Altering Diffusivity in Biological Solutions through Modification of Solution Structure and Dynamics

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Mass transfer by diffusion is an important component of biological systems and has been proposed to limit the physical size and volume of the metabolizing mass of living cells.[1] Diffusion has been proposed to be important in the transport of oxygen from capillaries to tissues of the body, and several experiments suggest that this is especially true during periods of maximal oxygen consumption.[2] Fick's law[3], $J = -D(\partial C/\partial x)$, relates the diffusive flux, J, across a reference plane to the product of the diffusion coefficient or diffusivity, D, and the concentration gradient perpendicular to the reference plane, $\partial C/\partial x$. Efforts to modify diffusive transfer rates have focused upon changing the concentration gradient, but an equivalent approach is to modify the diffusion coefficient.[4] While often considered to be a constant, the diffusion coefficient has been shown to be dependent upon the nature of the solution.[3] This sensitivity to solution composition suggests that controlled alteration of the medium could result in desired changes in the diffusivity of solutes.

Absolute rate theory provides a model of the transfer process, envisioning diffusion as the "jumping" of solute molecules, with an associated activation energy, between the vacancies within the solvent lattice.[5] Absolute rate theory can be further extended to predict the ratio of the diffusivity of a solute in a dilute solution to its rate in pure bulk liquid.[6,7] This approach allows the difference in activation energy to be related to the intermolecular distances between the molecules in solution. Thus, the diffusive behavior of a solute is dependent upon the spacing of the solution around it. This relationship provides a strategy for the modification of diffusivity; change the average intermolecular spacing in solution through the addition of solutes[7] and cosolutes[4,8] that change the local solution structure. This strategy has been used to both decrease[9] and increase[10] diffusion coefficients in dilute polymer and biological solutions.

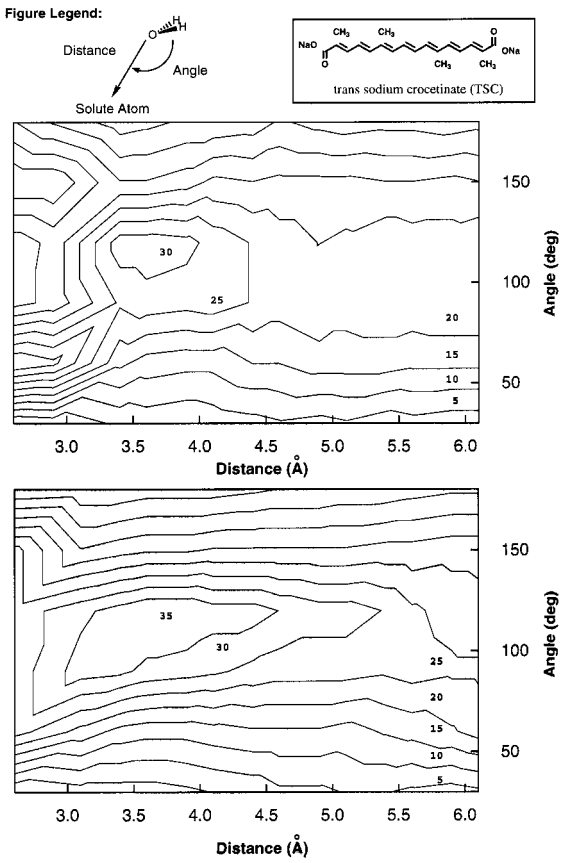

Figure 1. Plots depicting the radial and angular distribution of water around (a) a probe water in pure water and (b) the heavy atoms of the TSC hydrophobic surface. The angular distributions are given as percentages for each radial distance.

The diffusion of oxygen in plasma is sensitive to the constituents within plasma.[9] For example, increases in the concentration of naturally occurring proteins and cholesterol, changes associated with aging,[10] can lead to a nearly 50% decrease in the diffusivity of oxygen.[9] Conversely, addition of the carotenoid *trans*-sodium crocetinate (Figure 1) to plasma and plasma-like solutions increases the diffusivity of oxygen up to 30%.[8,11] Absolute rate theory predicts that the positive volume of mixing found for crocetin and plasma should also result in an enhancement in the diffusivity of other solutes through these solutions.[8]

If diffusion is a dominant mechanism controlling oxygen usage by the tissues (e.g., during certain stressed conditions), then altering the liquid structure of the blood plasma with crocetin offers a novel method of therapy. Sodium crocetin is an effective treatment for hemorrhagic shock, sharply decreasing mortality where increased survival has been correlated with higher oxygen consumption rates.[11,12]

The ability of sodium crocetin to increase diffusion of oxygen in solution[11] provides a macroscopic change in behavior based upon a microscopic change in solution structure. We have performed MD simulations[13] in order to investigate the consequences of this compound upon aqueous solution structure and dynamics at the atomic level. Sodium crocetin may exist as several different isomers; here, we focus on *trans*-sodium crocetinate (the sodium salt of *trans*-8,8′-diapo-$\psi\psi$-carotenedioic acid) or TSC (Figure

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1). Herein, we provide an atomic view of the interactions, upon addition of TSC, responsible for the observed increases in diffusivity of oxygen through aqueous solutions.

Simulations of 3 ns were performed of pure water, water with O_2, and with TSC both with and without O_2. Diffusion constants were calculated over the last ns.[13] The average diffusion coefficient for pure water is $(2.61 \pm 0.01) \times 10^{-5}$ cm^2 sec^{-1} (experiment: $2.1-2.7 \times 10^{-5}$ cm^2 sec^{-1}).[16] The average diffusivity of oxygen is $(2.21 \pm 0.19) \times 10^{-5}$ cm^2 sec^{-1} (experiment: $1.9-3.4 \times 10^{-5}$ cm^2 sec^{-1}).[8,17] Our simulations also recover the observed consequences of the addition of TSC, the diffusivity of oxygen is greater with TSC in solution than in pure water ($2.99 \pm 0.18) \times 10^{-5}$ cm^2 sec^{-1}), by 35% in comparison to the observed enhancement of 25 to 30%.[8] In contrast, the diffusion of water in the TSC solution is the same as in pure water.

The increased diffusion of oxygen is not the result of increased diffusion of water in the immediate vicinity of TSC. Water diffusivity within a 4.5 Å shell of TSC is slightly retarded in comparison to that of the bulk, with an average diffusion coefficient of 2.3×10^{-5} cm^2 sec^{-1}. This effect is reasonable given that a hydrophobic solute, such as TSC, causes a reorientation of the surrounding water layer.[18]

Reorientation of water is the primary consequence of adding TSC to an aqueous solution, modifying the structure and dynamics of the water solvating the solute. For pure water, there is a preferential clustering of water at a distance of 3.5−4.0 Å from the probe and at an angle of 110−120° (Figure 1) as expected based upon the tetrahedral hydrogen bonding pattern of water and reflected in the O··O RDF of water from both experiment[19] and simulation.[15c] Water solvating the hydrophobic atoms of TSC shows an altered distribution; the range of radial distances is larger, from 3.0 to 4.7 Å, and the angular distribution is also broader, from 90 to 120°. The differences arise from the attempt to maximize water−water interactions within the surrounding solvation shell of the perturbant.[18] The hydrophobic surface area of TSC, ∼980 Å^2,[20] coupled with the molecule's rigidity results in a "projection" of the ordering of water into the solvation shell. For example, the molecular volume of TSC is 325 Å^3,[21] which increases to ∼3300 Å^3 upon inclusion of a 6 Å hydration shell.

Figure 2. Two snapshots of water within 8 Å of TSC.[22] On the left is TSC and its hydration shell of water with an O_2 molecule in blue. On the right is a cutaway looking down the molecular axis after a 90° rotation. The water molecules that appear to be near TSC are actually behind the molecule in this view, as can be seen in the rotated figure on the left.

This increased order does not translate directly into a change in the average water−water intermolecular distance. The RDF between pairs of water oxygens [G(O··O)] within a layer of 4.5 Å of TSC was compared with that found in bulk water. There was a small decrease in the first intermolecular G(H··H) peak of 0.1 Å, signaling the decrease intermolecular distances resulting from the flattening of water against the hydrophobic surface. This is reasonable since the observed positive volume of mixing for TSC in aqueous solution[8] corresponds to an excess solution expansion of only ∼0.5 Å^3 per molecule of TSC added to solution.

Nonetheless, the ordering of water has an effect upon the transport of oxygen through the aqueous solution. The diffusivity of oxygen within the shell defined by the higher water orientational ordering, 3 to 5 Å from TSC, was found to be larger than the bulk value. The largest increase in diffusion was seen in the shell from 4 to 5 Å with an average oxygen diffusivity of 3.7×10^{-5} cm^2 sec^{-1}. The increased order of water can also be seen in the snapshot of the hydration shell around TSC in Figure 2. The waters of the hydration shell flatten out, with respect to the hydrophobic surface, to minimize interactions with TSC and maximize interactions with other water molecules.

Hydrophobic hydration reduces the disorder of water within the volume of solution spanned by TSC and its hydration shell, creating an "orderly channel" through which oxygen can diffuse more efficiently. For example, imagine oxygen diffusion through water as the passage of a person through a series of turnstiles. An orderly and regular arrangement of these turnstiles would facilitate passage in comparison to a situation in which the turnstiles are randomly oriented. In much the same manner, hydrophobic hydration acts to align water in the hydration shell as well as gaps between water molecules in comparison to bulk.

The simulations show that oxygen diffusivity in an aqueous system can be increased through strategic modification of solution structure and dynamics. When TSC is dissolved in blood plasma, this same phenomenon is thought to occur. The success of this prediction and the determination that sodium crocetin apparently has no effect on any other factors known to increase oxygen delivery and consumption[11] provides further evidence for the proposed mode of action. While this does not prove that TSC acts exclusively though this mechanism, our observations lend support to the hypothesis that changes in liquid structure can occur even at very low concentrations of solutes, which can lead to an increase in the diffusion rate of other solutes such as oxygen.

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(13) The program ENCAD was used for the simulations.[14a] The protocols, potential functions, and F3C water model used are described in detail elsewhere.[15] Periodic boundary conditions were employed and water molecules were added around the solute to fill a rectangular box with edges at least 20 Å away from the solute. An 8 Å force-shifted nonbonded truncation was used.[15b,c] Geometric parameters for TSC to yield alternating double and nonrotating single bonds for a planar molecule in the all-trans conformation were determined using the AM1 parametrized semiempirical methods implemented in SPARTAN.[14b] A typical concentration of TSC in experiments is 4 μg/mL, while the solubility of oxygen in water is 1.38 mmole/L, yielding an approximate molecular ratio of 1:100:5 000 000 for TSC/oxygen/water. Corresponding ratios used here were 1:30:14 745 an effective concentration of oxygen ∼110 times the solubility at standard conditions. Simulations using a smaller box, with a 10 Å water layer and a concentration of oxygen ∼250 times the solubility at STP, gave results comparable to those presented here.

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