THE JOURNAL OF PHYSICAL CHEMISTRY Letters

Letter

pubs.acs.org/JPCL

Ligand Diffusion on Protein Surface Observed in Molecular Dynamics Simulation

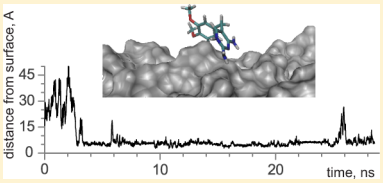
Dmitry Nerukh,*[,†] Noriaki Okimoto,[‡] Atsushi Suenaga,[§] and Makoto Taiji[‡]

[†]Nonlinearity and Complexity Research Group, Aston University, Birmingham, B4 7ET, U.K.

[‡]RIKEN Advanced Institute for Computational Science, 7-1-26, Minatojima-minami-machi, Chuo-ku, Kobe, Hyogo, 650-0047, Japan

[§]Computational Biology Research Center, National Institute of Advanced Industrial Science & Technology, 2-4-7 Aomi, Koto-ku, Tokyo 135-0064, Japan

ABSTRACT: The process of binding of small ligands to dihydrofolate reductase protein has been investigated using all-atom molecular dynamics simulations. The existence of a mechanism that facilitates the search of the binding site by the ligand is demonstrated. The mechanism consists of ligand diffusing on the protein's surface. It has been discussed in the literature before, but has not been explicitly confirmed for realistic molecular systems. The strength of this nonspecific binding is roughly estimated and found to be essential for the binding kinetics.



SECTION: Biophysical Chemistry and Biomolecules

Small molecules (ligands) interact with (bind to) proteins and change their biological functionality sometimes with medically desirable consequences. The interaction is usually described as a chemical reaction between the ligand and the protein that form the ligand-protein complex. The strength of the interaction is measured by the affinity constant, the inverse of the equilibrium dissociation constant. The latter is the ratio of the dissociation rate (the number of complexes dissociated into the components per unit time) and association rate (the inverse process) constants. Thus, the effectiveness of the ligand as a drug candidate is determined by the *rates of binding* and unbinding processes. Both rate constants can be measured experimentally; however, the specific molecular mechanisms can be best revealed in computer simulations. State of the art hardware and modern simulation techniques allow realistic simulations of small proteins on time scales of hundreds of nanoseconds to microseconds, on which elementary events of association occur.

Substantial efforts have been made in calculating the binding constants from molecular dynamics simulations. One of the most challenging tasks in this research is obtaining the kinetics of binding directly from simulations avoiding the use of thermodynamic approximations that could mask rate-limiting mechanisms. In other words, in addition to modeling initial (unbound) and final (bound) states, it is important to follow the complete route of the ligand between the states as it can contain rate limiting steps.

One of such rate-changing mechanisms of binding is associated with ligand "diffusing on the surface" of the protein. It is well-known that ligand diffusion on the surface of the protein accelerates ligand search for the binding site[1] and, thus, facilitates binding (see review[2] and references therein). Evidences supporting this mechanism have been reported for protein−DNA binding and cell surface receptor binding.[3,4] However, the surface diffusion mechanism has not been discussed in the context of proteins binding *small ligands*. This process is in the same conceptual framework, the association rate enhancement by the diffusional search in the reduced dimensional space (the surface instead of the volume), and as such can provide a chemically important mechanism of binding. The decisive question "do small ligands diffuse on the surface when binding to proteins?" remains, to the best of our knowledge, unanswered. The answer is not immediately obvious as the interaction of a small, but many-atom ligand molecule with complex structure of the protein surface mediated by atomistically represented water molecules is very intricate. It produces a delicate balance of forces that may or may not result in the effect of ligand attraction to the protein. Because the ligand is small, the forces are fundamentally different from the already investigated cases of protein−DNA and cell binding.

Here we report the results of *direct* simulations of DHFR-TMP and DHFR-BrWR systems (*Mycobacterium tuberculosis* dihydrofolate reductase with its cofactor NADPH complex binding trimethoprim (TMP) or Br-WR9920 (BrWR), an analogue of antimalarial agent WR99210) for almost a microsecond using all atom molecular dynamics with explicit water. This protein is a popular target for antibacterial agents research.[5,6] [Mouawad et al. conducted a study of CO migration pathways in cytochrome $P540_{cam}$.[19] Thirty-seven trajectories of only 1 ns each were sufficient to identify several intermediate docking sites of the ligand as it diffused to the solvent after a modeled flash photolysis dissociation. The statistics were too poor to calculate the rates, but good enough to identify the metastable states and complex water rearrangements inside the protein. There are a few more publications

Received: October 11, 2012
Accepted: November 6, 2012
Published: November 6, 2012

that attempt to calculate ligand binding rates from molecular dynamics. Kondrashov et al. studied the release of NO from nitrophorin 4 (a heme protein).[20] However, multiple assumptions and short simulation times did not provide a meaningful comparison with the experimental rate constants. Ligand escape from myoglobin was modeled by Sheu.[21] An elaborate and rather specialized model of the process was employed, even though only the direct statistics for the trajectories were considered. In the same framework Camacho et al. reported simulations[22] performed using Brownian dynamics. A limited representation of the atomistic motions did not allow revealing the atomistic details of the binding mechanism. The simulation of CO migration between solvent and binding site in myoglobin in atomistic detail reported by Ruscio et al.[23] These authors estimated the rate from 90 binding/unbinding trajectories of total length of 7 μs. The agreement with experiment was reasonably good. Very recently two publications provide estimates of the binding constants calculated directly from the results of simulations.[24,25]]

We have found that the ligands are attracted to the protein's surface. The attraction is, however, not strong enough to immobilize the ligand, so that the ligand can move along the surface before it detaches. Thus, the simulations clearly demonstrate the existence of a long discussed reaction channel: nonspecific binding with subsequent surface diffusion to the binding site.[1,2] This is the main result of our work, which implies the facilitation of the binding process. In addition, we provide a rough estimation of the strength of the nonspecific binding that keeps the ligand on the surface. We use a model by Berg and Purcell[1] describing the search of the binding site on a sphere taking into account the diffusion on the surface of the sphere. Our estimations in the framework of this model show that binding is indeed strong enough for essentially changing the binding rate.

The structure of the unbound (starting) state was constructed based on the X-ray crystallographic structure of bacterial DHFR-NADP-TMP (PDB ID: 1DG5) and DHFR-NADP-BrWR (PDB ID: 1DG7) ternary complexes.[7] It has been reported[7] that the presence or absence of a ligand on the folate binding site has little effect on the overall structure of *M. tuberculosis* DHFR (in contrast to *E. coli* DHFR for which subdomain movements are observed upon substrate binding[8]). We, thus, placed the ligand molecule 25 Å away from the crystallographic position in the starting conformation and replaced NADP by NADPH. This complex was solvated in a rectangular box containing TIP3P water molecules.[9] The box dimensions were chosen such that the minimum distance of any solute atom from the walls of the box was 15 Å. We would like to stress that this is the minimal distance, whereas on average the ligands could be as far as ≈50 Å away from the protein's surface. The initial structures were carefully equilibrated before collecting data for analysis.

Ten separate simulations differed in the initial distribution of the momenta of all atoms in the system. The simulation time ranged from 20 ns to 100 ns, and the total simulation time was approximately 800 ns. All the simulations were performed using the AMBER 8.0 package[10] modified for MDGRAPE-3.[11,12] The SHAKE algorithm[13] was applied to the bonds involving hydrogen atoms using the integration time step of 2.0 fs. The long-range Coulomb interactions were treated with the particle mesh Ewald (PME) method.[14] The real space component of the PME method was calculated using MDGRAPE-3, while the wavenumber space component for this method and the bonded interactions were calculated by the host computers. To optimize the balance between the calculation times for these components, a cutoff distance of 14 Å was used for the real space component. Each system was gradually heated to 310 K for the first 50 ps. The Berendsen temperature and pressure control methods[15] were used to maintain the temperature and pressure constant at 310 K and 1 atm. The AMBER ff03 force field was used for the DHFR molecule. Since NADPH, TMP, and BrWR were not included in the standard ff03 force field, their force field parameters were determined using the antechamber module version 1.27 of AMBER 8.0[10] by utilizing the general atom force field (GAFF),[16] and the atomic charges were determined by quantum chemical calculations using the Hatree-Fock method/6-31G** basis set.

We have found that in a few nanoseconds after the start of the simulation the ligand finds the surface of the protein and weakly binds to it. Figure 1 shows several representative plots of the distance between the centers of mass of the ligand and

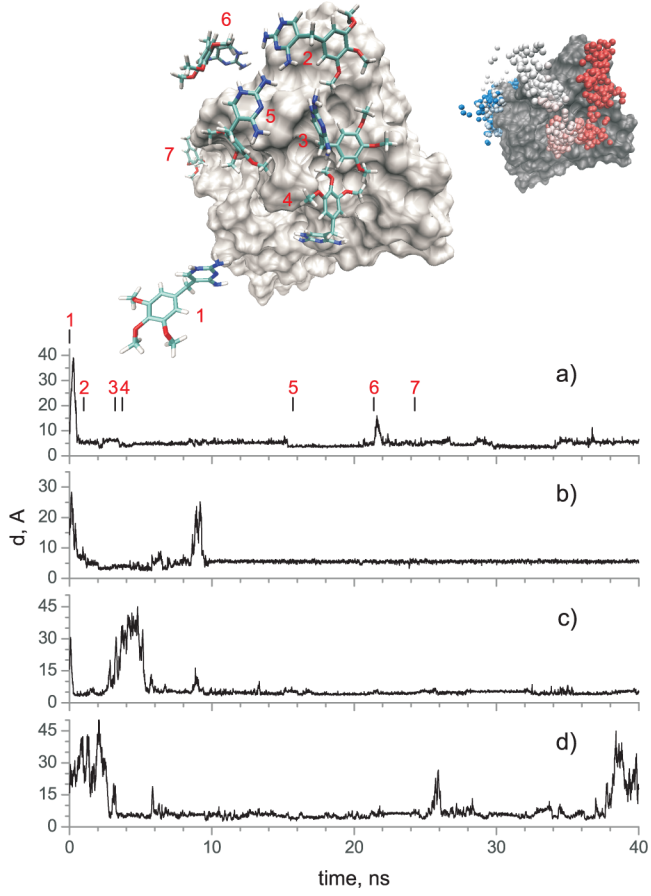


Figure 1. The distance d between the centers of mass of TMP (a, b, c) or BrWR (d) ligand and the nearest residue of the protein, the low, constant values of d correspond to the time intervals when the ligands are on the surface (and there is no water molecules between the ligand and the surface) while the values higher than ≈7 show the diffusion of the ligands in the bulk away from the protein; several representative positions of the TMP ligand on the surface (and, occasionally, away from it) are shown at the top, the numbers on the time plot (a), from 1 to 7, correspond to the locations of the ligand on the surface; top-right figure shows the diffusion of TMP on the surface from position 2 to position 7, the ligand is represented as a single ball gradually changing its color along the trajectory from red (position 2) through white (approximately position 5) to blue (position 7).

dx.doi.org/10.1021/jz301635h | J. Phys. Chem. Lett. 2012, 3, 3476−3479

the closest residue of the protein. This corresponded to TMP or BrWR making several hydrogen bonds with the protein surface and no water between the ligand and the protein. Evidently, the ligand spends most of the time on the surface of the protein. It also moves along the surface (slowly diffuses on it); a few typical locations of TMP are shown in Figure 1 together with a trajectory of diffusion on the surface. Finally, the ligand occasionally detaches from the protein and freely moves in water before attaching to the surface at some other location.

We would like to stress that in all 10 runs, the locations at which the ligand initially bound and rebound if detached were different. The binding was, therefore, nonspecific, in contrast to a few events of ligand binding to the experimentally known binding site where it was eventually trapped.

A simplified model of the described interaction between the ligand and the surface can help to estimate the effect of the surface diffusion on the binding rate. Let us suppose that the interaction is realized through a square well potential of depth ΔU and width l such that at the distances from the surface less than l the ligand is attracted to the surface[17] (Figure 2). Then,

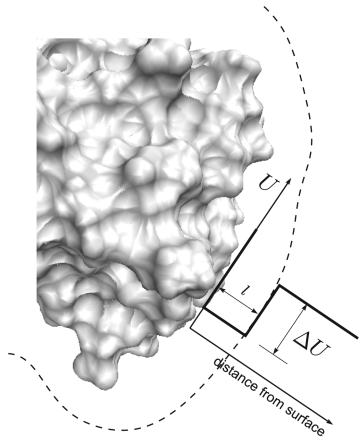


Figure 2. A square well potential model of the ligand−surface interaction.

the ratio τ of times spent by the ligand in the l-thick layer around the protein, t_s, and in the bulk, t_b, is $\tau = t_s/t_b$. If the system is in equilibrium, this ratio should be equal to the ratio of the corresponding volumes $\beta = (sl)/(V - sl)$ multiplied by the Boltzmann factor: $\tau = \beta e^{-\Delta U/kT}$, where s is the surface area and V is the volume of the system excluding the volume of the protein. From this the depth of the potential well can be found

$$\Delta U = -kT \ln \frac{\tau}{\beta} \tag{1}$$

The time spent by the ligand on the surface depends on l. This is all the time when the distance shown in Figure 1 is less than l. The values of $V = 367640$ Å^3 and $s = 9020$ Å^2 resulted in ΔU increasing from $1.2kT$ to $2.1kT$ as l changing from 7 to 10 Å. We have chosen these values of l somewhat arbitrarily based on the values of d in Figure 1 at the times when the distance d is approximately constant, which corresponds to the location of the ligand directly on the surface of the protein (the "surface diffusion" state).

These values of ΔU can be compared to the theoretical model of searching the binding site by the same mechanism of surface diffusion. The model is developed in the classical work by Berg and Purcell.[1] The authors estimated the current of ligands to the binding site represented by a small absorbing patch of radius r_s on a spherical protein of radius r_p. The ligand could search the binding site either by diffusing in water with the diffusion constant D and bouncing off the surface or, in addition, by weakly attaching to the surface and diffusing on it for some time with the diffusion coefficient D'. The latter is exactly the mechanism that we discuss here. Berg and Purcell have shown[1] that in order for the surface diffusion mechanism to be prevalent, the potential well ΔU should be greater than

$$\Delta U > kT \ln\left(\frac{D}{D'}\frac{r_s}{\pi l}\ln\frac{r_p^2}{r_s^2}\right) \tag{2}$$

Our protein can approximately be represented by a sphere of radius $r_p = 15$ Å having the binding site of radius $r_s = 6.3$ Å. The ratio D/D' can be assumed to be on the order of 10. For these values and l ranging in the same interval 7−10 Å, ΔU varies from $2.0kT$ to $1.6kT$. These values are effectively the same as our estimations above. Thus, this shows that the described mechanism (the surface diffusion) is indeed important for the binding kinetics.

The obtained values of ΔU both using formula 1 or formula 2 should be understood as very rough estimates, within the order of magnitude tolerance. For example, the variation in ΔU from eq 1 can be within $\approx 2-5kT$ if the surface area s varies by ≈ 3000 Å^2. Similarly, the same tolerance of $\approx 2-5kT$ results from eq 2 if the ratio of the diffusion constants D/D' changes in the range 1−1000. Therefore, even thought the given estimates provide the correct order of magnitude values, they should not be taken too literally.

Is is also worth noting that we have found a similar behavior for a different system. The interaction of trehalose with lysozyme exhibits a preferential positioning of the trehalose molecules next to the protein's surface.[18] The purpose of the study was to examine the stability of the protein's structure, and the dynamics of the ligand on the surface was not investigated.

Summarizing, we have demonstrated the existence of a mechanism that facilitates the search of the binding site by the ligand. The mechanism consists of ligand diffusing on the protein's surface, and it has been discussed in the literature before, but has not been shown explicitly in realistic molecular systems. We also roughly estimate the strength of this nonspecific binding and find it essential for the binding kinetics.

AUTHOR INFORMATION

Corresponding Author

*E-mail: D.Nerukh@aston.ac.uk.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The authors are grateful to Alexander Berezhkovskii for helpful discussions.

REFERENCES

(1) Berg, H.; Purcell, E. Physics of Chemoreception. *Biophys. J.* **1977**, *20*, 193−219.

(2) Zhou, H.-X. Rate Theories for Biologists. *Q. Rev. Biophys.* **2010**, *43*, 219−293.

(3) Alsallaq, R.; Zhou, H.-X. Protein Association with Circular DNA: Rate Enhancement by Nonspecific Binding. *J. Chem. Phys.* **2008**, *128*.

(4) Holyst, R.; Blazejczyk, M.; Burdzy, K.; Goralski, G.; Bocquet, L. Reduction of Dimensionality in a Diffusion Search Process and Kinetics of Gene Expression. *Physica A* **2000**, *277*, 71−82.
(5) Hawser, S.; Lociuro, S.; Islam, K. Dihydrofolate Reductase Inhibitors as Antibacterial Agents. *Biochem. Pharmacol.* **2006**, *71*, 941−948.
(6) Argyrou, A.; Vetting, M. W.; Aladegbami, B.; Blanchard, J. S. Mycobacterium Tuberculosis Dihydrofolate Reductase is a Target for Isoniazid. *Nat. Struct. Mol. Biol.* **2006**, *13*, 408−413.
(7) Li, R.; Sirawaraporn, R.; Chitnumsub, P.; Sirawaraporn, W.; Wooden, J.; Athappilly, F.; Turley, S.; Hol, W. G. Three-Dimensional Structure of M. Tuberculosis Dihydrofolate Reductase Reveals Opportunities for the Design of Novel Tuberculosis Drugs. *J. Mol. Biol.* **2000**, *295*, 307−323.
(8) Sawaya, M. R.; Kraut, J. Loop and Subdomain Movements in the Mechanism of *Escherichia coli* Dihydrofolate Reductase: Crystallographic Evidence. *Biochemistry* **1997**, *36*, 586−603.
(9) Jorgensen, W. L.; Chandrasekhar, J.; Madura, J. D.; Impey, R. W.; Klein, M. L. Comparison of Simple Potential Functions for Simulating Liquid Water. *J. Chem. Phys.* **1983**, *79*, 926−935.
(10) Case, D. A.; Darden, T. A.; Cheatham, T. E., III; Simmerling, C. L.; Wang, J.; Duke, R. E.; Luo, R.; Merz, K. M.; Wang, B.; Pearlman, D. A. et al. *AMBER 8*; University of California: San Francisco, CA, 2004.
(11) Narumi, T.; Ohno, Y.; Okimoto, N.; Koishi, T.; Suenaga, A.; Futatsugi, N.; Yanai, R.; Himeno, R.; Fujikawa, S.; Taiji, M. et al. A 55 TFLOPS Simulation of Amyloid-Forming Peptides from Yeast Prion Sup35 with the Special-Purpose Computer System MDGRAPE-3. 2006; http://doi.acm.org/10.1145/1188455.1188506.
(12) Taiji, M. *MDGRAPE-3 Chip: A 165 Gflops Application Specific LSI for Molecular Dynamics Simulations*; IEEE Computer Society: Washington, DC, 2004; pp. in CD-ROM
(13) Ryckaert, J.; Ciccotti, G.; Berendsen, H. Numerical-Integration of Cartesian Equations of Motion of a System with Constraints − Molecular-Dynamics of *N*-Alkanes. *J. Comput. Phys.* **1977**, *23*, 327−341.
(14) Darden, T.; York, D.; Pedersen, L. Particle Mesh Ewald: An *N*·log(*N*) Method for Ewald Sums in Large Systems. *J. Chem. Phys.* **1993**, *98*, 10089−10092.
(15) Berendsen, H. J. C.; Postma, J. P. M.; van Gunsteren, W. F.; DiNola, A.; Haak, J. R. Molecular Dynamics with Coupling to an External Bath. *J. Chem. Phys.* **1984**, *81*, 3684−3690.
(16) Wang, J.; Wolf, R. M.; Caldwell, J. W.; Kollman, P. A.; Case, D. A. Development and Testing of a General Amber Force Field. *J. Comput. Chem.* **2004**, *25*, 1157−1174.
(17) Zhou, H.-X.; Szabo, A. Enhancement of Association Rates by Nonspecific Binding to DNA and Cell Membranes. *Phys. Rev. Lett.* **2004**, *93*, 178101.
(18) Fedorov, M. V.; Goodman, J. M.; Nerukh, D.; Schumm, S. Self-Assembly of Trehalose Molecules on a Lysozyme Surface: the Broken Glass Hypothesis. *Phys. Chem. Chem. Phys.* **2011**, *13*, 2294−2299.
(19) Mouawad, L.; Tetreau, C.; Abdel-Azeim, S.; Perahia, D.; Lavalette, D. CO Migration Pathways in Cytochrome P450cam Studied by Molecular Dynamics Simulations. *Protein Sci.* **2007**, *16*, 781−794.
(20) Kondrashov, D. A.; Montfort, W. R. Nonequilibrium Dynamics Simulations of Nitric Oxide Release: Comparative Study of Nitrophorin and Myoglobin. *J. Phys. Chem. B* **2007**, *111*, 9244−9252.
(21) Sheu, S.-Y. Selectivity Principle of the Ligand Escape Process From a Two-Gate Tunnel in Myoglobin: Molecular Dynamics Simulation. *J. Chem. Phys.* **2006**, *124*, 154711.
(22) Camacho, C. J.; Kimura, S.; DeLisi, C.; Vajda, S. Kinetics of Desolvation-Mediated Protein−Protein Binding. *Biophys. J.* **2000**, *78*, 1094−1105.
(23) Ruscio, J. Z.; Kumar, D.; Shukla, M.; Prisant, M. G.; Murali, T. M.; Onufriev, A. V. Atomic Level Computational Identification of Ligand Migration Pathways Between Solvent and Binding Site in Myoglobin. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 9204−9209.
(24) Shan, Y.; Kim, E. T.; Eastwood, M. P.; Dror, R. O.; Seeliger, M. A.; Shaw, D. E. How Does a Drug Molecule Find Its Target Binding Site? *J. Am. Chem. Soc.* **2011**, *133*, 9181−9183.
(25) Buch, I.; Giorgino, T.; De Fabritiis, G. Complete Reconstruction of an Enzyme-Inhibitor Binding Process by Molecular Dynamics Simulations. *Proc. Natl. Acad. Sci.* **2011**, *108*, 10184−10189.