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Inhomogeneity of viscous aqueous solutions

G. Barshtein, A. Almagor, S. Yedgar,* and B. Gavish
Department of Biochemistry, Hebrew University—Hadassah Medical School, Jerusalem 91120, Israel

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Brownian principles for homogeneous media fail to explain the effect of viscous cosolvents on reaction rates. We examined the possibility that solvent viscosity in the microenvironment of a probe η^* (microviscosity) differs from the bulk viscosity η (macroviscosity). η^* was derived from the diffusion constant D of water tracers, measured in the presence of viscous cosolvents. We found that (a) $\eta^* \propto \eta^\alpha$, where $0 \le \alpha \le 1$, and (b) the temperature dependence of D is insensitive to the presence of these cosolvents. We propose that viscous aqueous solutions have an inhomogeneous microstructure with waterlike domains.

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There is a growing interest in the effect of solvent viscosity (η) on kinetic coefficients (k) of chemical [1] and biochemical [2] processes, in aqueous solutions. This effect is believed to probe the molecular dynamics involved in the process of crossing the reaction barrier. The phenomenological relation [1,2]

$$k \propto 1/\eta^p \tag{1}$$

with $0 \le p \le 1$, varies between two extremes: (a) A viscosity-independent $k(p=0)$ is derived by the transition state theory (TST), which assumes no collisions of the rectants with the solvent molecules during the process of crossing the reaction barrier. (b) A viscosity-dependent reaction rate, $k \propto 1/\eta$, i.e., $p=1$, as derived by Kramers [3]. This theory assumes that during the process of barrier crossing many collisions with the solvent molecules take place, leading to a diffusionlike motion of the reactants along the reaction coordinate.

Equation (1) has never been derived theoretically [4]. However, by combining the free-volume theory of Cohen and Turnbull for diffusion and Doolittle's phenomenological expression for the solvent viscosity, Gegiou *et al.* obtained Eq. (1) as an expression for the rate of structural rearrangements in a solute molecule surrounded by solvent molecules [5]. They interpreted p as a fraction of the minimal free volume required for translatory motions of a solvent molecule, when this minimal free volume is taken as 1. Another interpretation of Eq. (1) was proposed by Gavish [6] for the effect of solvent viscosity on protein reactions, where only a fraction of the bulk viscosity is sensed by the protein microenvironment; an N-fold increase of the bulk viscosity induces an N^p increase in the viscosity of the protein interior. Thus, in a more generalized sense, $0 \le p \le 1$ is a measure of the extent by which microviscosity is affected by macroviscosity.

In the above-mentioned studies, it was taken for granted that the solvent is seen by the solute as a continuum, to which the Brownian concepts of molecular dynamics are applicable. In practice, the viscosity of aqueous solutions is varied by adding viscous cosolvents of different sizes, from ethylene glycol to dextran polymers, which are all larger and less mobile than water. As illustrated in Fig. 1, for each solute-solvent system there is a cosolvent concentration below which the solute does not see a continuum, but an inhomogeneous medium. Since (a) structural rearrangements of the solute surface require displacements of the solvent molecules, and (b) water is the most mobile solvent component, we may expect that a solute of *small enough size* will experience the viscosity η^* of the surrounding water (microviscosity), which is smaller than the bulk viscosity η (macroviscosity). Thus, η^* is the *lowest microviscosity value* which can affect the reaction site. However, the state of the surrounding water may be affected by the presence of viscous cosolvents. This study was undertaken to explore the dependence of η^* or η. For this purpose we determined the diffusion coefficient of water in viscous aqueous solutions, as ra-

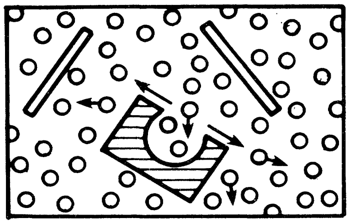

FIG. 1. Illustration of the inhomogeneity of aqueous solutions with viscous cosolvents. A solute performs small-scale structural rearrangements, which follow displacements of surrounding water molecules (open circles) but not of the less mobile cosolvent molecules (bars). Thus, the "reaction site" (concave region) senses mainly the water viscosity η^* in its microenvironment. For a high-enough cosolvent concentration and large-enough size ratio between the reaction site and the cosolvent molecule, the solution is viewed by the reaction site as a continuum.

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*Corresponding author. FAX: 972-2-784010.
Electronic address: YEDGAR@MD2.HUJI.AC.IL

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tionalized in the following.

Following the principles of the Brownian theory, the diffusion constant (D) of a particle, and the viscosity η^* of its surroundings are linked by the Stokes-Einstein relation

$$D\eta^* = AT \qquad (2)$$

where A is a geometrical factor and T is the absolute temperature. Experimental evidence supports the validity of this relation for the diffusion of a single molecule, including water [7]. This relation was applied here to obtain η^* from measurements of water diffusion in viscous solutions. The water isotope tritium oxide (T_2O) was chosen as a small "molecular probe."

$D_r = D(c,T)/D(0,T)$ is defined as the relative diffusion coefficient of T_2O in the presence of cosolvents at concentration c, relative to that in water ($c=0$). D_r was measured using the system depicted schematically in Fig. 2. The relative solvent viscosity $\eta_r = \eta(c,T)/\eta(0,T)$ was measured using an Oswald Capillary Viscometer. Using these notations, Eq. (2) can be expressed as $D_r\eta_r^* = 1$. Thus, by measuring D_r as a function of η_r, the relation between η_r^* and η_r can be determined.

The solvent viscosity was modified by the addition of viscous cosolvents which differ in size and chemical nature. This enables the differentiation of the viscosity *per se* from other solution properties, such as component concentrations and chemical interactions, which might be affected by the cosolvents. The cosolvents used were ethylene glycol (m.w. 62), glucose (m.w. 180), sucrose (m.w. 342), polyethylene glycol [(PEG), m.w. 4000], and bovine serum albumin [(BSA), m.w. 68 000]. All cosolvents were purchased from Sigma, St. Louis, MO. Measurements were performed at 25 and 50 °C.

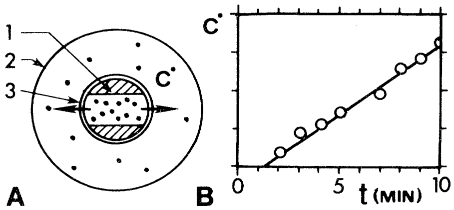

FIG. 2. Experimental setup for measuring the relative diffusion coefficient D_r of a radioactive tracer. The system, described in (a), consists of two compartments—a cell (1) and a beaker (2) containing, respectively, 0.6 and 50 ml of the same mixed solvent. The cell was formed by mounting a sleeve (3) (dialysis tubing MVCO1000, Spectrum Medical Ind.), permeable to molecules of water size, on a 7 mm diameter plastic cylinder that has a hole in the center (1). The tracer T_2O (closed circles) was placed in the cell at 5 μCi/ml (diluted 1/5000 from stock solution). The outward tracer diffusion (arrows) was monitored by measuring the radioactivity C^* of 100 μl samples taken from the well-stirred and thermoregulated ($\pm$0.5 °C) beaker compartment at desired times. For the first few minutes C^* depends linearly on time t, as shown in (b), reflecting a constant diffusion current, which is proportional to the tracer diffusion constant. Thus, $D_r = S/S_0$, where S and S_0 are the slopes of the regression line obtained for the mixed solutions and pure water, respectively.

Figure 3(a) shows, for all tested cosolvents, that the relation between D_r and η_r fits the equation

$$D_r\eta_r^\alpha = 1 \ , \qquad (3)$$

where $0 < \alpha < 1$.

Since, as noted above, $D_r\eta_r^* = 1$, it follows that

$$\eta_r^* = \eta_r^\alpha \ . \qquad (4)$$

Taking M and M_0 to be the molecular weight of the cosolvent and water, respectively, Fig. 2(b) depicts the dependence of α on the water/cosolvent size ratio $(M_0/M)^{1/3}$, suggesting that

$$\alpha = f(T)(M_0/M)^{1/3} \ , \qquad (5)$$

where $f(T)$ denotes a temperature dependence, to be determined. Here $f(25\,°C) = 0.68$ and $f(50\,°C) = 0.96$. In the limit of $(M_0/M)^{1/3} \rightarrow 0$, α vanishes within the experimental error.

Equation (5) shows that with increasing cosolvent molecular weight α decreases. In the limit of $\alpha \rightarrow 0$, $\eta_r^* \rightarrow 1$, corresponding to the microenvironment of pure water, i.e., the probe does not sense the cosolvent. With decreasing cosolvent molecular weight, a large but still finite fraction of the bulk viscosity is probed by the tracer. The limit $\alpha = 1$ is obtained when M(tracer) $\gg M$(solvent); in that case the solvent can be considered as a continuum to which the Stokes-Einstein equation [Eq. (2)] can be applied, and the microviscosity becomes identical with the bulk viscosity. Obviously, this is not the case for our system, in which M(tracer) $\leq M$(water or cosolvent). Therefore, $\alpha = 1$ cannot be obtained as a limiting case of Eq. (5). In fact, analysis of Marinin's data [8] concerning the diffusion of tracers of increasing molecular weight in water-glycerol solutions,

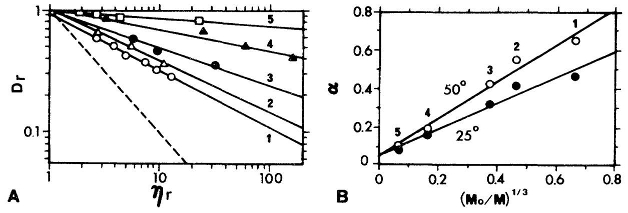

FIG. 3. The relation between the relative tracer diffusion coefficient D_r and the relative solvent viscosity η_r. (a) Linear regression of D_r vs η_r obtained at 25 °C, for the aqueous solutions of ethylene glycol (1), glucose (2), sucrose (3), PEG (4), and BSA (5). This analysis assesses the phenomenological relation $D_r\eta_r^\alpha = 1$ [Eq. (3)], where α is the slope of an individual line. $r > 0.984$ was obtained for all lines. The dashed line marks the Stokes-Einstein relation, $D_r\eta_r = 1$ [Eq. (2)]. A similar plot was obtained at 50 °C (not shown). (b) α values, derived from (a), plotted vs the water/cosolvent size ratio $x = (M_0/M)^{1/3}$. The regression slopes (A_1) and intercepts (A_0) at 25 and 50 °C are, respectively, $A_0 = 0.05 \pm 0.04$ and 0.04 ± 0.04, $A_1 = 0.69 \pm 0.10$ and 0.99 ± 0.10, with correlation coefficient of 0.975 and 0.985. The α values for cosolvents 1–5 were, respectively, 0.465, 0.421, 0.308, 0.176, and 0.063, at 25 °C, and 0.650, 0.543, 0.423, 0.209, and 0.068 at 50 °C.

fits Eq. (3) with $\alpha \rightarrow 1$, as the tracer size increases. It should be mentioned that Eq. (3) was already reported in the literature [9]. However, the relation of α to microviscosity, the cosolvent molecular weight, and the temperature was not elaborated.

In order to obtain a further insight into the cosolvent effect on the probe's immediate environment we examined the possibility that the presence of the cosolvent changes the diffusion energetics of the tracer. It is generally accepted that the temperature dependence of a quantity X, such as diffusion coefficient or solvent viscosity, is related to the molecular dynamics involved, and thus can be expressed in a narrow temperature range, by the "activation energy" E_x, defined by $-\partial \ln x/\partial(1/RT)$. Since $D_r = D/D_0$ we obtain $E_{D_r} = E_D - E_{D_0} \equiv \delta E_D$. A similar definition is applied for δE_η. Thus, by measuring D_r and η_r at 25 and 50 °C, we can determine the changes in the activation energies of D_0 and η_0 caused by the presence of the cosolvent. With all tested cosolvents we have found δE_η to be 4–6 kcal/mol at the maximum cosolvent concentration used. Surprisingly, in the same mixtures, δE_D was found to vary between -0.35 to 0.30 kcal/mol. These values are smaller than the thermal energy characterizing translatory motions (ca. 0.6 kcal/mol), and thus cannot be considered significant. Thus, the energetics of water diffusion was found to be unaffected by the presence of the cosolvent, at the applied concentration range.

It should be mentioned that previous studies have already pointed out the deviation of $D\eta$ from the Stokes-Einstein relation by means of its dependence on the cosolvent concentration, and the distinction between η and η^* [9–11], the relation of the results to local inhomogeneity [12], and the independence of E_D on the cosolvent concentration [11]. However, these studies made no attempt to quantify the observed relation between the solvent viscosity and the solute diffusion, or the microviscosity. The present work provides a systematic study of the relation between macro- and microviscosity, as a function of the cosolvent size, formulated by Eq. (5).

The present approach suggests that the diffusion coefficient of small solutes of atomic size in liquid mixtures can still be related to the solvent properties using the Stokes-Einstein equation, but with an "effective viscosity." This "effective viscosity" concept intuitively fits the Brownian interpretation of "friction" by collision of the solute with the solvent molecules. However, the question if cosolvents affect the geometrical factor A [see Eq. (2)] also in the case of larger and more complicated solutes is still open.

We may conclude that the energetics of water diffusion seem not to be appreciably modified in the presence of cosolvents at the applied concentration range. The decrease observed in D_r upon increasing the cosolvent concentration, could be attributed to the spatial constraints that the relatively immobile cosolvent molecules probably impose on the fast-diffusing water molecules. This observation supports our proposed view concerning the inhomogeneous nature of aqueous solutions of viscous cosolvents as depicted in Fig. 1.

The results of the present studies suggest that in studying the effect of solvent viscosity on chemical kinetics, a power law of the type given in Eq. (1) characterizes the difference between the reaction microenvironment and the bulk. This is consistent with the local nature of Kramers law, which can be written as $k \propto 1/\eta^*$ [6]. Thus, studying reaction kinetics in the presence of viscous cosolvents of varying molecular weight provides a tool for measuring the extent by which the reaction site "senses" its environment. It seems that a better understanding of molecular dynamics in viscous liquids requires the modeling of diffusion in noncontinuous and inhomogeneous systems.

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