT) human muscle FBPase and its E69Q mutant were carried out as described previously (Rakus et al., 2003; Zarzycki et al., 2007). AMP was removed from protein samples by extensive dialysis and FPLC gel filtration on a HiLoad Superdex 200 16/60 column (GE Healthcare), as described previously (Barciszewski et al., 2016).

Crystallization experiments were carried out at 292 K using the hanging-drop vapor-diffusion method. Protein and precipitant solutions were mixed at 1:1 volume ratio to form 3 μl drops. Samples of catalytically active WT FBPase and its E69Q mutant without any ligands were concentrated to 6 mg ml\(^{-1}\) and crystallized using 10 mM Tris buffer pH 7.4, containing 10 mM MgCl\(_2\), 2 M NaCl and 10% (v/v) PEG6000. The best crystals grew to the dimensions of 0.20×0.15×0.15 mm within three months. Two soaking experiments were performed using crystals of both WT and E69Q FBPase. In the soaking experiment the mother liquor was supplemented with 5 mM fructose-6-phosphate (F6P) or 10 mM fructose-1,6-bisphosphate (F1,6BP), with soaking times of 2.5 h. Each soaking solution was additionally supplemented with 100 mM CaCl\(_2\) to inhibit the enzyme activity.

**X-ray data collection and processing**

For data collection, the crystals were cryoprotected by immersion for a few seconds in the mother liquor supplemented with 20% (v/v) glycerol and, where appropriate, with the soaking ligands, and then flash-vitrified at 100 K in a cold nitrogen-gas stream. X-Ray diffraction data were collected for five crystals (maximum resolution in parentheses): (i) WT with F6P (1.92 Å), (ii) WT with F1,6BP (1.98 Å), (iii) E69Q with F6P (1.98 Å), (iv) E69Q with F1,6BP (2.19 Å), (v) E69Q with F6P (2.30 Å). In all cases, synchrotron radiation was used, provided by MX beamlines of the BESSY II (Berlin, Germany) synchrotron, equipped with Rayonix MX-225 (square) or Ryonix SX-165 (round) CCD detectors. The diffraction data for E69Q in complex with F1,6BP were processed and scaled with the HKL-2000 package (Otwinowski & Minor, 1997) while XDS-APP (Kabsch, 2010; Krug et al., 2012) was used in all other cases. The data collection statistics are presented in Table 1.

**Structure determination and refinement**

Both, the WT and mutant E69Q muscle FBPase crystallized in the same space group \( \text{I}_{4}1_{22} \), isomorphously with the wild-type human muscle FBPase (PDB: 5ET5) described previously (Barciszewski et al., 2016), with one protein molecule in the asymmetric unit, located near the crystallographic 222 site. In several rounds of manual rebuilding in COOT (Emsley et al., 2010), starting with the coordinates of 5ET5, the models were corrected according to the electron density maps, with special emphasis on the N-terminal fragment of the protein molecules.

The refinement of all structures was carried out in *phenix.refine* (Afonine et al., 2012). Riding hydrogen atoms of the protein molecules were included in *Fc* calculations for all structures. TLS parameters (Winn et al., 2001; Painter & Merritt., 2006) were refined for four (WT/F6P), seven (WT/F1,6BP), three (E69Q/F6P) and three (E69Q/F1,6BP) rigid groups per subunit, as suggested by the refinement program. Water molecules (94, 25, 59, 61 and 12, respectively) were added to the models after manual validation of their electron density and hydrogen bonding. The refinement statistics are presented in Table 1.

**PDB accession codes and raw data deposition**

Atomic coordinates (.pdb) and processed structure factors (.mtz) corresponding to the final models presented in this work have been deposited with the Protein Data Bank (PDB) under the accession codes 5ET8 (R-state muscle FBPase in complex with F6P); 5K56 (R-state muscle FBPase in complex with F1,6BP); 5K55 (R-state E69Q mutant FBPase in complex with F6P);...
5L0A (R-state E69Q mutant FBPase in complex with F1,6BP); and 5K54 (R-state E69Q mutant FBPase with no any ligands). The corresponding raw X-ray diffraction images have been deposited in the RepOD Repository at the Interdisciplinary Centre for Mathematical and Computational Modelling (ICM) of the University of Warsaw, Poland, and are available for download with the following Digital Object Identifiers (DOI): http://dx.doi.org/10.18150/3279048 (R-state E69Q mutant FBPase in complex with F1,6BP); http://dx.doi.org/10.18150/7369446 (R-state muscle FBPase in complex with F1,6BP); http://dx.doi.org/10.18150/2073150 (R-state E69Q mutant FBPase in complex with F6P); http://dx.doi.org/10.18150/3279048 (R-state E69Q mutant FBPase without any ligands).

SAXS experiments in solution

Wild-type FBPase for the experiments in solution was prepared in the same way as the sample for crystallization experiments described previously (Barciszewski et al., 2016). The SAXS measurements were performed at three different concentrations (1 mg/ml; 4 mg/ml; 8 mg/ml) using 25 mM Hepes buffer pH 7.0. Small-angle X-ray scattering data were collected on the bio-SAXS beamlines P12 (Petra-III, EMBL/DESY, Hamburg, Germany) and I911-4 (MAX-Lab, Lund, Sweden). Samples of 20 μl protein solution and of the corresponding matching buffer were analyzed. SAXS data were collected at 300 K over the q range of 0.00088-0.5 Å⁻¹ (DESY) or 0.01–0.45 Å⁻¹ (MAX-Lab), and overlays of the merged data sets were used to detect concentration-dependent scattering in the lowest q region. All SAXS data were processed using the ATSAS package (Petoukhov et al., 2012). The CRYOL system (Svergun et al., 1995) was applied for the evaluation of the solution scattering patterns using the crystallographic FBPase models. Ab initio modelling was performed with DAMMIN (Svergun et al., 1999). The SAXS data collection and structural parameters are presented in Table 2.

### RESULTS

#### Overall structure

All five structures of the WT and E69Q mutant protein presented in this report were solved in space
group J4,22. They are isomorphous with the structure of muscle FBPase in the R-state (PDB: 5ET5) described previously (Barciszewski et al., 2016). In each crystal, there is one protein subunit in the asymmetric unit and the 222-symmetric homotetramer is generated by crystallographic symmetry. The overall structures are similar to that described previously (Barciszewski et al., 2016), as illustrated by the r.m.s.d. of their Cα superpositions (0.15–0.18 Å) when compared with the reference R-state structure 5ET5 of human muscle FBPase. The topology of the α-helices and the β-sheet is also conserved. Several amino acid residues (including the E69Q mutation) could not be traced in the electron density maps. In particular, the catalytic loop L2 (residues 50–73) is in the disordered state in all the structures presented here.

Tetramer architecture

Superpositions of all five WT and E69Q mutant FBPase tetramers from this study onto the reference human muscle R-state tetramer show no change in the tetramer conformation. The upper C1•C2 dimer is rotated by x=−85° relative to the lower C3•C4 dimer in all five structures presented here.

The active site

Among the presented structures, there are two types of active-site complexes, with the catalytic reaction substrate (F1,6BP) or product (F6P) at full occupancy. The excellent electron density leaves no doubt that all of these ligand molecules are the β anomers of the fructose furanose ring (Fig. 1). Both ligand molecules are stabilized in the active site by a network of direct hydrogen bonds formed by residues Asn212, Tyr215, Tyr244, Met248, Tyr264 and Lys274, as listed in Table 3. Additionally, Asp251 forms a water-mediated hydrogen bond with the fructose hydroxyl O4 atom (Fig. 2). Each ligand molecule is also hydrogen-bonded with the side chain of Arg243 from the complementary subunit within the tight dimer (upper or lower).

Table 2. SANS data collection and structural parameters obtained from SANS scattering derived parameters.

| FBPase state | R | T | R | T |
|--------------|---|---|---|---|
| Data collection parameters | | | | |
| Instrument | P12 Petra-III | 1911-4 MAX-Lab | | |
| Wavelength (Å) | 1.24 | 0.91 | | |
| q range (Å⁻¹) | 0.00088-0.5 | 0.01-0.45 | | |
| Exposure time (s) | 1 | 120 | | |
| Concentration range (mg/ml) | 1-8 | 1-8 | | |
| Temperature (K) | 293 | 293 | | |
| Structural parameters | | | | |
| I(0) (arbitrary units) [from P(r)] | 46.35±1 | 46.37±1 | 163.7±1.7 | 163.2±1.7 |
| Rg (Å) [from P(r)] | 35.9 | 34.3 | 35.9 | 34.3 |
| Rg (Å) (from Guinier) | 35.9 | 34.3 | 35.9 | 34.3 |
| Dmax (Å) | 101 | 115 | 101 | 115 |
| Porod volume estimate (Å³) | 180015 | 180201 | 180120 | 180191 |
| Dry volume calculated from sequence (Å³) | 179516 | 179516 | 179516 | 179516 |
| Molecular mass determination | | | | |
| Contrast (Δρ x 10¹⁰ cm⁻²) | 3.047 | | | |
| Molecular mass M, from [I(0)] (kDa) | 148 | | | |
| Monomeric M, calculated from sequence (kDa) | 36.842 | | | |
| Software used | | | | |
| Primary data reduction | PRIMUS | | | |
| Data processing | PRIMUS | | | |
| Ab initio analysis | DAMMIN | | | |
| Validation and averaging | DAMAVER | | | |
| Computation of model intensities | CRYSOL | | | |
| 3D graphics representation | PyMOL | | | |
The experimental SAXS curves collected for the FBPase sample in 25 mM Hepes buffer pH 7.0 are shown in Fig. 3. The SAXS analysis and structural parameters confirmed the existence of the R and T states in solution. Low-resolution SAXS models of both states superimposed with the crystal structures are shown in Fig. 4. The conformational transformation from the T to R state is induced by removal of AMP, in agreement with the previous crystallographic study (Barciszewski et al., 2016).

**DISCUSSION**

Despite a number of crystallographic and biochemical studies, the structure of FBPase in solution has not been investigated before. The studies of Wisniewski and others (Wisniewski et al., 2017), which used analytical centrifugation, demonstrated that muscle FBPase exists in equilibrium of tetramers, dimers and monomers, when the allosteric inhibitor AMP is absent. Addition of AMP induces tetramerization of practically the whole population of the enzyme molecules (Wisniewski et al., 2017). Our SANS studies unambiguously show that muscle FBPase exists in solution in both, the T and R states that are identical as in the crystallized enzyme (Barciszewski et al., 2016).

The crystal structures of human muscle WT and E69Q FBPase in the R state presented here show that upon removal of AMP from the crystallization solution, the enzyme assumes the cruciform conformation regardless of the presence or absence of the substrate or the product of the catalytic reaction.

The binding mode of the ligands (F6P, F1,6BP) in the active site of the muscle isozyme closely resembles the situation known for the liver isozyme. Additionally, we have analyzed the structure of the mammalian enzymes available in the PDB and found that the residues involved in substrate/product binding are conserved among all vertebrate FBPases. Specifically, Asn212, Asn215, Arg243, Tyr244, Leu248, Tyr264 and Lys274 (human muscle FBPase residue numbers) form a hydrogen bond network with the C6 phosphate group and the furanose ring of the ligand molecules in the active site (Fig. 2). Residues involved in the catalytic metal (Mg\(^{2+}\)) coordination (Glu68, Glu97, Glu98, Asp118, Asp121, Glu280) are also conserved in vertebrate FBPases. Together with the phosphate group at the C1 atom of the F1,6BP substrate molecule, these residues form a huge acid cluster which is essential for divalent cation recognition. However, despite their presence in the crystallization solution, no Mg\(^{2+}\) ions could be detected in the presented crystal structures. This might have been presumably caused by the presence of calcium ions in the soaking solutions, which impede transition of loop

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**Quaternary structure in solution**

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L2 (residues 50–72) into the engaged state, in which it would take part in the formation of the acid cluster.

An intriguing, albeit still open, question is which fructose anomer is the natural substrate of FBPase when the catalytic reaction actually takes place. In solution, fructose-1,6-bisphosphate, like other similar carbohydrates, exists in an equilibrium of the α-anomer (15%), β-anomer (81%) and small amounts of some other forms (4%) (Frey et al., 1977). Based on such equilibrium binding studies, it was shown that an analogue of β-d-fructose-1,6-bisphosphate binds to the enzyme ten times more tightly than its α-anomer (Marcus, 1976). However, it was also shown that high concentration of β-fructose-6-phosphate inhibits the activity of FBPase (Benkovic & deMaine, 1982; Villeret et al., 1995). The studies of Benkovic and colleagues, also revealed that liver FBPase may utilize the β-anomer of F1,6BP as substrate, however, at a rate that was 5- to 10-times lower than what was measured for the α-anomer (Benkovic et al., 1974). The latter finding might suggest that the α-anomer is the preferred physiological substrate of FBPase, and this hypothesis seemed to be supported by the crystal structure of the E69Q mutant of human muscle FBPase in complex with AMP and F6P, which was interpreted with the α-anomer of the F6P product in the active site (PDB deposit 3IFC) (Kolodziejczyk et al., 2011). However, a re-refinement of that 3IFC model indicates that the F6P ligand molecule can fit the 1.9 Å electron density maps equally well in the α and β configurations (Barciszewski et al., unpublished results) (Fig. 5).

On the other hand, experiments on complex formation between muscle FBPase and muscle aldolase apparently supported the hypothesis that only the α-anomer is the physiological substrate of FBPase. These experiments demonstrated that only aldolase-associated FBPase will catalyze the reaction of F1,6BP hydrolysis in the presence of physiological concentration of the AMP inhibitor (Rakus et al., 2003), and that the aldolase may directly cascade the newly synthesized fructose-1,6-bisphosphate down to the FBPase in a process called substrate channeling (Rakus et al., 2004). Since it has been conclusively shown that aldolase synthesizes the α-anomer of F1,6BP (Penhoet et al., 1969) it might be speculated that the α-anomer is the main physiological substrate of FBPase.
Figure 2. Comparison of the F6P (A) and F1,6BP (B) binding modes in WT FBPase.
The ligand binding modes in the E69Q mutant protein are identical with those illustrated here. Residues from subunits C1/C2 are colored green/blue, main-chain atoms have been omitted for clarity (except for M248).

Figure 3. Experimental SAXS curves (A), Guinier plots (B), and pair distribution functions (C) for R- (red) and T-state (black) muscle FBPase.

Figure 4. Comparison of the crystal structures (cartoon model) and low-resolution SAXS structures (semitransparent surface) of muscle FBPase in the T-state (A) and R-state (B).
The crystal structures of the T-state (SET6) and R-state (SETS) were presented previously (Barciszewski et al., 2016).
Table 4. Comparison of hydrogen bonds (Å) between residues of WT (this work) and E69Q mutant (PDB ID 3IFC) FBPase C1 subunit and anomers of fructose-6-phosphate. Additional contacts with subunit C2 are in italics.

| E69Q FBPase – α-F6P (3IFC) | WT FBPase – β-F6P |
|---------------------------|------------------|
| P6P[O3]                   | 2.81             | Met248[N]        | Met248[N] | 2.78   | F6P[O3] |
| P6P[O5]                   | 2.67             | Lys274[Nε]      | Lys274[Nε] | 2.78   | F6P[O5] |
| P6P[O6]                   | 2.90             | Lys274[Nε]      | Lys274[Nε] | 2.90   | F6P[O6] |
| P6P[O3P]                  | 2.88             | Asn212 [Nγ2]    | Asn212 [Nγ2] | 2.96   | F6P[O1P] |
| P6P[O3P]                  | 2.71             | Tyr244[OH]      | Tyr244[OH] | 2.63   | F6P[O1P] |
| P6P[O1P]                  | 2.74             | Tyr264[OH]      | Tyr264[OH] | 2.59   | F6P[O2P] |
| P6P[O1P]                  | 2.65             | Tyr215[OH]      | Tyr215[OH] | 2.46   | F6P[O2P] |
| P6P[O3P]                  | 3.65             | Arg243[Nη1]     | Arg243[Nη1] | 3.68   | F6P[O1P] |
| P6P[O2P]                  | 2.68             | Arg243[Nη2]     | Arg243[Nη2] | 2.74   | F6P[O3P] |
Taking into account all of the above (sometimes contradictory) observations and hypotheses, it might be speculated that both anomers of F1,6BP may serve as a substrate for FBPase. However, the β-anomer of fructose-6-phosphate, because of its higher affinity for the enzyme, will be preferably observed in the FBPase crystal structures, even though under physiological conditions the α-anomer of F1,6BP might be the true substrate of FBPase.

There is only one structure of mammalian FBPase in complex with fructose-1,6-bisphosphate deposited in the PDB. That structure of the porcine liver isozyme (1FBBH) was modeled with the active site occupied by a superposition of the α- and β-anomers in 0.2:0.8 ratio. It is difficult to believe that a ligand molecule with only light atoms could be reliably modeled at 0.2 occupancy, especially at 2.5 Å resolution. It is rather likely that the authors were trying to mimic the active site occupancy with the natural abundance of the α- and β-anomers known from solution. Unfortunately, it is not possible to validate this conclusion of the original authors (Zhang et al., 1993) in the electron density maps because no structure factor data were deposited together with the atomic coordinates. Moreover, the spatial arrangement of the two isomers in that model closely resembles the situations known from the structures containing the 2,5-anhydro analog of the substrate (Villeret et al., 1995). In contrast, in the structures presented in this work the β-anomer of the substrate was modeled in excellent and unambiguous electron density maps with the resolution as high as 1.92 Å. The anomeric configuration of the F1,6BP substrate is easy to determine with the help of the phosphate group at the C1 atom. Modeling of the α-anomer of F6P and the β-anomer of F6P in complex with the F1,6BP substrate is straightforward.

In summary, in this paper we have presented, for the first time, the crystal structure of human muscle FBPase in complex with its F1,6BP substrate, as well as its F6P product at full occupancy. Also, to the best of our knowledge, this is the first SAXS evidence for both, the R- and T-states of this enzyme in solution, so far known only from crystal structures.

Author contribution
JB: conducted structure refinements, analyzed the results, wrote the manuscript. KS: conducted the SAXS experiments and interpreted their results. JW: carried out protein expression and purification. RK: conducted most of the crystallographic experiments and calculations. DR: interpreted the biological context of the results, wrote the manuscript. MJ: coordinated the crystallographic part of the project, analyzed the results, wrote the manuscript. AD: conceived and coordinated the project, analyzed the results, wrote the manuscript.

Acknowledgments
We thank HZB for the allocation of synchrotron radiation beamtime. We are indebted to SAXS beamlines: A, B, and C of the EMBL at the Petra-III storage ring.

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