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ORIGINAL PAPER

Studies of conformational changes of an arginine-binding protein from *Thermotoga maritima* in the presence and absence of ligand via molecular dynamics simulations with the coarse-grained UNRES force field

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Abstract The arginine-binding protein (ArgBP) from the hyperthermophilic eubacterium *Thermotoga maritima* (TmArgBP) is responsible for arginine transport through the bacterial cell membrane. The protein binds a single molecule of L-arginine, which results in conformational changes due to hinge bending. Thereby, TmArgBP acquires one of two possible conformations: open (without the presence of the arginine ligand) and closed (in the presence of the arginine ligand). Here we report a molecular dynamics study of the influence of the presence or absence of the ligand on the dynamics of TmArgBP, using the coarse-grained UNRES force field. The results of our studies indicate that binding of the arginine ligand promotes a closed conformation, which agrees with experimental data. However, the sensitivity of the TmArgBP conformation to the presence of arginine decreases and the protein becomes more flexible with increasing temperature, which might be related to the functionality of this protein in the thermophilic organism *T. maritima*.

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Introduction

ATP-binding cassette (ABC) transporting systems, which play a crucial role in nutrient gathering and the excretion of toxic molecules, are common among living organisms in all kingdoms of life [1]. These systems exhibit a high degree of conservation of amino acid sequence and in arrangement of the domains [2, 3]. ABC systems are composed of two transmembrane domains (TMDs) responsible for ligand transport across the membrane, and two nucleotide-binding domains that are located in the cytoplasm and hydrolyze ATP. These systems rely on the presence of periplasmic binding proteins (PBPs) capable of binding a specific solute [1]. Changes in the TMDs and the transport process are initiated by conformational changes in the solute-bound form of PBPs [1].

The structure of a typical PBP is composed of two easily distinguishable domains connected by a hinge region, and a ligand-binding site located between them [1, 4]. These proteins are capable of binding and transporting many naturally occurring ligands (e.g., sugars or amino acids) and are, therefore, targeted as possible scaffolds in the design of fluorescent and electrochemical protein biosensors [5–10].

The subject of this study is the arginine-binding protein (ArgBP) from the hyperthermophilic eubacterium *Thermotoga maritima* (TmArgBP); this protein is a typical PBP. TmArgBP comprises 229 amino acid residues and has the typical two-domain structure characteristic of PBPs. Domain I is composed of the N-terminal and the C-terminal ends

of the protein (residues 1–92 and residues 193–229) and domain II consists of N-terminal residues 100–187 [11, 12]. Previous studies on this protein have demonstrated its ability to bind the L-arginine ligand, leading to an increase in the stability of the protein [11, 13, 14]. Moreover, TmArgBP exists as a dimer at room temperature [12, 15]. Very recently, the crystal structures of its ligand-free (PDB ID: 4PRS) and ligand-bound (PDB ID: 4PSH) conformations were determined and compared [12]. It was found that, whereas the secondary structures of the arginine-free and arginine-bound forms of the protein are preserved, massive changes in tertiary structure were observed. The presence of the arginine ligand brings the two domains together, so the protein acquires a closed conformation, while in the arginine-free form the protein is in an open conformation (Fig. 1a and b, respectively) [12]. Furthermore, the observed changes in TmArgBP structure at the tertiary level caused by arginine-ligand binding lead to changes in the arrangement of monomers in the dimer [12].

At the time of this study, the crystallographic structures were not available, therefore the previously prepared tertiary structures of TmArgBP for both ligand-free and ligand-bound conformations obtained by the homology modeling method of Scirè et al. [11] were used in this study. These models are very similar to the respective crystal structures (Fig. 1c–f).

The purpose of this study was to investigate the influence of the arginine ligand on the dynamics of TmArgBP by using the coarse-grained United Residue (UNRES) force field. Domain movement occurs at the microsecond time scale [16], therefore coarse-graining was used to speed up simulations. Coarse-grained molecular dynamics (MD) simulations with the UNRES force field have been used successfully in similar studies investigating the motions of protein domains such as, e.g., computation of the binding between different large domains in PICK1 [17], opening of the Hsp70 chaperone [18], the prediction of multichain protein structures [19], and the prediction of protein structures [20]. In the 10th Community-Wide Experiment on the Critical Assessment of Techniques for Protein Structure Prediction (CASP10), UNRES predicted the domain packing of the domains of target T0663; the structure of each of the domain of this target was predicted correctly by homology modeling but the domain packing was not [20]. MD with

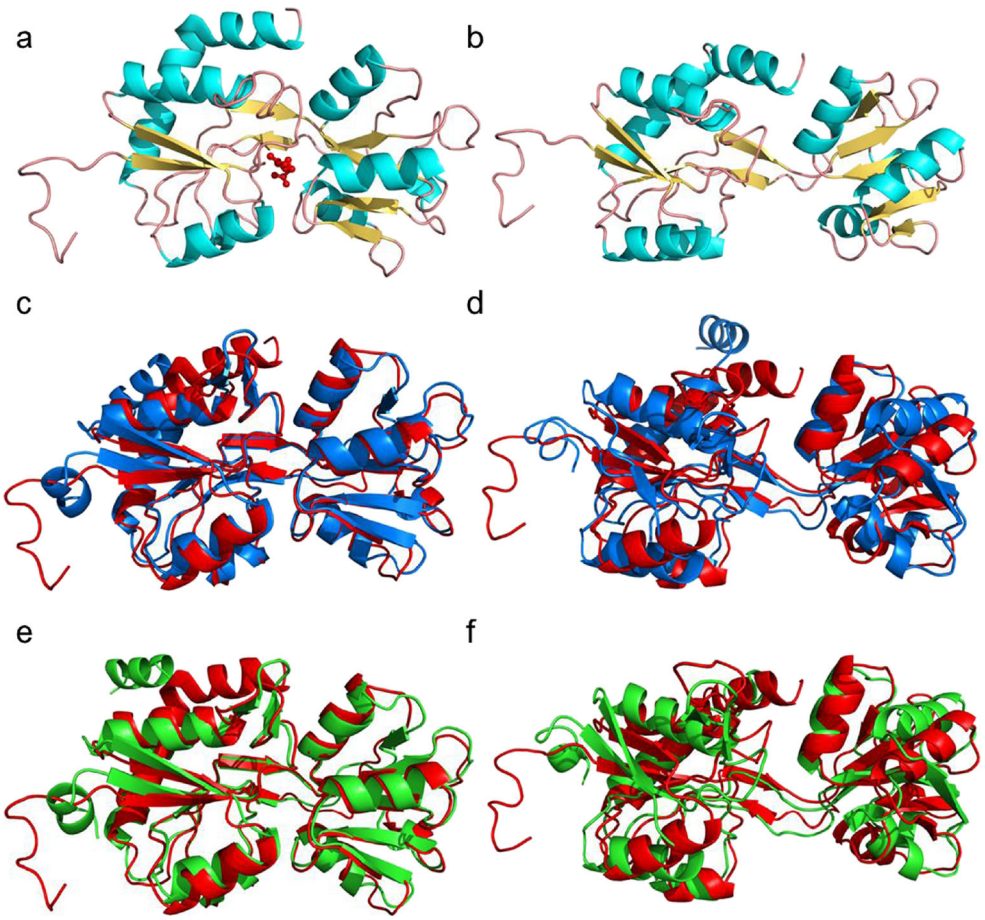

Fig. 1 **a** Homology model of the arginine-bound closed conformation of the arginine-binding protein (ArgBP) from the hyperthermophilic eubacterium *Thermotoga maritima* (TmArgBP, *red* arginine) [11]. **b** Homology model of the arginine-free *open* conformation of TmArgBP [11]. **c**, **e** Superpositions of the homology model of the *closed* conformation on the corresponding experimental structure of chain A (**c**) and chain B (**e**) of the experimental structure of closed TmArgBP [12]. **d**, **f** As in **c** and **e** but with the homology models superimposed on chains B of the experimental structures. Panels **c**–**f**: *red* homology models, *blue* A chains of experimental structures (**c**, **e**), *green* B chains of experimental structures (**d**, **f**)

UNRES has also simulated the open structure of the DnaK bacterial chaperone (starting from the closed structure) [18]; this structure turned out to be very close to the experimental structure [21] solved after the calculated structure was published. All these facts indicate that UNRES is capable of simulating the structure and dynamics of multi-domain proteins.

Methods

Modeling the closed and open structures of TmArgBP

As mentioned earlier, the crystallographic structures of the open and closed conformations of TmArgBP were unavailable at the time our study was carried out. Therefore, high quality homology-modeling structures [11] were used; it should be noted that these structures are very similar to the recently published experimental structures [12]. The root mean square deviation (RMSD) of the models from the experimental structures and the deviations of the C^{α} atoms of the models from their counterparts in the corresponding experimental structures are analyzed in subsection Quality of the starting structures of the Results and discussion section.

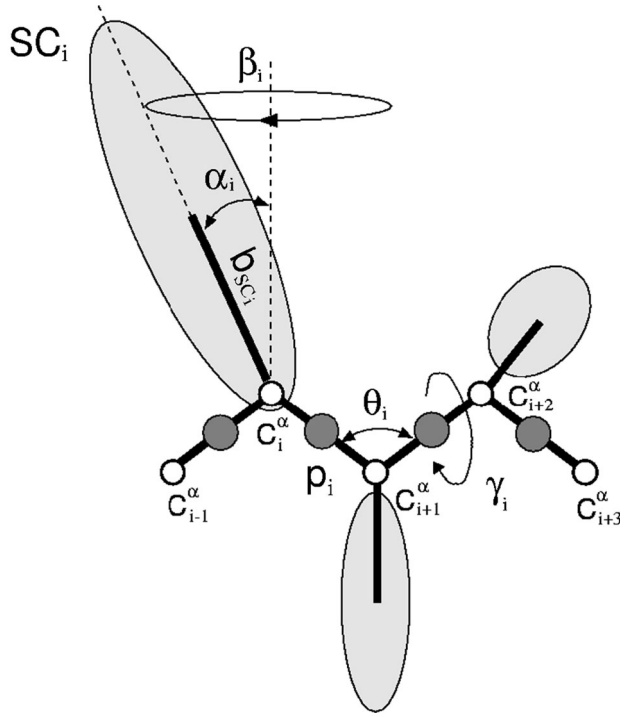

Fig. 2 United residue (UNRES) model of a polypeptide chain. Two types of interaction centers are present: peptide-bond centers (*p*), and side-chain centers (*SC*), both linked to the respective C^{α} atoms. The p centers are located halfway between two consecutive C^{α} atoms. θ Virtual-bond angles; γ virtual-bond dihedral angles; α_{SC}, β_{SC} angles that define the location of a side chain with respect to the backbone

The UNRES model of polypeptide chains

All simulations were carried out with the UNRES package [22]. In the UNRES model [22–26], a polypeptide chain is represented as a sequence of C^{α} atoms with united side chains (SC) attached to them and united peptide groups (p) positioned halfway between the consecutive C^{α}s (Fig. 2). The resulting reduction in the number of degrees of freedom and use of implicit water model results in a speed-up of calculations of over 1,000-fold compared to the all-atom treatment [27]. We used the version of the force field calibrated with the albumin-binding GA module (PDB ID 1GAB [28]) [29].

The UNRES energy function is expressed by Eq. 1:

$$\begin{aligned} U = {} & w_{SC}\sum_{i<j} U_{SC_iSC_j} + w_{SC_p}\sum_{i\neq j} U_{SC_ip_j} + w_{pp}^{VDW}\sum_{i<j-1} U_{p_ip_j}^{VDW} \\ & + w_{pp}^{el} f_2(T)\sum_{i<j-1} U_{p_ip_j}^{el} + w_{tor} f_2(T)\sum_i U_{tor}(\gamma_i) \\ & + w_{tord} f_3(T)\sum_i U_{tord}\left(\gamma_i,\ \gamma_{i+1}\right) + w_b\sum_i U_b(\theta_i) \\ & + w_{rot}\sum_i U_{rot}\left(\alpha_{SC_i},\beta_{SC_i}\right) + w_{bond}\sum_i U_{bond}(d_i) \\ & + w_{corr}^{(3)} f_3(T) U_{corr}^{(3)} + w_{corr}^{(4)} f_4(T) U_{corr}^{(4)} + w_{turn}^{(3)} f_3(T) U_{turn}^{(3)} \\ & + w_{turn}^{(4)} f_4(T) U_{turn}^{(4)} \end{aligned} \tag{1}$$

where the U values are the energy terms, each multiplied by an appropriate weight, w; θ_i denotes the ith backbone $C^{\alpha}\ldots C^{\alpha}\ldots C^{\alpha}$) virtual-bond angle; γ_i denotes the ith backbone $C^{\alpha}\ldots C^{\alpha}\ldots C^{\alpha}$) virtual-bond dihedral angle; and α_{SCi} and β_{SCi} are

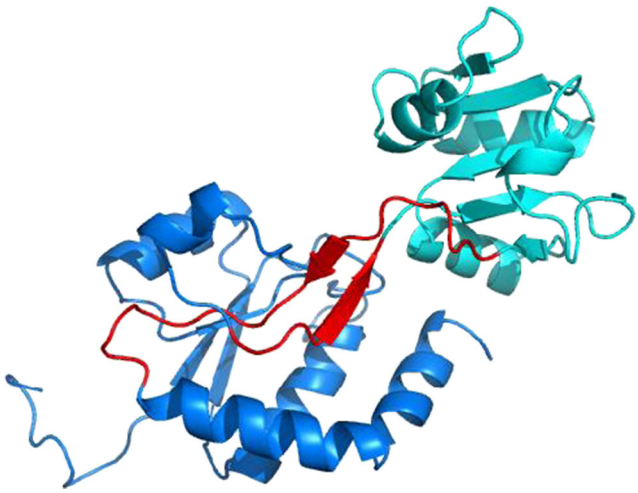

Fig. 3 Domain structure of TmArgBP. The N-terminal domain (residues 1-90 and 98-182) is colored blue, the C-terminal domain (residues 202-229) is colored cyan and the hinge region (residues 91-97 and 183-201) is colored red. The Ca-distance restraints were imposed on the N-terminal and on the C-terminal domain

the spherical angles that define the location of the *I*th side chain with respect to the backbone (Fig. 2).

The terms $U_{SC_iSC_j}$ denote the mean free energy of the hydrophobic (hydrophilic) interactions between the side chains, which implicitly contain the contributions from the interactions of the side chains with the solvent. The terms $U_{SC_ip_j}$ represent the excluded-volume potential of the side chain–peptide group interactions. The terms $U^{VDW}_{p_ip_j}$ and $U^{el}_{p_ip_j}$ are the peptide-group interaction potential for the Lennard-Jones interaction energy between peptide-group centers and the average electrostatic energy between peptide-group dipoles, respectively. The terms U_{tor} and U_{tord} correspond to the torsional and double-torsional potentials, respectively. The terms U_{b}, U_{rot} and U_{bond} are the virtual-bond angle-bending terms, the side-chain rotamer and the virtual-bond-deformation terms, respectively. The terms $U^{(\mathrm{m})}_{\mathrm{corr}}$ are correlation or multibody contributions from the coupling between backbone-local and backbone-electrostatic interactions, while the terms $U^{(\mathrm{m})}_{\mathrm{turn}}$ are correlation contributions involving m consecutive peptide groups; they are, therefore, termed turn contributions [22–26].

Molecular docking

To obtain the initial structures of TmArgBP with the arginine ligand, flexible molecular docking of arginine to the TmArgBP molecule in the open conformation was performed using AutoDock 4.2 and AutoDockTools from MGL Tools 1.5.7 Ri 1 [30]. The method of conformational searching was a Lamarckian genetic algorithm [31], with the following parameters: 150 individuals in the population, 100 hybrid GA-LS runs, 44×40×40 grid points defining the box size with all torsion angles of the heavy ligand's atoms free to change. The resulting conformations were clustered with a 2 Å RMSD cut-off and subsequently two conformations were used as starting structures for MD simulations with UNRES.

Table 1 Root mean square deviation (RMSD) values for C^{α} atoms of the homology modeled open and closed conformations of the hyperthermophilic eubacterium *Thermotoga maritima* (TmArgBP) from chains A and B, respectively, of the corresponding experimental structures [12]

Open TmArgBP		Closed TmArgBP	
4PRS chain A	6.547 Å	4PSH chain A	5.842 Å
4PRS chain B	7.024 Å	4PSH chain B	5.860 Å

MD simulations with UNRES force field

In order to maintain the secondary structure of the TmArgBP during the simulations, following the procedure of our previous work [17, 18], weak harmonic restraints were imposed on the $C^{\alpha}\cdots C^{\alpha}$ distances of both domains, as shown in Fig. 3. The weight of the distance-penalty function was w=0.05.

Simulations were performed using five different starting TmArgBP structures: (1) open structure without arginine; (2) closed structure without arginine; (3) open structure 1 with

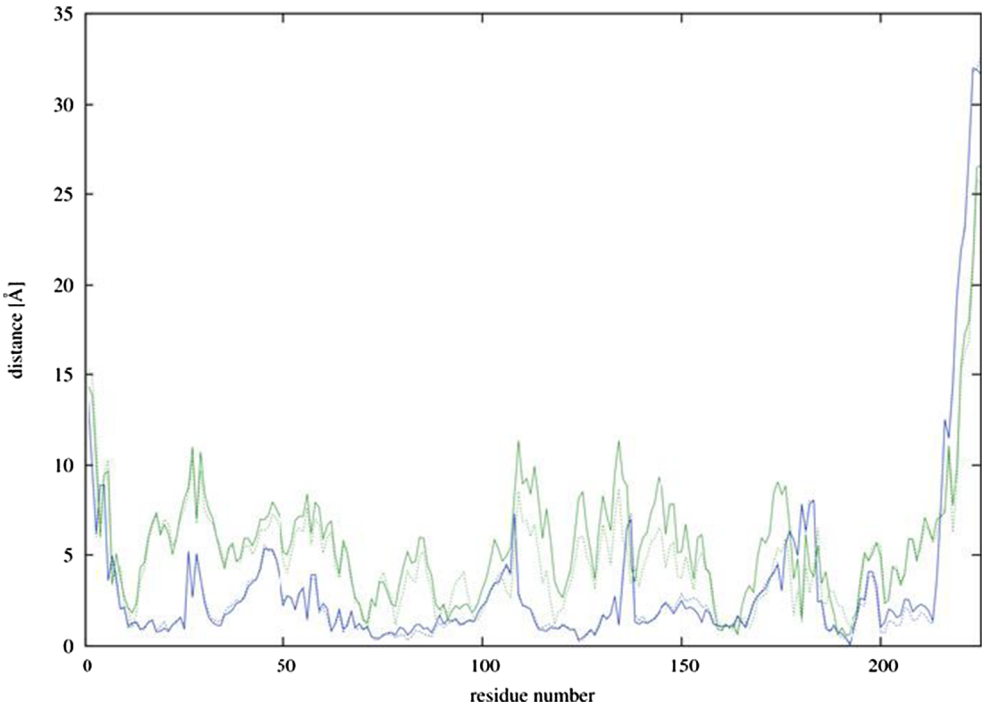

Fig. 4 Plots of the distances between the C^{α} atoms of the homology models of TmArgBP and the corresponding experimental structure in residue index after superposition of these structures onto the experimental structures. *Green* Open conformations, *blue closed* conformations, *solid lines* superposition of a model on chain A of the corresponding experimental structure, *dashed lines* superposition on chain B

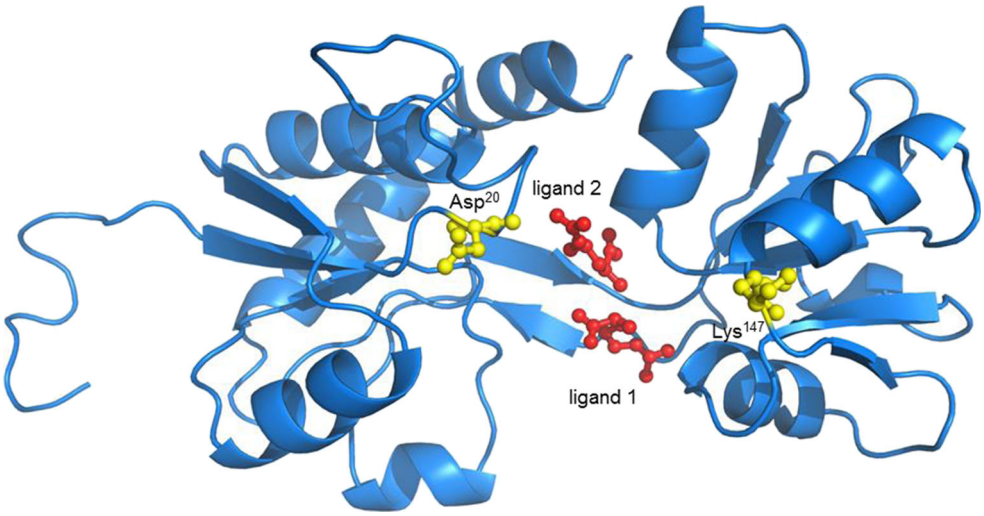

Fig. 5 Starting structures for UNRES simulations of the TmArgBP open conformation (*blue*) with arginine ligand (*red*) obtained by molecular docking: open structure 1 and open structure 2. Two amino-acid residues, Asp^{20} and Lys^{147}, are marked in *yellow*

arginine obtained by molecular docking; (4) open structure 2 with arginine obtained by molecular docking; (5) closed structure with arginine bound. Each starting structure was run at two temperatures: T_A=300 K (room temperature) and T_B= 353 K. The total number of trajectories without the arginine present was 32 (16 trajectories starting from the open conformation and 16 starting from the closed conformation). The runs with the ligand were started from three initial structures: the closed structure, open structure 1, and open structure 2. Each starting structure was run using two means of preventing the arginine ligand from escaping from the system: distance restraints (between the arginine ligand and the C end) or periodic boundary conditions (PBC), recently introduced into UNRES [32]. Thus, the total number of trajectories was 96 for the ligand-bound structures. Altogether, 16 series of runs were carried out.

The temperature T=300 K was chosen because many experimental data were obtained at room temperature [11–15], while the second temperature of 353 K was selected because it is the optimum growth temperature for *T. maritima* [33].

The total length of each trajectory was 10,000,000 steps (except for the series with the closed conformation without arginine, where 20,000,000 steps were run at 300 K), with a time-step length of 5 fs. It should be noted that, because of averaging of the fine-grain degrees of freedom, one time unit of UNRES simulations corresponds to at least 1,000 units of real time; [34] therefore the real time of each trajectory is about 50 μs (100 μs for the closed conformation without arginine at 300 K).

Initially, short equilibration simulations with a small time step (0.5 fs) and restraints imposed on the whole protein were performed until the system thermalized. Subsequently, production MD simulations with a longer time step of 5 fs were run.

The distances between the two amino acid residues Asp^{20} and Lys^{147} (Fig. 5), which served to anchor fluorescence markers, were computed to monitor the relative domain arrangements, represented in the form of histograms with distances binned every 0.5 Å, and normalized. The data from the second part of the production section of each trajectory was used for analyses.

The free energy differences of the transition from the closed to the open conformations were calculated from Eq. 2:

$$\Delta G = -RTln\left(\frac{n_{open}}{n_{closed}}\right) \quad (2)$$

where R is the universal gas constant, T is the simulation temperature, and n_{open} and n_{closed} are the numbers of the open and the closed conformations in given simulations, respectively. A conformation was considered closed when the distance between the geometric centers of the domains (calculated over the C^{α} atoms) was less than 11.86 Å, which is the arithmetic average of the distance of the open and of the closed conformation. The data from the second half of the production section of each trajectory were used in the calculations.

Results and discussion

Quality of the starting structures

As mentioned in the Introduction, homology models of TmArgBP were used to generate the starting structures for UNRES simulations. As can be seen from Figs. 1 and 4, the most significant differences between the homology-modeled and the experimental structures are in the N-terminal and C-terminal regions. Moreover, the domains of the open conformation are shifted more than in the closed conformation, with the main difference being in the position of the C-terminal helix. The differences between the homology models and

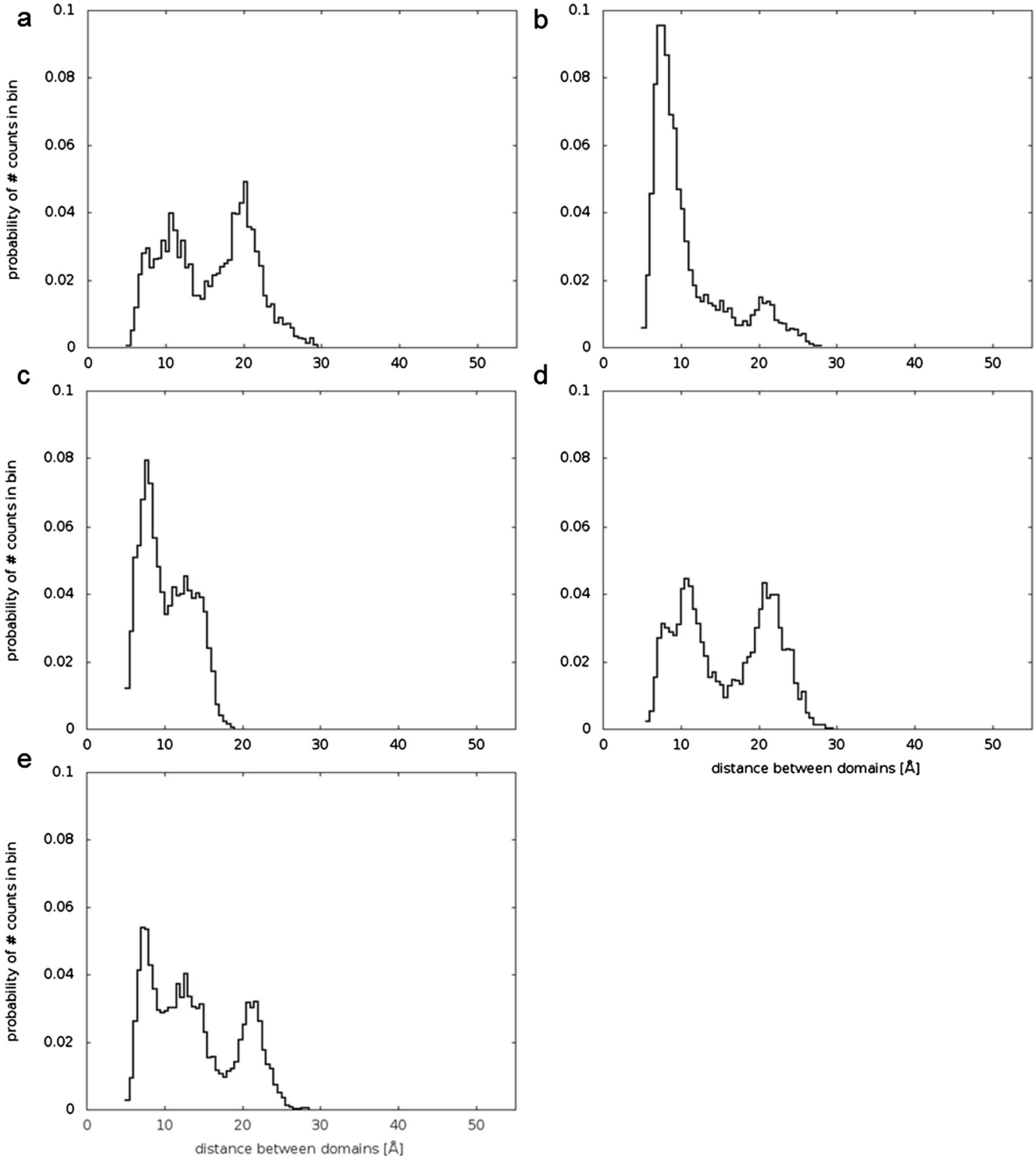

Fig. 6a–e Histograms of the distances between the domains obtained from UNRES/MD simulations at 300 K, without periodic boundary conditions (PBC). **a** Starting from the open structure, no arginine bound. **b** Starting from the closed structure, no arginine bound. **c** Starting from open structure 1, with arginine present. **d** Starting from open structure 2, with arginine present. **e** Starting from the closed structure, with arginine present

the experimental structures along the chain are visualized in Fig. 4, in which the distances between the corresponding C^{α} atoms are plotted. For the closed conformation, these distances are less than 2 Å; for the open conformation the deviations are larger (up to 8 Å). The probable reason of this shift is that dimers, in which the loops preceding the C-terminus interact with each other, are present in the crystal structures. However, those difference should not affect the

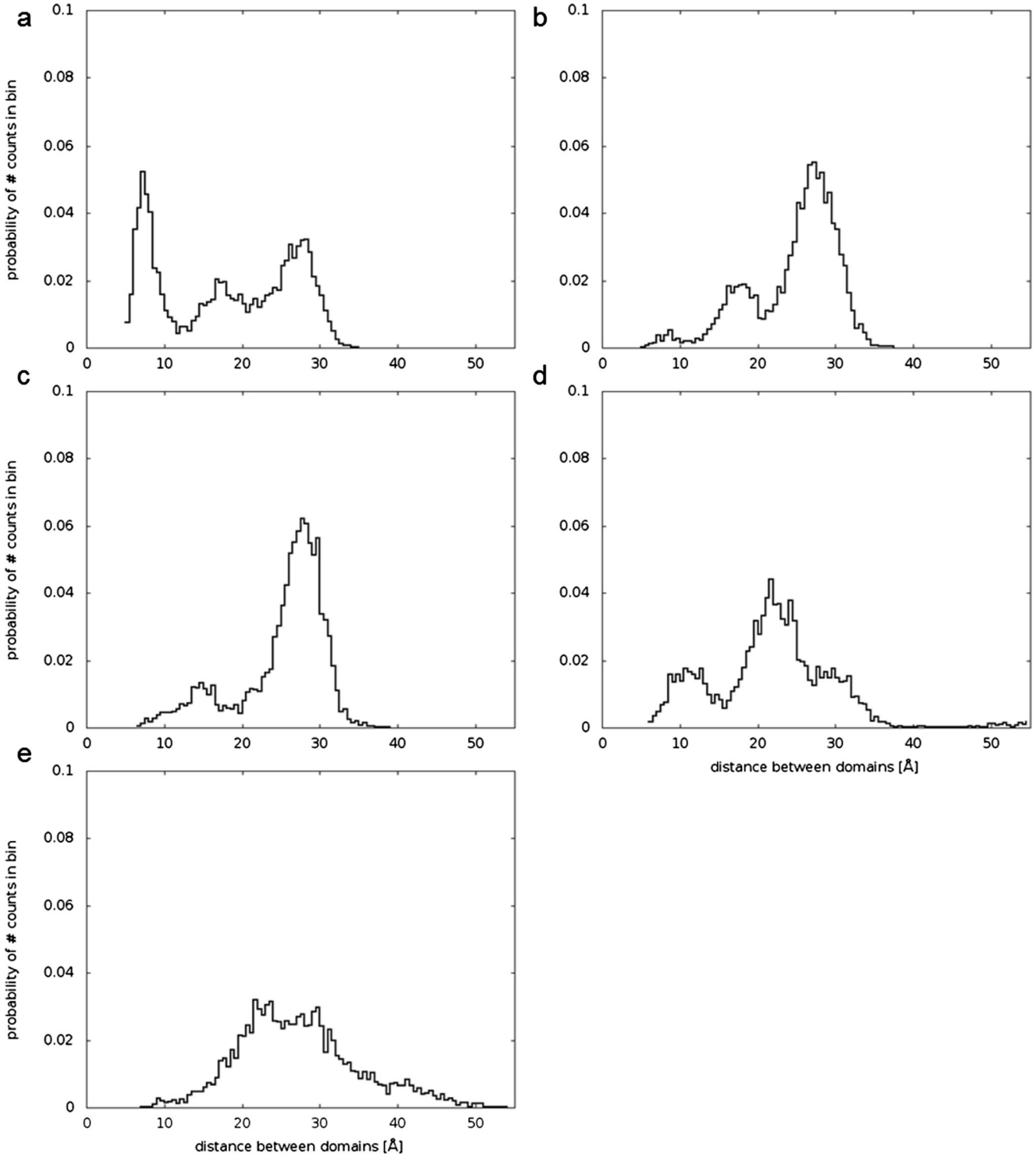

Fig. 7a–e Histograms of the distances between domains obtained from the simulations at 353 K, without PBC. **a** Starting from the open structure, no arginine bound. **b** Starting from the closed structure, no arginine bound. **c** Starting from the open structure 1, with arginine present. **d** Starting from the open structure 2, with arginine present. **e** Starting from closed structure, with arginine present

performed simulation significantly as the homology modeled structures are still very similar. The C^{α} RMSD values, given in Table 1, are in the range of UNRES resolution for proteins with the size of TmArgBP [20], which means that the structures used in this research are of sufficiently good quality to be used as starting structures.

The values of the $C^{\alpha}(Asp^{20})\cdots C^{\alpha}(Lys^{147})$ distances for the open and the closed form of the TmArgBP-homology-

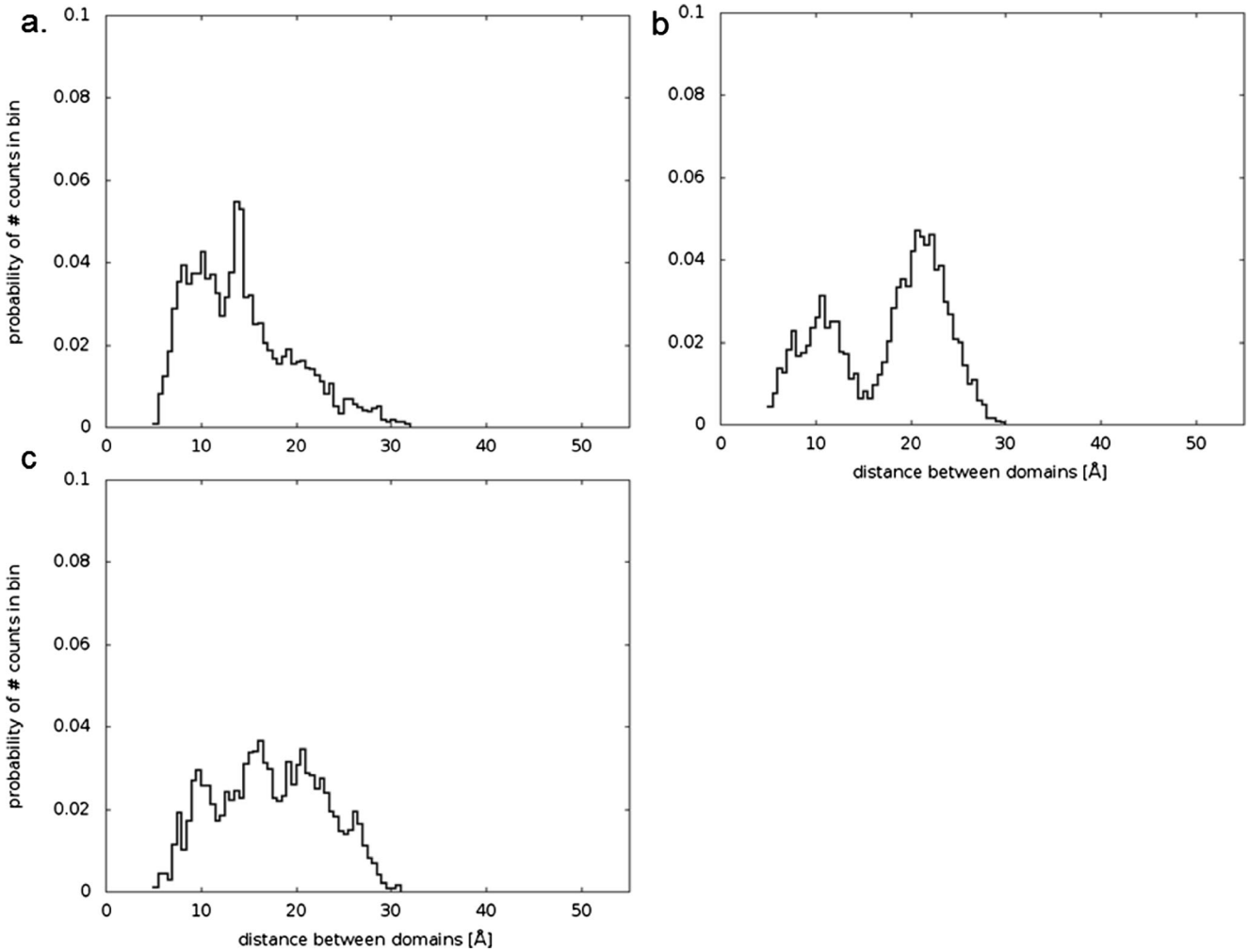

Fig. 8a–c Histograms of the distances between domains obtained from the series at 300 K, with PBC. **a** Starting from the open structure 1, with arginine present. **b** Starting from the open structure 2, with arginine present. **c** Starting from closed structure, with arginine present

modeled structures [11] are 17.57 and 6.15 Å, respectively, which are close to those corresponding to the experimental structures, i.e., 21.38 Å (chain A) and 21.29 Å (chain B) for the open conformation and 6.47 Å (chains A and B) for the closed conformation, respectively.

Molecular docking

Two starting positions of the open TmArgBP conformation with the arginine ligand are shown in Fig. 5. These two structures were chosen from the nine clusters obtained during molecular docking in respect of the placement of the acidic amino acids near the ligand.

Molecular dynamics simulations with the UNRES force field

Histograms depicting the $C^{\alpha}(Asp^{20})\cdots C^{\alpha}(Lys^{147})$ distances for each of the 16 series (averaged over eight trajectories) are shown in Figs. 6, 7, 8 and 9. The free energies of the transition between the open and the closed conformations are shown in Table 2. Because individual domains were restrained, the corresponding open and closed structures obtained in simulations are similar to the structures of the homology models and the experimental structures shown in Fig. 1.

Figure 6 shows histograms computed from the data from the simulations performed at T=300 K without PBC. The results of simulations carried out without the presence of the arginine ligand, starting from both the open and the closed conformations of the TmArgBP are shown in Fig. 6a and b, respectively. Two peaks are observed in the histograms for the open conformation: one with a maximum near 10 Å interdomain distance and the other for longer distances. As can be seen from the plots and from the free energies summarized in Table 2, the conformations obtained in simulations starting from the closed state remain closed. These data suggest that the closed conformation is more stable and less prone to change than the open one at 300 K.

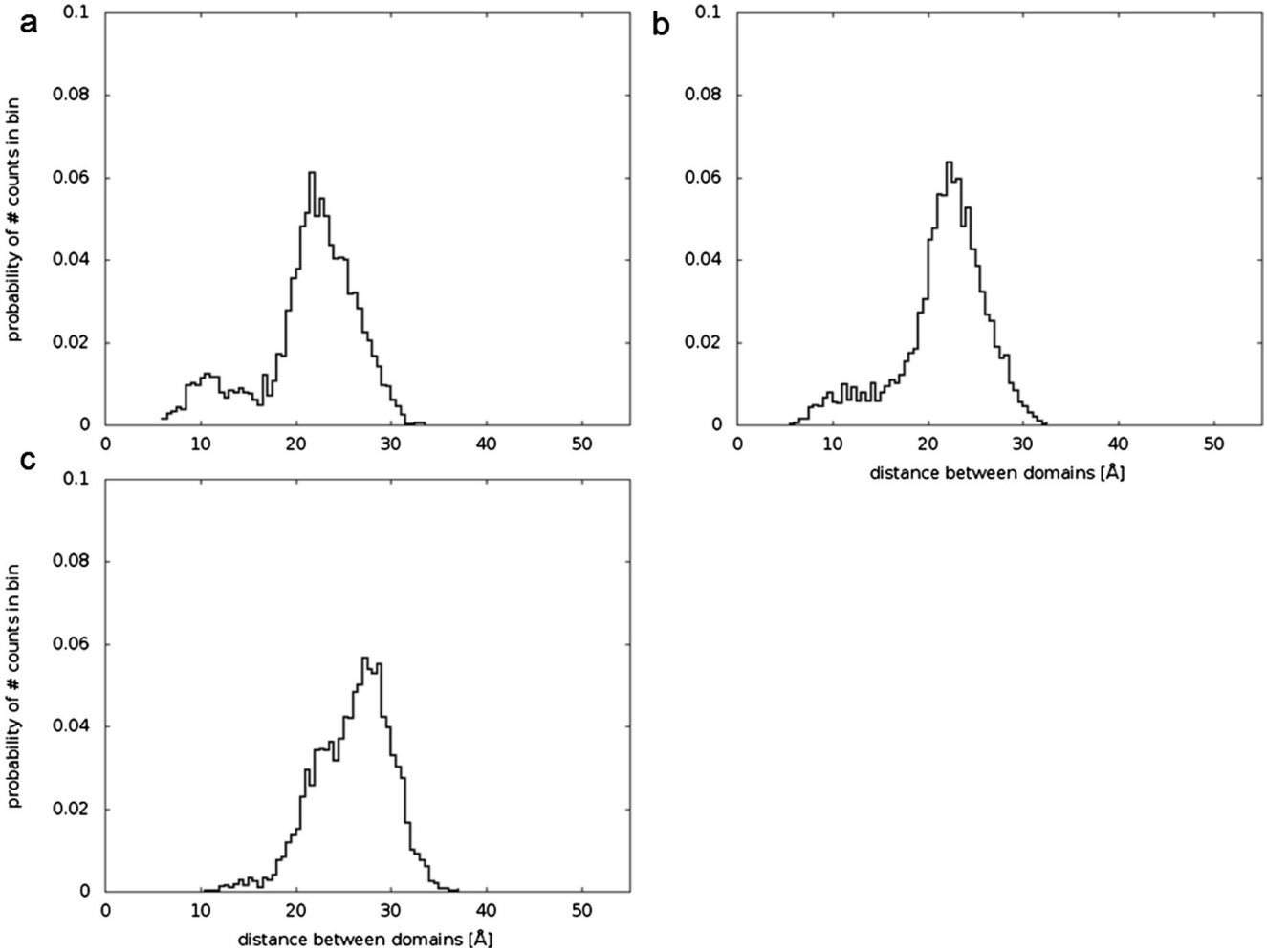

Fig. 9a–c Histograms of the distances between domains obtained from the series at 353 K, with PBC. **a** Starting from open structure 1, with arginine present; **b** Starting from open structure 2, with arginine present; **c** Starting from the closed structure, with arginine present

The histograms of the simulations performed with the arginine ligand present are shown in Fig. 6c–e. The results for open structure 1 with arginine and open structure 2 with arginine, both obtained from molecular docking (Fig. 6c, d), as well as the free energies summarized in Table 2, show that the closed conformations appear frequently and are observed to be in the majority for open structure 1. This may suggest that this position is more accurate compared with open structure 2. For simulations starting from the closed arginine-bound conformation (Fig. 6e), it can be observed that a significant fraction of the protein stays in the closed conformation (Table 2). Thus, the open conformations are most likely to close when the arginine ligand is present. This observation suggests that the presence of the arginine lowers the free-energy barrier between the open and closed conformations. This result is in agreement with experimental data that demonstrated the ability of the protein to bind the arginine ligand, which increases the stability of the protein [11, 13, 14], by converting into the closed form.

Table 2 Free-energy differences, ΔG (kcal mol^{-1}) (Eq. 2) between the open and closed conformations of TmArgBP calculated from UNRES/MD simulation data. *PBC* Periodic boundary conditions

Starting conformations	With restraints on arginine		With PBC	
	300 K	353 K	300 K	353 K
Open without arginine	−0.434	−0.482	–	–
Closed without arginine	0.615	−2.446	–	–
Open with arginine: open structure 1	0.385	−2.379	−0.269	−1.646
Open with arginine: open structure 2	−0.370	−1.353	−0.644	−1.985
Closed with arginine	−0.145	−3.113	−0.786	−5.447

The results of the simulations at 353 K without PBC are shown in Fig. 7. A broader distribution of the interdomain distance values and a more balanced ratio of the open to the closed form during the simulations without the presence of the arginine ligand can be observed (Fig. 7a, b). Moreover, comparison of the number of conformational changes from the open to the closed conformation, and vice versa during the

entire simulation time for each trajectory demonstrates more significant changes for the simulations performed at a higher temperature (150 at 353 K and 115 at 300 K). Taking into account the fact that the flexibility of a protein is fundamental for its functionality, more frequent transitions of TmArgBP between the open and closed conformations at 353 K suggest that the protein functions better at a higher temperature. This observation agrees with the fact that the optimal growth temperature of *T. maritima* is relatively high (T=353 K).

In the series of simulations with the arginine ligand present, it was observed that the ligand left the protein very early during the simulation. This might be an actual effect, taking into account the transporting function of the protein [1], but it might also be due to the fact that the version of the UNRES used in this study does not have specific potentials for salt bridges, even though the Arg-Asp, Arg-Glu, Lys-Asp and Lys-Glu potentials possess deep minima corresponding to salt bridges. Nevertheless, the initial period during which the arginine ligand was bound to the protein was sufficient to bring the two domains together.

In order to avoid the escape of the arginine ligand too far from the protein in simulations without using harmonic restraints on the protein–arginine distance, a series of simulations was carried out in the PBC mode. The results of MD simulations at T=300 and T=353 K are shown at Figs. 8 and 9, respectively. The same general trend as that found in the previous simulations, performed without PBC (but with arginine restrained to bind the protein) can be observed. At elevated temperature, the fraction of open structures generally increases (Figs. 8, 9; Table 2). From the free energies summarized in Table 2, it can also be gathered that the fraction of open structures is larger compared to simulations in which arginine was restrained to bind to the protein; this observation suggests that arginine binding results in the formation of the closed conformation of TmArgBP.

The experimental data show that TmArgBP has an open conformation in the absence of the arginine ligand and a closed conformation in the presence of the arginine ligand [11, 13–15]. In our simulations, the arginine-free protein often formed closed conformations, while the initially arginine-bound protein often formed open conformations (Table 2) (however, as already mentioned, ligand loss occurred quite frequently). However, the protein forms a dimer at room temperature [12, 15] and for two other PBPs: TM0322 from *T. maritima* [35] and from *Rhodobacter sphaeroides* [36], it is thought that the conformational changes induced by ligand binding lead to multimerization, and that these changes are necessary for ligand transport [35, 36]. It has been proved that both the ligand-free and the ligand-bound forms of TmArgBP are dimeric and the former shows a more packed quaternary structure [12]. Thus, it is possible that the proper domain arrangement can be achieved only in the dimer.

Conclusions

This work investigated conformational changes in the tertiary structure of TmArgBP, a two-domain protein [11], in the presence and the absence of the arginine ligand using coarse-grained MD with the UNRES force field [22–26]. It was found that the protein jumps between the closed and the open conformation, the two conformations differing in the separation of the two domains (Fig. 1). The presence of arginine, which interacts with acidic residues, was found to promote the formation of the closed conformation at 300 K (Figs. 6, 7) but at the higher temperature of 353 K (optimal for the growth of *T. maritima*—the host organism of this protein) no apparent effect of the presence of arginine could be observed on the frequency of occurrence of the open and closed conformations (Figs. 7, 9), The different simulation results of TmArgBP at two different temperatures might reflect the behavior of the protein; however, it should be kept in mind that part of the difference might result from low resolution of coarse-grained simulations. A hybrid representation of the system, in which regions requiring more detailed consideration (the arginine molecule and the residues of TmArgBP that bind it) would probably be required to settle the question. Extension of the UNRES model in this direction is now being undertaken in our laboratory. Nevertheless, it can be concluded that the sensitivity of the conformation of TmArgBP to arginine decreases with increasing temperature. At elevated temperature, the protein also becomes more flexible, i.e., the transitions between the closed and the open conformation occur more frequently, which might be related to the functionality of this protein at relatively high temperatures.

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