Are Membrane Enzymes Regulated by the Viscosity of the Membrane Environment? †

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ABSTRACT: We have examined the idea that membrane enzymes are regulated by the viscosity of surrounding lipids using data compiled from the literature for the effect of the change in membrane viscosity (η) at the gel- to liquid-crystal-phase transition on the activities of several enzymes. The analysis was not extended explicitly to the problem of viscosity-dependent regulation of membrane enzymes in liquid-crystalline lipids because of the absence of exact data for values of η in liquid-crystalline phases of variable composition. For most membrane enzymes studied, energies of activation are discontinuous, while k_{cat} is continuous, at the main-phase transition. We consider that the energy of activation contains terms related to the height of the chemical barrier to reaction and terms due to the mechanical properties of the bilayer, such as the work of expansion during the catalytic cycle and the temperature dependence of η. We find that the differences in energies of activation, above and below the break points in Arrhenius plots, are orders of magnitude larger than can be accounted for by the above mechanical factors. Thus, discontinuities in energies of activation at the phase transition appear to reflect changes in the chemical barrier to reaction, which is independent of η. The theoretical analysis indicates too that values of η for bilayers in the liquid-crystalline phase would have to be several orders of magnitude larger than those for gel phases in order to provide a basis for viscosity-dependent regulation of membrane enzymes in liquid-crystalline phases. We conclude that a minority of lipid-dependent membrane enzymes are regulated by the viscosity of the lipid environment. We note that the basis for viscosity-dependent regulation is unclear even in these cases. We examined next the possibility that key motions leading to the activated state of a membrane phase could be damped not by the membrane phase but by the aqueous phase surrounding the membrane. These experiments, which were carried out with the microsomal enzyme UDP-glucuronosyltransferase, showed that membrane enzymes can be sensitive to the viscosity of the aqueous phase. In fact, the activity of UDP-glucuronyltransferase was inhibited completely at an aqueous-phase viscosity below that for gel-phase bilayers in which the enzyme is known to be active.

Pervasive in the literature is the idea that membrane fluidity,[1] which is the inverse of viscosity (η), regulates the activities of integral membrane enzymes (Dornmair et al., 1989; Kimelberg & Papahadjopolous, 1974; Machtiger & Fox, 1973; Mavis & Vagelos, 1972; Silvius & McElhaney, 1980; Zakim et al., 1985) analogous to the behavior of aqueous-soluble proteins (Beece et al., 1980; Doster, 1983; Gavish & Werber, 1979; Frauenfelder et al., 1988; Somogyi et al., 1984). The best support for this idea is the correspondence between discontinuities in energies of activation and/or activities of lipid-dependent, membrane-bound enzymes and the main-phase transitions of the membrane (Table I and references therein). There are several reasons, however, for considering that a causal link between membrane fluidity and enzyme function is problematic. First, properties of membrane lipids other than fluidity change discontinuously at the main-phase transition (Cevc & Marsh, 1987). Also, many proteins are immiscible with lipids in the gel phase, which is a property not dependent on η per se. Second, discontinuities in Arrhenius plots are seen for some membrane enzymes in the absence of lipids (Carruthers & Melchior, 1984; Dean & Tanford, 1978; Houslay & Gordon, 1983). Third, correspondences between discontinuities in Arrhenius plots and the main-phase transitions of surrounding lipids occur for several membrane enzymes for which correlations between membrane viscosity and enzyme function cannot be demonstrated in other experimental settings (Burns, 1969; Carruthers & Melchior, 1984; Cheng et al., 1985; Dean & Tanford, 1978; East et al., 1984; Thorneley et al., 1975).

The above studies show that the effect of viscosity on the activity and other properties of membrane enzymes is not understood. In this paper, we have approached this problem by predicting, quantitatively, the extent to which k_{cat} and the energy of activation (E_a) of membrane enzymes should be altered by the large increase in viscosity that occurs with the transition from the liquid-crystal to the gel phase. We then compare these predicted changes to reports of the effects of the lipid-phase transition on k_{cat} and energies of activation of nine well-studied membrane enzymes. We find from this analysis that changes in the viscosity of membrane lipids at the phase transition cannot account for the associated changes in the properties of membrane enzymes. This result does not mean, however, that the activities of membrane enzymes are insensitive to the viscosity of the solvent. We also present data showing that k_{cat} of the lipid-dependent, membrane enzyme UDP-glucuronylsyltransferase is highly dependent on the viscosity of the aqueous phase, not the membrane phase.

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[1] Viscosity cannot be defined adequately in a quasi-two-dimensional fluid like a membrane. However, the physical characteristics on which it usually is based (i.e., free volume and flexiblity of chains) can be determined experimentally. This allows us to discuss changes in viscosity in an operational sense.

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Thus, membrane viscosity appears to have secondary importance at best for regulating the function of UDP-glucuronosyltransferase and perhaps other membrane enzymes. We propose a mechanism to explain the differential effects of aqueous and membrane viscosity on the properties of this enzyme.

MATERIALS AND METHODS

Microsomes from rat liver were prepared as in Zakim et al. (1985). UDP-glucuronosyltransferase was assayed using *p*-nitrophenol as aglycon (Hochman et al., 1981). Unless noted otherwise, assays were at 30 °C and contained 1.0 mM UDP-glucuronic acid and 0.05 mM *p*-nitrophenol. Activities at V_{max} were determined graphically for an enzyme with two substrates and a rapid equilibrium random kinetic mechanism (Vessy & Zakim, 1972). Enzyme in microsomes was converted to the activated state (Zakim et al., 1985) by adding palmitoyllysophosphatidylcholine at a detergent/microsomal protein ratio (w/w) of 5/1 (Zakim et al., 1985). Protein was measured with the method of Lowry et al. (1951).

THEORY

Explicit Relation between Solvent Viscosity and the Activity of Enzymes. If a protein can exist in two different conformational states separated by an energy barrier, then collisions with solvent molecules will transfer energy from the solvent to the protein to facilitate transitions between the lower and higher energy states. The general effect of viscosity on reaction rates was modeled (Kramers, 1940) as the resistance to diffusion (or rotation) of reacting molecules across a potential barrier, i.e., the rate of transition between energy states. The predicted relationship between transitions across an energy barrier and the viscosity of the solvent was given by eq 1, in

$$k = (A/\eta)e^{-E_a/RT} \quad (1)$$

which E_a is the height of the chemical barrier (Kramers, 1940; Gavish, 1978; Gavish & Werber, 1979). There is now ample experimental verification for the applicability of eq 1 to the effects of η on the rates of transitions of polymers between conformational states (Bullock, 1974; Helfand, 1971), the dynamics of proteins (Beece et al., 1980; Doster, 1983), and the activities of enzymes (Gavish, 1978; Gavish & Werber, 1978; Gogaudze et al., 1991; Somogyi et al., 1984). It has been found for some enzyme-catalyzed reactions that k is proportional to $1/\eta^m$, where m is less than 1 (Beece et al., 1980; Gavish & Werber, 1979). In a few instances, k has no dependence on solvent viscosity (Gogaudze et al., 1991). We focus here only on the general applicability of eq 1 because this is the basis for the idea that membrane fluidity regulates membrane enzymes. Since we will be dealing with enzymes, k_{cat} will be used in place of k.

Inspection of eq 1 indicates that an increase in η will decrease the activity of a viscosity-dependent enzyme. Since the effect of viscosity on the activities of membrane enzymes usually is analyzed in the context of Arrhenius plots of rate data, we depict in Figure 1 the expected effect of a discontinuous change in η on the Arrhenius plot of a membrane enzyme that is sensitive to the change in η at the main-phase transition of a membrane. For the case of the liquid-crystal- to gel-phase transition of membrane bilayers, the change in η of the lipids will be discontinuous. As noted below, the change in η at the phase transition is on the order of 10-fold. Assuming that $m = 1$ in the expression $1/\eta^m$, we show a discontinuous change in k_{cat} of 10-fold at the phase transition of the membrane.

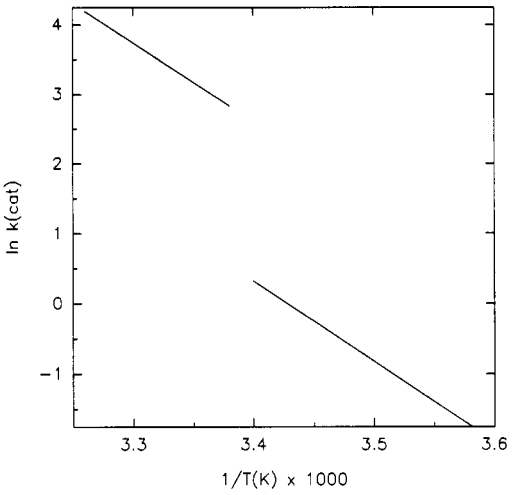

FIGURE 1: Schematized Arrhenius plot for a membrane enzyme. The Arrhenius plot is drawn according to eq 1 to show a discontinuous change in k (or k_{cat}) at the temperature (23 °C) of the phase transition of a bilayer or DMPC. The discontinuous change in η of the bilayer, at the phase transition, is assumed to be 10-fold. The slope of the plot corresponds to an E_a of about 22 kcal/mol.

Also, since E_a is independent of η, the slopes of the Arrhenius plot above and below the temperature for the phase transition of the lipids are shown to be identical. Equation 1 is not strictly applicable to the probelm at hand, however, because η is temperature-dependent. To account for this, we substitute $\eta = \eta_0 e^{E_\eta/RT}$, where E_η is the activation energy for viscous flow (Kramers, 1940; Gavish & Werber, 1979) to give eq 2.

$$k = (A/\eta_0)e^{-E_a/RT}e^{-E_\eta/RT} \quad (2)$$

If we consider too that there is work of expansion against the lipid bilayer during the catalytic cycle (Jahnig & Bramhall, 1982), then a work term can be added to eq 2 (eq 3).

$$k = (A/\eta_0)e^{-E_a/RT}e^{-E_\eta/RT}e^{-E_w/RT} \quad (3)$$

Dissipation of energy by rotation is incorporated in the preexponential term.[2] Equation 3 assumes that the rate of collisions between solvent and protein is much faster than the catalytic rate.

The chemical barrier (E_a) to reaction is not the slope of the Arrhenius plot in Figure 1. The energy barrier includes terms due to the temperature dependence of η of the medium and the work of expansion, which will differ in gel and liquid-crystalline phases. We refer to the slope of an Arrhenius plot

[2] It has been proposed (Squier et al., 1988) that energy dissipation due to protein rotation can influence activity. In our treatment, rotational effects are included in the preexponential term of eq 4, as a direct consequence of η. In the absence of changes in protein–protein contacts during catalysis in a multisubunit enzyme, the physical basis for an effect of rotational rate on catalysis is unclear. Nevertheless, we include below an analysis of the amount of energy that might be dissipated through rotation. We assume that the protein is a cylinder. The rate of energy loss due to the friction around the sides of the cylinder, assuming the top and bottom are frictionless, is given by

$$dE_r/dt = 4\pi\eta LU^2$$

where L is the length of the cylinder and U is the angular velocity. We note that allowing energy loss on the all sides of the cylinder is an extreme case. A more appropriate model would be one where the top and bottom ends of the cylinder experience friction (the head-group region) but the middle does not. We can also calculate the rate of energy dissipation per unit volume using the expression for linear bulk flow and inserting the protein circumference for the length (l) by $dE_r/dt = \eta U^2/l^2$ and obtain similar results.

Table I: Association between the Main Lipid-Phase Transition and the Properties of Membrane Enzymes[a]

enzyme	source	lipids	discontinuity of Arrhenius plot	E_{tot} above/below phase transition (kcal/mol)	ref
Ca–Mg-ATP'ase	SR	reconst mito lipids	E_{tot}	11.8/23	1
Na–K-ATP'ase	microsomes	PS	E_{tot}	16.6/49.8	2
Na–Mg-ATP'ase	*Acholeplasma laidlawii* B	*	E_{tot}	1.56/19.8	3
ATP'ase	yeast PM	DMPC	E_{tot}	96/170	4
Lac permease	*E. coli*	DMPC	k_{cat}		5
cyt *c* reductase	mitochondria	mito from summer active	E_{tot}	28.7/99.1	6
acetylcholin esterase	rat brain synaptosomes	intact synaptosomes	E_{tot}	17.5/57.4	7
succinic oxidase	sheep mito	intact mito	E_{tot}	3.4/50	8
β-hydroxybutyrate dehydrogenase	heart mito	DMPC	k_{cat}		9
glucose transport	RBC	intact RBC	E_{tot}	23.8/51.1	10

[a] References to original data are as follows: (1) Squier et al. (1988); (2) Kimelberg & Papahadjopoulos (1974); (3) Silvius & McElhaney (1980); (4) Dufour & Tsong (1981); (5) Dornmair et al. (1989); (6) Augee et al. (1984); (7) Foot et al. (1983); (8) McMurchie & Raison (1979); (9) Houslay et al. (1975); (10) Whitesell et al. (1989). Asterisk: organism was grown in the presence only of isoheptadecanoic acid, and values of E_{tot} were calculated from data plotted in reference 3. Experiments in the references cited were not necessarily limited to the lipid listed in the table, but these lipids are those for which a discontinuity in Arrhenius plots was seen and for which values of E_{tot} are reported.

as $E_{tot} = E_a + E_\eta + E_w$. The slopes of the Arrhenius plot in Figure 1, which is based on eq 1, need to be adjusted for the energy terms in eq 3. Therefore, estimates of the differences between E_η and E_w in liquid-crystalline- and gel-phase bilayers are essential for examining experimental data for the effects of η on the activities of membrane enzymes.

Temperature Dependence of η in Liquid-Crystalline- and Gel-Phase Bilayers. E_η is 2-fold larger for liquid-crystalline- versus gel-phase bilayers of DMPC (Scarlata, 1989); i.e., the bilayer is more compressible in the liquid versus the gel state. However, the absolute values of E_η in either phase are small. They are approximately 8 cal/mol in the liquid-crystal phase and 4 cal/mol in the gel phase. The absolute values of E_η are quite small as compared with typical values of E_a for enzyme-catalyzed reactions in general and in the context of values of E_{tot} in Table I in particular. We note too that E_{tot} will be slightly larger in the liquid-crystalline phase as compared with the gel phase due to the contributions of E_η in these phases.

Change in E_W at the Phase Transition. Expansion of an enzyme–substrate complex as it moves along the reaction pathway requires that work be done against the environment. This energy can be calculated directly on the basis of the viscosity of the environment against which work of expansion occurs and the volume of activation ($V^\#$) of a given enzyme. $V^\#$ of an aqueous soluble enzyme can be obtained from plots of ln k_{cat} vs $1/P$, but this relation will not apply for a membrane enzyme for which activity is sensitive to η of the bilayer. This is so because in contrast to water, to about 1.5 kbar (Isaccs, 1981), pressure increases the viscosity of bilayers (Scarlata, 1991). As a result, there may be pressure-dependent effects on the k_{cat} of membrane enzymes secondary to the variation of η of a bilayer with pressure. Therefore, we choose a value for $V^\#$ (40 mL/mol) for a model membrane enzyme. This value is typical of $V^\#$ for an aqueous-soluble enzyme (Morild, 1981). Moreover, the calculations below show that any reasonable value of $V^\#$ will yield the same result as the value of 40 mL/mol. Obviously, negative activation volumes will not contribute a E_w term. To maximize the work of expansion against the bilayer during the catalytic cycle, we assume that $V^\#$ is due only to a change in the actual volume of the enzyme, noting that changes in $V^\#$ due to changes in hydration of the protein will not be associated with expansive work. For the same reason, we select a small value for expansion (1 cm/mol) of the enzyme against the surrounding water phase, which is expansion in the z plane (parallel to the polymethylene chains). Expansion in the x and y planes, which is against the viscosity of the bilayer, then will be 40 cm^2/mol.

For large, unilamellar bilayers of egg PC (in the fluid phase), the modulus of compressibility is about 0.14 J m^{-2} at 25 °C (Evans & Needham, 1982). The same value is obtained for fluid-phase DMPC vesicles (Kwok & Evans, 1981). From this, we calculate a value of 5.6×10^{-2} J/mol for the work of expansion against the bilayer, under the conditions just given. Since the modulus of compressibility of DMPC in the gel phase is about an order of magnitude larger than that for the fluid phase (Kwok & Evans, 1981), differences in E_w for enzyme in fluid or gel phases will be a small component of E_{tot}. The contributions of E_η and E_w to the energy in eq 3 are so small that eq 1 will apply to the effects of η on the activity of a membrane enzyme, and the Arrhenius plot in Figure 1 depicts accurately the response of a membrane enzyme to a discontinuous change in η at the phase transition of a bilayer. Divergence from the behavior in Figure 1 cannot be explained on the basis of the viscosity dependence of an enzyme.

RESULTS AND DISCUSSION

Comparison of Theory with Data for Membrane Enzymes. Relevant data are in Table I. We have not listed reports for which a transition temperature was not reported for a probe molecule dissolved in the membrane of interest. We also have not included data from reports in which there were large discrepancies between temperatures for discontinuities in Arrhenius plots (discontinuitites of slope for enzymes in intact membranes) and the putative phase changes in the lipid matrix. For example, Esfahani et al. (1972) found that temperatures for discontinuitites in Arrhenius plots of succinic reductase (from *Escherichia coli*) changed as membrane lipids were modified. However, these temperatures did not correspond to the phase transitions in the lipids.

Table I is not meant to be complete [see Linden and Fox (1975) and McElhaney (1982) for reviews] but to be representative of the following points. For most membrane enzymes with discontinuous Arrhenius plots, the discontinuity is in E_{tot} not k_{cat}, E_{tot} being smaller in the liquid versus the gel state of the lipid matrix. As noted above, this change cannot be due to the discontinuous change in E_η, which is larger in the liquid-crystalline as compared with the gel phase (Scarlata, 1989). Also, for enzyme with discontinuities in k_{cat} at the phase change, no enzyme activity could be measured in the gel phase. Viscosities in gel versus liquid-crystalline bilayers are about 10-fold larger for bilayers of phosphatidylcholines comprised of saturated polymethylene chains [e.g., see Scarlata (1989)]. A change in η of this magnitude is expected to give rise to a finite discontinuity of k_{cat}, which

cannot be larger than 10-fold (eq 2). Yet we can find no well-studied system for which there was a finite discontinuity in activity at the main-phase transition.

The data in Table I show that the membrane enzymes studied to date fall into two classes. The more abundant class displays continuity of k_{cat} at the phase transition but a change of slope of the Arrhenius plot above and below this temperature. The less abundant class shows no measurable activity in the gel phase; i.e., the discontinuity in k_{cat} at the phase transition is infinite. These data do not correspond with theoretical expectations for the behavior of viscosity-sensitive enzymes at the phase transition. The divergence between real behavior and theoretical predictions includes the following problems.

Enzymes with Activity in Gel-Phase Lipids. The data in Table I show for these enzymes that k_{cat} is continuous at the phase transition and the differences between E_{tot} in liquid-crystalline and gel phases are too large to be accounted for by viscosity-dependent parameters, e.g., the temperature dependence of η and the work of expansion against media of different η. Also, the sign of the change in the former parameter, which we have shown above is greater than the latter, is opposite to the effects of the lipid-phase transition on measured values of E_{tot}. Therefore, the behavior of this group of membrane enzymes at the phase transition does not fit in any way with the effects expected as secondary to the discontinuous change in η at the phase transition. It could be argued that the value of $V^{\#}$ chosen under Theory for calculations of E_w is inappropriate or that our assumptions about the distribution of expansion in the x, y, and z planes of the membrane are unrealistic. These assumptions were chosen, however, to maximize the size of the work term and thus to favor the hypothesis that membrane enzymes are responsive to η of the bilayer. It appears, further, that no reasonable change in the value of $V^{\#}$, in the difference between the modulus of compressibility in liquid and gel phases, or in the distribution of expansion of a membrane enzyme in the x, y, and z planes of the membrane will alter the conclusion that the work of expansion, even against highly viscous membranes, will be small and insufficient to account for continuity of activity at the main-phase trransition of bilayers. We think it is an inescapable conclusion that changes in E_a must be the reason that Arrhenius plots are discontinuous with regard to slope at the phase transition of the bilayer for membrane enzymes with activity in gel-phase lipids. Since E_a is not viscosity-dependent, it follows that η of the lipids is not a primary determinant of activity. There are several potential mechanisms by which lipids could modulate the stabilities of different conformational states of membrane enzymes and thereby modulate the chemical pathway for reaction. We note too, however, that large decreases in E_{tot} in the liquid-crystalline versus the gel phase could compensate for possible discontinuous effects of η on activity at the phase transition. If we consider that the size of the discontinuity in η is 10-fold at the phase transition, then a chnage of ln 10, or about 2.3-fold, in E_{tot} will maintain continuity of k_{cat} at the phase transition. The observed changes in E_{tot} at the phase transition, for enzymes with activity in gel-phase lipids, are large enough to provide this degree of compensation (Table I).

The data in Table I and the analysis under Theory do not address directly that membrane enzymes in intact organisms function in lipids in the liquid-crystalline state. That is, independent of the organism studied, physiological temperatures are above those for the main-phase transitions of biological membranes. Unfortunately, there are no quantitative data for differences in η between phospholipid bilayers in the liquid-crystal phase as a function of the composition of the polar and apolar regions. We hence cannot calculate values of E_w for an enzyme in, for example, a series of bilayers of phosphatidylcholine with variable acyl chain compositions. In addition, we have to consider the validity of extrapolating the analysis of bilayers comprising a single species of lipid to the problem of enzyme function in heterogeneous biological membranes. Again, we are aware of no data for values of η in such membranes. On the other hand, the Theory section shows that E_w will be an exceedingly small component of E_{tot} in liquid-crystal phases (Table I) for values of η orders of magnitude larger than those of bilayers in the gel phase. Therefore, it appears reasonable to propose that variations of η in liquid-crystalline bilayers of different composition are unlikely to modulate k_{cat} of membrane enzymes. We note that this will apply even to enzymes in Table I that are inactive in gel-phase lipids.

Enzymes with No Activity in Gel Phases. Table I contains two examples in this category. The Kramers equation predicts that the maximum change in k_{cat} should be 10-fold for the 10-fold difference in η between liquid-crystalline- and gel-phase bilayers. Our analysis of the effects of the temperature dependence of η and the work of expansion on k_{cat} show that such effects will be too small to measure. Thus, it is not immediately clear why some membrane enzymes have no measurable activity in gel-phase lipids. It is interesting too that we could find no enzyme for which there was a finite and discontinuous change in k_{cat} at the phase transition of lipids.

Effect of the Viscosity of the Aqueous Phase on the Function of Membrane Enzymes. The data in Table I show that the viscosity of the lipids is unlikely to be the primary property of the membrane for regulating membrane enzymes, but we cannot be certain of the extent to which viscosity modulates activities independent of the differential effects of gel and liquid-crystal phases on the conformational states of membrane enzymes. Data for ATPases suggest that hydrolysis of ATP is catalyzed by a region of the enzyme that is completely solvated by water (Andersen et al., 1985; Castellani et al., 1985; Maunsbach et al., 1988; Taylor et al., 1986). The catalytic regions of other membrane enzymes could have a similar organization, which could lead to a function that is independent of the viscosity of the membrane. An alternative possibility is that critical motions leading to catalysis by membrane enzymes occur predominantly in a plane subject to damping by the viscosity of the aqueous phase rather than that of the bilayer. In addition, energy exchange between solvent and enzyme could be predominantly via enzyme–water interactions, not enzyme–bilayer interactions. These possibilities were examined by testing the effect of changes in the viscosity of water on the function of the lipid-dependent, microsomal enzyme UDP-glucuronosyltransferase (Hochman & Zakim, 1983; Hochman et al., 1981, 1983; Magdalou et al., 1982; Rotenberg & Zakim, 1989, 1991).

The data in Figure 2 show that the activity of UDP-glucuronosyltransferase decreased with addition of increasing amounts of glycerol to water. Measurements of activity extrapolated to infinite concentrations of both *p*-nitrophenol and UDP-glucuronic acid (V_{max}) are shown in Figure 3 as a function of temperature for enzyme in water or 20% (v/v) glycerol. Glycerol decreased the activity of UDP-glucuronosyltransferase at V_{max} (Figure 3), but did not change the slope of the Arrhenius plots (e.g., E_{tot}) when the data were corrected for the effect of temperature on η of glycerol solutions. It has been reported previously that 55% glycerol in water (v/v)

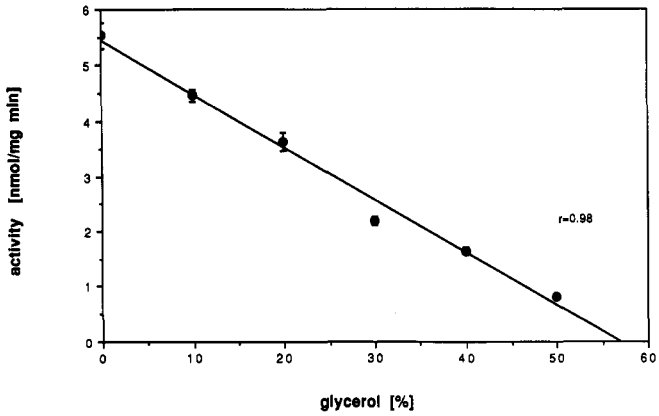

FIGURE 2: Effect of aqueous-phase viscosity on the activity of UDP-glucuronosyltransferase. Assays conditions were as described under Materials and Methods except that the indicated amounts of glycerol were present.

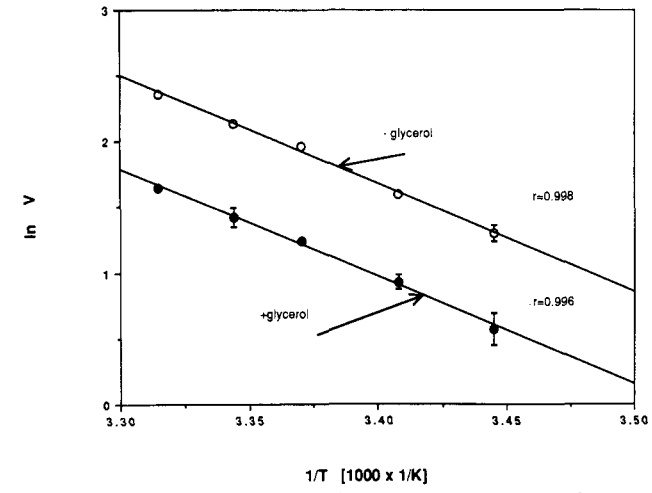

FIGURE 3: Energy of activation of UDP-glucuronosyltransferase in water or in 20% glycerol/water (w/w). Activities at V_{max} were measured at the indicated temperatures, as described under Materials and Methods, for an enzyme with two substrates and a rapid equilibrium random kinetic mechanism.

does not affect the rotational motions of fluorescent probes embedded in bilayers (Scarlata, 1989). Therefore, the effects of glycerol on the activity of UDP-glucuronosyltransferase (Figures 2 and 3) were mediated via its effect on η of water. Another imortant point illustrated by the data is that the activity of UDP-glucuronosyltransferase approached zero (Figure 2) in 55% glycerol/water. UDP-glucuronosyltransferase is active in gel phases of DMPC and DPPC and DSPC (Hochman et al., 1983; Rotenberg & Zakim, 1991), which like 55% glycerol/water mixtures are phases with very high viscosities.

Interactions of UDP-glucuronosyltransferase with the polymethylene chain region of lipid matrices regulate activity at V_{max} (Hochman et al., 1981; Hochman & Zakim, 1984; Magdalou et al., 1982), binding of substrates (Hochman et al., 1981, 1983), interactions between binding sites (Hochman et al., 1983), the response to allosteric effectors (Hochman & Zakim, 1983), and the thermal stability (Rotenberg & Zakim, 1989). In addition, infrared spectra of UDP-glucuronosyltransferase reconstituted into bilayers of DMPC show only one structural motif, which was α-helix (Zakim and Wong, unpublished data). This finding makes it unlikely that there are extensive globular portions of UDP-glucuronosyltransferase in the aqueous phase. Therefore, it appears that UDP-glucuronosyltransferase is not organized in membranes in the manner of ATPases (Andersen et al., 1985;

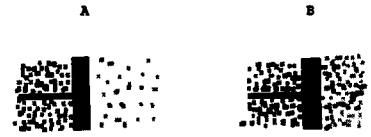

FIGURE 4: Representation of the effect of density on the ability of a protein to push against the solvating medium. The backbone motions of the protein are represented by a piston. In (A), the density of the medium is less than that of the protein. In (B), the density of the medium equals that of the protein.

Castellani et al., 1985; Maunsbach et al., 1988). We think instead that transfer of energy between solvent and enzyme might be predominantly via collisions between enzyme and water and that the backbone motions critical for generating the activated state of the enzyme–substrate complex are not damped extensively by the membrane. Motions in the x and y planes of the membrane could occur, but the primary effect of this sort of membrane–enzyme interaction might be to determine the reaction pathway, which would appear as a change in E_a in association with the main-phase change of the membrane. If this change behaved like a first-order-phase transition, we would expect discontinuities in E_a and k_{cat} for at least some of the enzymes in the Table. Reports that Arrhenius plots of membrane enzymes, when studied carefully, tend not to display sharp breaks as much as continuous bending with temperature are compatible with this idea (McElhaney, 1982).

Mechanism for Loss of Activity in Gel-Phase Lipids. Aside from aggregation of proteins, there are other possible mechanisms by which these enzymes could become nonfunctional in gel phases. We show in Figure 4 a scheme in which the loss of activity is a direct consequence of the viscosity of the bilayer. Movement of the peptide backbone is depicted here to occur against a protein domain that expands subsequently against the solvent. In Figure 4a, a certain force is required to move a densely packed protein domain of length L a certain distance in a solvent with low viscosity. In Figure 4B, η of the solvent is similar to that of the protein. In this case, the length of domain that must be moved by the expanding protein is infinite because there is no clear difference between the molecular density of the solvent and the protein. Therefore, the force required to move the protein in the highly viscous solvent becomes infinite, and catalysis cannot occur. This result leads to the idea that membrane proteins differing in packing density will have different responses to the abrupt change in viscosity of a bilayer at the phase transition. For example, for enzymes with densities less than those of gel-phase lipids and key backbone motions in the x and y planes of the bilayer, activity will be zero in gel-phase lipids. The question then is whether we can expect the packing density of some membrane enzymes to be less than gel-phase lipids. For aqueous-soluble proteins in isotropic solvents, the freezing of backbone motions occurs between 0.5 and 2 P (Rholam et al., 1984; Scarlata et al., 1984). If aqueous-soluble and integral membrane proteins have similar packing densities [but the density decreases as the content of hydrophobic amino acids increases (Gekko & Hasegawa, 1986)], then the viscosities reported for gel phases are large enough to completely inhibit the function of all membrane proteins.

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