Studies of Ligand Diffusion Pathways over a Protein Surface

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Studies were conducted on the behavior of simulated molecules diffusing within organized water, postulated to form from the hydropathic states of protein surface amino acid side chains. This organization is postulated to facilitate the diffusion of ligands across the protein surface to their effector. These studies reveal that the organized water can be disrupted in their diffusion facilitating function by the presence of some other solute in high concentration. It was also found from cellular automata simulations that chiral isomers behaved in a slightly different manner when in an asymmetric enclosure simulating a fragment of the organized water pathway. These findings have relevance to observations about the mechanism of action of nonspecific anesthetic agents.

INTRODUCTION

The subject of ligand passage to an active site has been of interest for many years. Questions of diffusion through bulk water in a synapse and diffusion across the surface of a cell or membrane have been examined and written about extensively.[1−6] A prominent view focuses on the surface of proteins and the involvement of nearby surface water as the influential realm where ligands move to the active site. A recent theory has been proposed invoking water on the surface of a protein, organized by the hydropathic states of amino acid side chains protruding into the bulk water.[7] The varying influence of the side chains on vicinal water create evanescent, preferred pathways through the voids between water molecules through which ligands pass. These pathways support and facilitate the diffusion across the protein surface from the point of contact to the active site. This results in a facilitated and directed diffusion faster than dependence on a direct hit from diffusion through bulk water. A number of phenomena can be explained by this theory. Supporting the theory are a number of experimental observations.[8−12]

To study the role of various side chains and their influence on diffusion, we have simulated, using cellular automata, pairs of amino acid side chains using stationary cells in a field of water.[7] These side chains were endowed with rules giving them hydropathic character, typical of five classes of amino acid side chains shown in Table 1. A diffusant was allowed to escape past paired side chains to evaluate the effect on diffusion rate due to the side chain hydropathic state. The study showed that the more hydrophobic side chains permitted a faster diffusion than the hydrophilic side chains. Another observation concerned the distance between the stationary cells, surrogate for a pair of side chains. A separation distance of three cells between side chains produced the most selective effect on diffusion rate, while a separation distance of four cells was less discriminating and a separation of five cells did not produce any discrimination in the rate of diffusion. The implication is that the side chains influencing pathways, must have some optimum distance between them in order to influence the water and the subsequent diffusion of ligands through the system.

Table 1. Rules Equivalent to the Hydropathic States of Amino Acid Side Chains

code	side chain	P_B(WS)/J	hydropathic state
A	Arg, Asp, Lys	0.1/2.80	very hydrophilic
B	Asn, Glu, Gln, Gly, Ser	0.3/1.40	hydrophilic
C	Ala, His, Pro, Thr	0.5/0.71	intermediate
D	Cys, Met, Tyr, Val	0.7/0.36	hydrophobic
E	Ile, Leu, Phe, Trp	0.9/0.18	very hydrophobic

With the information gleaned from the modeled side chain studies and hydropathic influences, we next explored the possibility of a guided or directed trajectory of the diffusant on the hydrodynamic landscape. In that study, we used a cellular automata grid located on the surface of a torus with a central region representing a target for a ligand, shown in Figure 1. A random distribution of the five cell types representing the five groups of amino acid side chains based on hydropathic state, Table 1, was introduced onto a cellular automata grid, Figure 1. All of these cells (A, B, C, D, E) were separated from each other by 3 cell spaces. The system contained water in the same proportion as used in earlier studies.[13−15] The water was allowed to move freely and to interact with the ligand and stationary gate cells. The average

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S	C	C	D	D	B	E	B	C	D
D	B	E	B	C	C	A	C	D	C
B	D	A	D	A	C	D	B	B	D
A	C	B	A	D	A	A	C	E	A
B	D	C	E	B	E	C	B	A	C
E	C	A	D	♥	A	B	E	B	B
C	A	C	A	C	E	A	C	D	C
C	A	B	D	A	E	C	B	A	D
A	D	A	B	B	A	B	A	C	B
C	A	C	A	D	B	A	E	A	C

Figure 1. A cellular automata model of a random surface on a torus. Movement of a cell off of an edge brings it to the opposite edge. The heart symbol is the active site position, S, the diffusant with an intermediate hydropathic state, and A, B, C, D, and E are the different subsets of side chains shown in Table 1. All side chains are 3 spaces apart with water molecules moving freely among them.

S	C	C	D	A	B	E	B	C	D
D	B	E	B	B	C	A	C	D	C
B	D	A	D	C	C	D	B	B	D
A	C	B	A	D	A	A	C	E	A
B	D	C	E	E	E	C	B	A	C
B	C	D	E	♥	E	D	C	B	A
C	A	C	A	E	E	A	C	D	C
C	A	B	D	D	E	C	B	A	D
A	D	A	B	C	A	B	A	C	B
C	A	C	A	B	B	A	E	A	C

Figure 2. A cellular automata model of a fragment of a water pathway (3-cell gates). The heart symbol is the active site position, S is the diffusant with an intermediate hydropathic state, and A, B, C, D, and E are the different subsets of side chains shown in Table 1. All side chains are 3 spaces apart with water molecules moving freely among them.

count of iterations necessary for the ligand, S, to traverse the grid and touch the central target was calculated to be 12 429.

A second model was created in which the A, B, C, D, and E side chains were randomly scattered as in Figure 1. Each of these cells was separated from another by a 3-cell space. Eighteen of the 100 cells were organized to form a pattern shown in Figure 2. The dynamics were run as before and the average time to diffuse to the center was calculated to be 8609 iterations. This model simulates the possible diffusion of a ligand across a protein surface with specifically positioned amino acid side chains coordinating their hydropathic states to facilitate diffusion toward the center of the grid. It was found that the rate of diffusion of a ligand is faster in the ordered side chain model as compared to a random distribution of these same side chains on the grid.

Our interest in these results and the possibility of this guided passage explaining some drug mechanisms has led us to consider several properties of the organized water. In particular we are interested in the possible influence of other molecules on the stability and functioning of the water patterns and the influence of chirality on the behavior of molecules within the organized water. Accordingly we have examined these aspects of the theory in studies using cellular automata simulations.

CELLULAR AUTOMATA

The Method. Cellular automata are dynamical computational systems that are discrete in space, time, and state and whose behavior is specified completely by rules governing local relationships. They are an attempt to simplify the often numerically intractable dynamic simulations into a set of simple rules that mirror intuition and that are easy to compute. As an approach to the modeling of emergent properties of complex systems it has a great benefit in being visually informative of the progress of dynamic events. From the early development by von Neumann,[16] a variety of applications ranging from gas phenomena to biological applications have been reported.[17,18]

Our model is composed of a grid of square spaces called cells on the surface of a torus to remove boundary conditions. Each cell i has four tessellated neighbors, j, and four extended neighbors, k, in what is called an extended von Neumann neighborhood. Each cell has a state governing whether it is empty or is occupied by a water or other molecule. The contents of a cell move, join with another occupied cell, or break from a tessellated relationship according to probabilistic rules. These rules are established at the beginning of each simulation. The rules are applied one after another to each cell at random, the complete application of the rules to all cells constituting one iteration. The rules are applied uniformly to each cell type and are local, thus there is no action at a distance. Our cellular automata model is kinematic, asynchronous, and stochastic. The initial conditions are random hence they do not determine the ultimate state of the cells, called the configuration. The same initial conditions do not yield the same set of configurations after a certain number of iterations except in some average sense. The configurations achieved after many iterations reach a collective organization that possesses a relative constancy in appearance and in reportable counts of attributes. What we observe and record from the cellular automata simulations are emergent attributes of a complex system.

The Rules. On a grid of cells, each cell can be either unoccupied or occupied by a molecule. The configuration of the entire system is defined by the state values of the cells in the grid. Molecules are allowed to move to a vacant cell and leave its original cell vacant, thus the configuration of the entire system changes accordingly. The movement of a molecule is determined by its state, the states of cells touching it and general rules of movement**.** The rules depend on two parameters, which allow the moving probabilities of a cell to be computed, and then a random choice according to those probabilities determines where a molecule will move if it moves at all.

Two parameters are selected for a simulation to control the probabilities for movement of the molecules in the grid. The breaking probability, $P_B(X,Y)$, is the probability for a molecule, of type X, in cell (i,j), to break away from a molecule, of type Y, in its immediate environment, when there is exactly one occupied cell. The value of $P_B(X,Y)$ lies between 0 and 1 (inclusive). The second parameter J(X,Y), describes the movement of the molecule, of type X, in cell (i,j), toward or away from the molecule, of type Y, in its environment when the intermediate touching cell is vacant.

Given that (i,j) cell is occupied by a molecule of type X, and all cells of (i,j) cell are vacant, J(X,Y) is defined to be the ratio A/B, where A is the probability that the molecule in cell (i,j) will move toward a cell occupied with a molecule of type Y and B is the probability that the molecule in cell (i,j) will move toward a vacant cell. The value of J(X,Y) is a positive real number. When $J(X,Y) = 1$, the molecule in cell (i,j) has the same probability of movement toward an occupied with molecule of type Y, as when that cell is empty. When $J(X,Y) > 1$, it indicates that the molecule in cell (i,j) has a greater probability of movement toward an occupied cell with molecule of type Y, than when that cell is empty. When $J(X,Y) < 1$, it indicates that the molecule in cell (i,j) has a lower probability of such movement.

The Influence of a Second Molecule on the Organized Water. In this study we have used the model, Figure 2, described above. In addition to the ligand, there was added 18 cells, simulating a second solute. These were endowed with an intermediate hydropathic state, C in Table 1, and random motion in the grid. The simulation was run 200 times, and the average time for the ligand to diffuse to the active site was recorded. The average number of iterations necessary for the passage of the ligand to the active site was found to be 12 594. This is almost exactly the time necessary for the ligand to traverse the grid when the organized pattern of water was not present. The presence of the second solute destabilized the organized pattern of water sufficiently to render it inoperative; that is, equivalent to a random distribution of amino acid side chains.

The Influence of Solute Chirality on Behavior in Organized Water. It is of interest to assess the possibility that chiral isomers may exhibit some difference in their behavior when in a segment of an organized water pathway. A corollary to this possibility, the influence of a solute on surrounding water, has been reported using experimental and theoretical methods.[19,20] One study examined the influence of chiral isomers on surrounding water using circular dichroism. The results indicated that the isomers produced a chiral ordering of solvent.[19] These results were supported by molecular dynamics simulations. A second study, using cellular automata, led to the same results.[20]

In the present study, we have modeled a fragment of a water pathway as a square arrangement of stationary cells representing four different amino acid side chains, as shown in Figure 3. The side chains are 3 cells apart from their neighbors and were endowed with parameters described in Table 1. Thus the simulated side chains present an asymmetric pattern to solutes that may enter the enclosure. A solute molecule was positioned in the center of the pathway fragment. It had a different set of parameters for each face, as shown in Figure 4. This pattern of parameters conferred chirality to the solute. The solute molecule and the surrounding water molecules were allowed to move freely according to their parameters. When the solute passed beyond the line of cells joining an adjacent pair, the run was stopped and the number of iterations recorded. The solute molecule was rotated 90 degrees for each study consisting of 2000 runs each. The average number of cells exiting the square by 100 iterations, from 8000 runs was summed from these four rotational presentations. This value was found to be 5870 molecules (73.4%) with a standard error of the mean of 1.5%. The study was repeated but with the chiral isomer of the solute molecule presented to the square as the mirror image, as shown in Figure 4. The results of this analysis showed that 5325 (66.5%) molecules exited the square by 100 iterations, with a standard error of the mean of 1.5%. The study revealed a 10% difference in the number of escapes of one chiral isomer relative to the other.

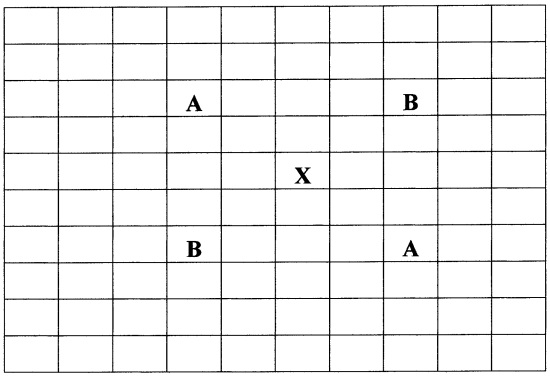

Figure 3. Simulation of a fragment of a water pathway using four amino acid side chains in a square arrangement.

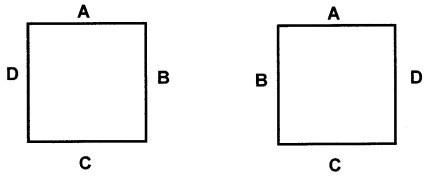

Figure 4. Simulation of chiral isomers of a diffusant.

DISCUSSION

The theory of facilitated, directed ligand passage over a protein surface is built around the proposition that the amino acid side chains are structuring the vicinal water in a pattern that creates evanescent passages or pathways through the water.[7] Depending upon the hydropathic state of each side chain, there is a variable influence on nearby water that ranges from a strong hydrophobic effect to a strong attraction leading to extensive hydration as in electrostriction. Recent experimental work on the relationship of water to walls of similar and different hydropathic states revealed that there is a marked influence when the walls are greatly different in this property.[21] This effect, called the Janus effect, leads to waves of cavities through the system, reminiscent of the effects described before[7] and in this study.

The present study reveals that these pathways are fragile in the sense that they can be altered or destroyed by the presence of some solutes in high concentration. This alteration manifests itself as a reduced capacity for them to form, to admit, and to make possible the rates of diffusion

encountered in an organized pathway through the water. The altering agent in this case is the presence of other solute molecules with sizes similar to the side chains. These intruding solutes bring about the formation of different patterns in the water due to their own hydropathic states. This effect disrupts the rate of diffusion of a ligand producing a rate very much like that found with a random distribution of side chains. This effect of this disruption by a second set of molecules may very well be the mechanism whereby the volatile general anesthetic agents act.

In the second part of the study, it was demonstrated that an asymmetric pattern of side chains could differentiate among simulated chiral isomers as far as the ability to retain the isomers. The difference, about 10%, is modest but real. It is reminiscent of the modest difference exhibited by chiral isomers of some halothanes in their general anesthetic potency. These findings further illuminate the possibility of the existence and role of the hydrodynamic surface water influencing ligand passage across a protein surface.

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