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Brownian dynamics simulation of the competitive reactions: Binase dimerization and the association of binase and barstar

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Abstract

A comparative study of the competitive reactions–the association reaction of binase with polypeptide inhibitor barstar and the reaction of binase dimerization–has been performed by the Brownian dynamics simulation method. It was shown that three types of the binase dimers could be formed and the dimerization reaction could compete with the inhibition reaction. The first type of the dimers leaves the active centre of binase free. During the formation of the dimers of the second and the third types the active centre of one or both binase molecules is blocked and ribonuclease becomes partially or fully inactive. Brownian dynamics simulation shown, that the ratio of competitive reaction rates depends on pH and ionic strength of solution.

Keywords: Binase; Barstar; Dimer; Electrostatic interactions; Brownian dynamics simulation

1. Introduction

The specific recognition of proteins and the interaction of two protein molecules underlie the most biochemical processes. That is why plenty of theoretical and experimental works are devoted to the study of this problem [1,2]. Increasing number of publications studying protein interactions using computer simulations confirms the actuality of the problem, as well as the applicability of these methods for such studies [3,4].

It is now accepted that the binding of two macromolecules proceeds in two stages. For electrostatically interacting proteins, the first process includes the region of free diffusion, when the proteins are far apart and the region of electrostatic steering. At the end of first stage, two molecules form an intermediate, a diffusion encounter complex (EC) [5–7]. At the second stage, this intermediate converts into the resultant bound complex under influence of the short-range forces and the subtle reorganization of the interface. If the transition of the EC into a bound complex is faster than the first stage, the association rate is determined by the rate of EC formation. Brownian dynamics simulation method (BD) is well suited for the study of such reactions. It was successfully applied for the protein–protein association [8,9], dimerization reactions [10] and antibody–antigen interaction investigations [11]. Gabdoulline and Wade [5] used BD for studying association reaction barnase with barstar. Authors of the works [5,12,13] shown that electrostatic interaction plays a key role in this reaction, investigated the effect of mutations at individual amino acid residues on the inhibition rate and observed a good agreement between calculated results and experimental data [14]. The leading role of electrostatic interactions in EC formation of binase–barstar complex was recently confirmed by Ababou A. et al. [15] by semi-empirical quantum chemical method.

The object of investigation of this work is a *Bacillus intermedius ribonuclease* (binase). Binase is a homologue of barnase with a degree of homology amounting to 85%; it has 17 amino acid substitutions located mainly on the surface of the globule. However, the activity of binase differs considerably from that of barnase [16,17]; the kinetic characteristics of the binase–barstar complex differ from the characteristics of barnase–barstar complex

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as well. It was demonstrated [18,19] that the association constant for the binase–barstar complex is by one order of magnitude lower than the constant for barnase inhibition by barstar.

Previously, we have shown [20] that binase could form two types of complexes with barstar. In type I complex, the amino acid residues of the binase active centre are involved in the formation of the complex; in type II complex, the active centre of binase remains free. It was suggested that temporary binding of free barstar into type II complex competes with the inhibition reaction. The goal of the present work is to study the binase dimerization reaction by Brownian dynamics simulation method. We suppose that binase dimerization reaction as well can compete with the association reaction of binase and barstar.

2. Material and methods

Brownian dynamics simulation method allows one to model the behaviour of two rigid macromolecules of irregular form in solution under the influence of an electrostatic interaction with each other. In this work, we used the program MacroDox, developed by S.H. Northrup [21]. This package was used to assign the titratable charges for protein, solve the linearized Poisson-Boltzmann equation and run the BD simulations. The details of the algorithms are given in Northrup et al. publications [22,23]. The general characteristics of the method and its advantages and shortcomings have been discussed in details in the literature [5,8]. Using protein atomic coordinates, charges of titratable amino acids were assigned for solvent ionic strength of 0.1 M, temperature of 298 K and pH values varied from 5.0 to 10.0 by Tanford–Kirkwood method [24,25]. Electrostatic interactions (long-range forces) between two reactants were included in this model as a basic feature along with short-range repulsive forces. Electrostatic potential around molecules was determined by solving the linearized Poisson–Boltzmann (PB) equation numerically using Warwicker–Watson [26] method, implemented in the program MacroDox. The PB equation was solved for two cubic lattices, and the centre of mass (COM) of the molecule was placed at the origin of lattice. The resolution was equal to 0.9 Å for inner grid and to 2.7 Å for outer grid, respectively. The dielectric constant used in the calculations was set at 4 for proteins and at 78 for solvent. Trajectories were calculated using Ermak-McCammon algorithm [27]. At the beginning of each trajectory, the centre of mass of second protein was randomly placed on the spherical surface with the radius of b (60 Å in this study) from the COM of first protein. Then, second molecule was allowed to rotate and translate. If the distance between COMs of two molecules reached escape radius c (200 Å) the trajectory was terminated. Coordinates of second particle at the time moment of $t+\tau$ were defined according to equation

$$r(t+\tau) = r(t) + \frac{D\tau}{\mathrm{kT}}F + R(\tau)$$

Here D is the spatially isotropic translation diffusion coefficient for relative motion, F is the direct interparticle (electrostatic) force, $R(\tau)$ is the random displacement vector used to simulate solvent-mediated Brownian translation motion. Similar equation was used to describe a rotational motion of particles. In our calculations D was equal to 0.029 $Å^2\ ps^{-1}$ for binase–barstar relative motion and 0.027 $Å^2\ ps^{-1}$ for dimerization reaction, both molecule can be rotated, rotational diffusion coefficients were $0.4 * 10^{-4}\ ps^{-1}$ and $0.45 * 10^{-4}\ ps^{-1}$ for binase and barstar, respectively.

Encounter complex is formed when two atoms from different proteins come as close as X , where X is the "reaction criterion". In that case, the trajectory is considered to be successful. The number of successful trajectories is used to estimate the rate constant for association. The rate constant for binase dimerization was calculated according to Northrup's equation [21,22], derived using Smoluchovski theory [28]:

$$k = k_D(b)\frac{p}{1-(1-p)k_D(b)/k_D(c)}$$

$$k_D(x) = 4\pi x \mathrm{D} N_A$$

Here p represents the number of successful trajectories as a fraction of collected trajectories, and N_A is the Avogadro's number.

As far as we know, a strict definition of the encounter complex, as well as the criteria for its determination, are still absent [4,7]. Several recently published papers [5,29] discuss this issue. Encounter complex is defined [5] in terms of an ensemble of configurations of the two proteins. Each structure from this ensemble suits some conditions. Spaar et al. [29] defined EC as the minimum of the free-energy landscape in the diffusional regime. Most frequently, the EC is considered formed if the distance between the prespecified contact groups is smaller than a certain critical distance. This distance is called a reaction criterion (RC). In our calculations RC was equal 7 Å. It is larger then 5 Å taken as RC in [13] and less the 10 Å, which was identified by Spaar et al. [29] as a minimum distance between contact groups in barstar–barnase EC. We believe that 7 Å are enough large to make simplifications of the BD simulation method unimportant. These simplifications become important only at small protein–protein separations: the proteins are modelled as rigid bodies and short-range interactions as van der Waals forces and the formations of hydrogen bonds and salt bridges are not modelled. Meantime this distance is enough little to transform the EC into the binding complex under the short-range forces. Moreover, the comparison of our calculated barnase-barstar association rate constants with experimental data gave the best result at RC equal to 7 Å [20]. If the complex structure is unknown it is useful to use energetic criterion [9] to analyse the most energetically favourable complexes. Two kinds of simulations were performed in this work. At first of them 250,000 trajectories were run and complexes with negative interaction energy were analysed without biasing the reaction criterion for particular types of complexes. Second kind of simulations was run for 3 different predefined encounter complexes and the rate constants of each complex formation were calculated. The choice of the contact groups for second kind of calculation was based on the results of the first one.

3. Results and discussion

Binase and barstar structures were taken from Protein Data Bank [30] (pdb entries are 1bue and 1btb). The distribution of electrostatic potential around the proteins was calculated, depending on pH and ionic strength of solution, using Tanford–Kirkwood theory [24]. There are two extended, positively charged regions on the surface of binase, formed by amino acid residues Lys26, Arg82, Arg86, Arg58 and Arg15, Lys17, Arg107, Arg109. Residues from the first group are directly involved in the complex formation with barstar during the reaction of inhibition. This complex (Type I) is characterized by the strong charge and the surface complementary of two interactive proteins. Previously, we have shown [20] that binase can also form second type of complexes with barstar (Type II), in which positively charged residues from the second group, Arg15, Lys17 and Arg109, interact with negatively charged residues of barstar, Asp35 and Glu80. In this case the active centre of binase remains unoccupied. The formation of the Type II binase–barstar complex competes with the reaction of inhibition.

In this paper we analyze the reaction of binase dimerization, which can also compete with the reaction of binase inhibition by barstar.

250,000 trajectories were collected to find the most energetically favourable orientations of two binase molecules in the dimer. The structures of the complexes with the electrostatic interaction energy below −2 kcal/mol were selected from each trajectory and stored for the further analysis.

The distribution of the centre of mass (COM) of the first binase monomer around the second molecule is presented in Fig. 1 for the most energetically favourable orientations of these proteins in the dimers. The surface of the first binase molecule is coloured according to electrostatic surface potential. Positively charged regions are dark blue. Dots represent centres of mass of the second molecule.

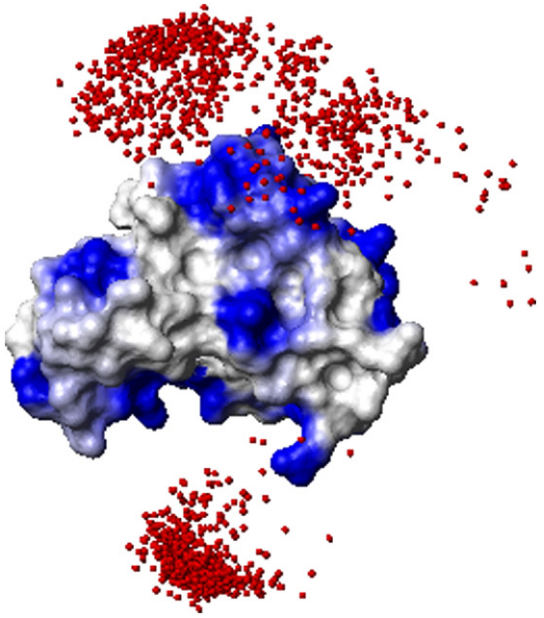

Fig. 1. The distribution of the first binase molecule centres of mass around the second molecule for the most energetically favourable orientations of these proteins in the dimers. The surface of the central molecule is painted according to surface electrostatic potential of binase. Positively charged regions are dark blue. Calculation of electrostatic potential and picture were carried out using MOLMOL [34] program.

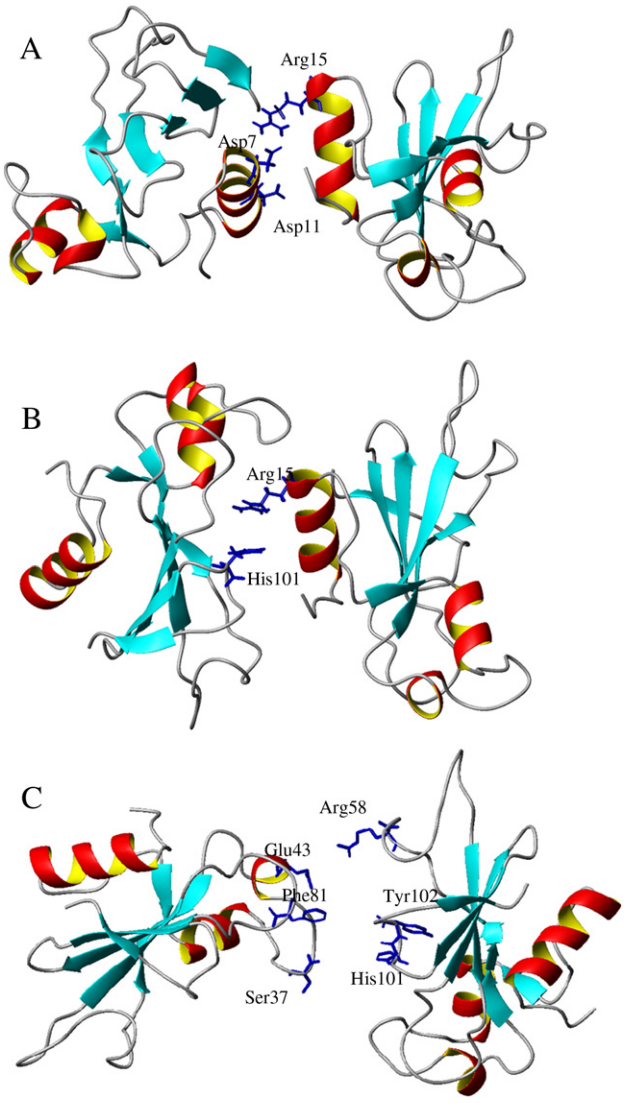

Fig. 2. Three types of binase dimers. Dimer of type I (A), dimer of type II (B) dimer of type III (C).

Analysis of the most energetically favourable dimer structures shows that the residues Asp7, Asp11, Arg15, Arg58, Glu59, Arg61, Lys97, Arg107, and Arg109 are frequently involved in the complex formation, and the pairs of residues Asp7-Arg15, Asp7-Arg107, Asp7-Arg109, Asp11-Arg107, Asp11-Arg109, Arg15-Arg109, Lys97-Arg109, Arg107-Arg109 and Arg109-Arg109 are formed most frequently. The complex surface charge distribution and good accessibility of charged amino acid residues to water allow two positively charged residues be placed in the close proximity from each other even if it is energetically unfavourable. The formation of contacts Asp7-Arg58, Asp7-Arg107, Asp11-Arg58, Arg15-Glu59, Asp11-Arg61, Arg58-Asp85, Arg71-Asp7, Lys97-

Asp21 gives a main contribution to the energetic stability of the dimers.

Three types of binase dimers can be formed. The typical structures of all three types of dimers, taking as snapshots during the trajectory construction, are drawn in Fig. 2, as an example. In the type I dimer, the active centres of both binase monomers are freely accessible. Residues Arg15, Arg107 and Arg109 from one monomer are in a close contact with residues Asp11, Asp7 from the second monomer. Additional stabilization of these complexes is achieved by the creation of the second or the third contact pairs with distances between contact atoms about 4-8 Å. The formation of the contacts of Asp7-Arg15, Asp7-Lys17, Asp7-Lys97, Asp7-Arg107, Asp11-Arg15, Arg15-Asp7, Arg15-Asp11 gives a main contribution to the energetic stability of the type I dimers. One of the typical dimer of type I is drawn in Fig. 2A. Some of the contact groups are labelled. Contact area is calculated as the decrease of the solvent accessible surface area (SASA) during the complex formation and for this complex it is equal to 310 $Å^2$.

In the type II binase dimer, one monomer has freely accessible active centre, whereas the access to the active centre of the second monomer is hampered. Amino acid residues Asp11, Arg15 of the first binase molecule contact most frequently with His101, Arg82, Glu72, Arg86 and Asp100 amino acids of the second molecule. For the complexes of type II the most intimate contact pairs are formed by Asp11-Arg58, Asp11-Arg82, Asp11-His101, Arg15-Arg86, Arg15-Glu72 and Arg15-Asp101. The formation of contacts Ala1-Asp100, Asp7 –Arg58, Asp11-Arg58, Asp11-Arg82, Arg15-Asp86, Lys17-Asp100 gives a main contribution to the complex energetic stability. One of the typical complexes of type II is represented in Fig. 2B. Contact area for this complex equals 395 $Å^2$.

During the formation of the type III dimers the active centres of both binase molecules are blocked. Amino acid residues Arg58, Arg61 or Ser37, Lys38, Glu43 of one binase molecule are in a close contact with amino acids His101, Arg82, Arg86, Asp100 of the second molecule. Additional stabilization of these complexes is achieved by a creation of the additional contacts between the opposite charged amino acids: Arg86-Glu59, Arg82-Asp100, Arg61-Glu59, Glu59-Arg107, Glu43-Arg58 and Lys38-Asp100. One of the typical complexes of type III is shown in Fig. 2C. Contact area for this complex equals 210 $Å^2$. Consequently, the formation of type II or type III binase dimers sterically masks the active centre of one or both monomers and molecule of binase becomes partially or fully inactive.

In order to calculate EC formation probability for all types of complexes, the second kind of calculations was run. Analysis of the most energetically favourable orientations of two binase molecules for all three types of complexes has allowed assigning the contact groups for these complexes. Amino acid residue Arg15 of the first molecule and Asp11 of the second molecule were chosen as a contact groups for the complex of the type I, amino acids Arg15 and His101 for the complex of the type II, respectively. For dimers of the type III amino acid residues Arg58 or Ser37 of the first molecule and His101 of the second molecule were taken as a contact groups.

Calculated association rate constants of the formation of encounter complexes are listed in the Table 1. These rate constants have been obtained for the ionic strength of solution of 0.1 M at pH 7 by using reaction criterion of 7 Å. For the III type dimers the average value of the reaction constants for two complexes is given. In the first kind of complexes, His101 of one binase molecule forms a contact with Arg58 of the second binase molecule. In the second kind of complexes, the same histidine contacts with Ser37 of the second binase molecule. The access to the active centres of both molecules is blocked in all complexes of the III type; and both complexes can be transformed into each other by the rotation of one or two binase molecules around their own rotation axes. This is why we classified these complexes as the same type of dimer.

The association rate constant of the reaction of inhibition of binase by barstar is also given in the Table 1, for comparison. The pH and ionic strength values were kept the same as for the reaction of binase dimerization. The reaction of association binase and barstar is more energetically favourable than the reaction of binase dimerization. The interaction energy of two monomers in dimers falls in the range of −4.0 to −6 kcal/mol, whereas the interaction energy of binase and barstar in a binase–barstar complex changes from −6.0 to −11 kcal/mol.

The existence of binase dimers has been detected experimentally — by hydrogen exchange and NMR relaxation methods [31], and by x-ray crystallography [32]. Pavlovskiy et al. [32] found the binase dimers in the crystal form and supposed that binase dimers could exist in solution also.

We fulfilled the analysis of macromolecular structures (http://pqs.ebi.ac.uk) that could be formally described as binase dimers (pdb entries 1GOU, 1GOV, 1GOY) [30]. Despite of the strong analysis does not allow defining these structures as dimers (solvent accessible surface area decreases significantly (661.2 $Å^2$ for 1GOU) upon complex formation but the interaction energy is positive (5.6 kcal/mol)), the comparison of these structures with calculated ones displays the similarity of the experimental structures with the dimer of type III. Encounter state as defined in this article and another publications [5,29] is not a single conformation like the bound state but rather a cloud of conformations. Backbone root-mean-square deviations (RMSD) of the calculated structures of type III from the experimental structures is in diapason from 13 Å to 21 Å. The RMSD is defined as follow: one binase molecule of the calculated complex is superimposed on the first binase molecule of the experimental

Table 1
EC formation rate constants for the different kinds of the binase dimers

Type of complex	First protein contact group	Second protein contact group	Rate constant (M^{-1} s^{-1})
Dimer of type I	NH2 Arg15	OD1 Asp11	$1.9*10^8$
Dimer of type II	NE2 Arg15	NE2 His101	$8.5*10^7$
Dimer of type III	NH2 Arg58 or OG Ser37	NE2 His101	$3.5*10^7$
Barstar–binase	OD2 Asp35	NE2 His101	$1.9*10^8$

complex and the RMSD is calculated between the second molecules of the complexes. For example, the RMSD of backbones for the structure shown in Fig. 2C equals 13 Å, distance between O atom of Ser37 of the first molecule binase and NE2 His101 of the second molecule binase equals 6.8 Å, between OE2 Glu43 and NH1 Arg58 — 5.1 Å, between CE1 Phe81 and O Tyr102 — 8.6 Å. (For comparison, these distances in the dimer equal 3.3 Å, 4.2 Å and 4.8 Å, respectively [32].) We believe that calculated dimers of type III can be transformed into experimental dimer as a result of moderate turning and specific orientation of two proteins and a small conformational realignment of the interface side chains. As a result of such reorganization, hydrogen bonds and ionic bridges between residues Ser37, Glu43 of one binase monomer and His101 and Arg58 of the second monomer, respectively, could be formed [32].

As it follows from Table 1, the rate constant of the formation of the type I dimers is the largest one and it is comparable to the rate constant of the reaction of inhibition. The rate constants of the EC formation of the II and III type dimers are much lower. The short-range forces determine the probability and the rate of the transformation of these complexes into the bound dimer. Obtained rate constants can be considered as an upper limit of the association rate constants of binase dimerization.

We assume that the reaction of binase dimerization can compete with the reaction of the association of binase and barstar. Formation of the type II and III dimers partially or fully blocks the access to the active centre of binase for barstar and decreases the activity of binase versus the activity of barnase [17,19].

The association rates of binase–barstar complex and binase dimer formation are determined by the electrostatic interactions. However, influence of the solution parameters on these association rate constants can be different and is determined by the change of the total charges of proteins. We assume that the ratio of the association rate constants of the competitive reactions can be changed by the alteration of reaction condition, such as pH and the ionic strength of solution.

Changing of solution pH from acid to neutral gives rise to deprotonation of Glu, Asp residues, further change of pH to alkaline gives rise to deprotonation of His, Lys, Arg amino acids. In this case, the total charge and its distribution over the surface also change, determining the change in the protein electrostatic properties with pH. The net charge of protein at any given pH is determined by the pK values of the ionizable groups [24–26]. The surface-accessibility-modified Tanford–Kirkwood method encoded in MacroDox was employed to determine the protonation status of each titratable residue in the protein at different pH and ionic strengths. Calculated pI for barstar equals 4.3; binase's pI equals 9.7. Increasing pH leads to the decrease of the total positive charge of binase and eliminates the unfavourable repulsive electrostatic interaction, causing the rate constant of the dimer formation to grow. The dependence of the rate constants of binase dimerization reaction on pH is shown in Fig. 3 for all three types of dimers. For the reaction of dimerization one can see a monotone growth of rate constants with pH. The dependence of the association rate constant for binase – barstar complex on pH is also shown for comparison. The curve of the inhibition reaction is bell-shaped [20].

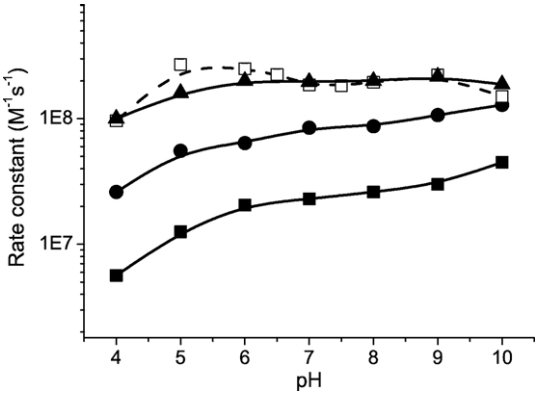

Fig. 3. Binase dimerization rate constant dependence on solution pH (ionic strength equals 0.1 M). Dimer of type I (▲), dimer of type II (●) dimer of type III (■). Association rate constant of the reaction of binase and barstar (□). Rates are given in units of M^{-1} s^{-1}.

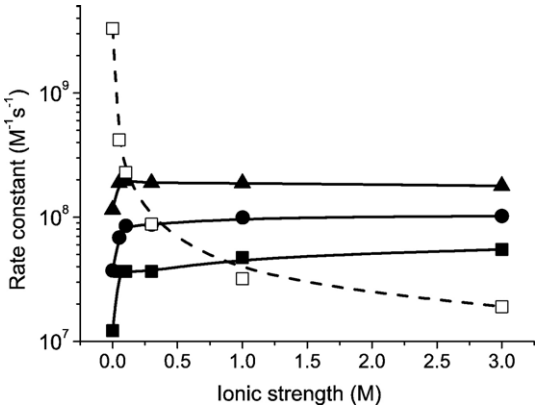

Fig. 4. Ionic strength dependence of binase association rate constant (pH equals 7.0). Rates are given in units of M^{-1} s^{-1}. Dimer of type I (▲), dimer of type II (●) dimer of type III (■) association rate constant of the reaction of binase and barstar (□).

The dependence of the rate constants of EC formation on the ionic strength of solution for both types of reactions is shown in Fig. 4. Increasing ionic strength screens the electrostatic interaction between oppositely charged binase and barstar and decreases the rate constant of the reaction of inhibition. On the contrary, decreasing electrostatic repulsion of positive charged binase molecules enhances the association rate constants of the binase dimerization. The rate constant of dimer formation for all three types of dimers grows sharply at ionic strength from 0 to 100 mM and slows off at higher ionic strength. Our calculations agree well with the conclusion of [33] that the dependence of rate constant on ionic strength is determined by the total charge of the molecules. At high values of the ionic strength the rate constants of the dimers formation overpass the rate constant of the reaction of inhibition.

In conclusion, we have shown that the reaction of the dimerization of binase can compete with the reaction of binase inhibition by barstar using Brownian dynamics simulation method. The ratio of competitive reaction rates depends on pH and ionic strength of solution.

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