Lipid Fluidity Directly Modulates the Overall Protein Rotational Mobility of the Ca-ATPase in Sarcoplasmic Reticulum*

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We have developed a quantitative and relatively model-independent measure of lipid fluidity using EPR and have applied this method to compare the temperature dependence of lipid hydrocarbon chain fluidity, overall protein rotational mobility, and the calcium-dependent enzymatic activity of the Ca-ATPase in sarcoplasmic reticulum. We define membrane lipid fluidity to be $T/\eta$, where $\eta$ is the viscosity of a long chain hydrocarbon reference solvent in which a fatty acid spin label gives the same EPR spectrum (quantitated by the order parameter $S$) as observed for the same probe in the membrane. This measure is independent of the reference solvent used as long as the spectral line shapes in the membrane and the solvent match precisely, indicating that the same type of anisotropic probe motion occurs in the two systems. We argue that this empirical measurement of fluidity, defined in analogy to the macroscopic fluidity ($T/\eta$) of a bulk solvent, should be more directly related to protein rotational mobility (and thus to protein function) than are more conventional measures of fluidity, such as the rate or amplitude of rotational motion of the lipid hydrocarbon chains themselves. This new definition thus offers a fluidity measure that is more directly relevant to the protein's behavior. The direct relationship between this measure of membrane fluidity and protein rotational mobility is supported by measurements in sarcoplasmic reticulum. The overall rotational motion of the spin-labeled Ca-ATPase protein was measured by saturation-transfer EPR. The Arrhenius activation energy for protein rotational mobility (11–12 kcal/mol/degree) agrees well with the activation energy for lipid fluidity, if defined as in this study, but not if more conventional definitions of lipid fluidity are used. This agreement, which extends over the entire temperature range from 0 to 40°C, suggests that protein mobility depends directly on lipid fluidity in this system, as predicted from hydrodynamic theory. The same activation energy is observed for the calcium-dependent ATPase activity under physiological conditions, suggesting that protein rotational mobility (dependent on lipid fluidity) is involved in the rate-limiting step of active calcium transport.

Since the general acceptance that biological membranes are fluid structures, there has been an active discussion of the possible role that changes in membrane fluidity could play in triggering or modulating membrane functions (reviewed by Kates and Manson, 1984; Shinitzky, 1984; Deuticke and Haest, 1987). Lipids in most biological membranes under physiological conditions are primarily in the liquid-crystalline phase (McElhaney, 1984); in fact, membrane-bound enzymes reconstituted into pure lipids have consistently demonstrated little or no activity below the phase transition (reviewed by Sandermann, 1978; Shinitzky, 1984; Hidalgo, 1985, 1987). Although head group composition (reviewed by Hidalgo, 1985) and fatty acyl chain length (and thus bilayer thickness) (Lewis and Engelman, 1983) have been shown to be critical to the functioning of membrane-bound enzymes (Bennett et al., 1980; Caffrey and Feigenson, 1981; Johannsson et al., 1981a, 1981b; Navarro et al., 1984; East et al., 1984), it is commonly proposed that an important general mechanism for the regulation of membrane function is the modulation of both specific interactions at the lipid-protein interface (Fong and McNamara, 1987; Bigelow and Thomas, 1987) and membrane fluidity, presumably through changes in fatty acid unsaturation and cholesterol content (reviewed by Benga and Holmes, 1984; Shinitzky, 1984; Yeagle, 1985). Many membrane activities have been correlated with membrane fluidity (Hidalgo et al., 1978; Sinensky et al., 1979; Blanquet, 1983; Bigelow and Thomas, 1987); however, examples in which a precise role for membrane fluidity has been identified are limited (reviewed by Marsh and Watts, 1982; Shinitzky, 1984; Marsh, 1985). We intend to provide more quantitative and useful measurements of membrane fluidity and to determine the relationship between fluidity and function. SR1 is an ideal system in which to study the role of membrane fluidity because the Ca-ATPase has little or no selectivity for different phospholipids (Roelfsema, 1981; Caffrey and Feigenson, 1981; East and Lee, 1982).

It has been proposed that the correlation of some membrane functions with lipid fluidity is the result of a general requirement for mobility of membrane-bound proteins (reviewed by Shinitzky, 1984). Support for this proposal has come from the previous findings in our laboratory that the overall rotational mobility of the Ca-ATPase of SR, as well as lipid hydrocarbon chain dynamics, measured by EPR correlates well with enzymatic function under conditions that perturb both functional properties and molecular motions (reviewed by Thomas, 1985, 1986; Hidalgo, 1985, 1987). In addition, it has been suggested that the rate-limiting step of calcium transport by this enzyme may be dependent on the fluidity of the bilayer (Moore et al., 1978; Almeida et al., 1982), as well as on the protein rotational mobility (Bigelow et al., 1986; Bigelow and

* The abbreviations used are: SR, sarcoplasmic reticulum; ST-EPR, saturation-transfer EPR; SASL, stearic acid spin label(s); MESL, methyl ester spin label(s); MOPS, 3-[(N-morpholino)propanesulfonic acid; EGTA, [ethylenebis(oxyethylenenitrilo)]tetraacetic acid.

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Thomas, 1987), although this point is controversial (East et al., 1984; Proux et al., 1986a, 1986b).

In this study, in order to help resolve this controversy regarding the role of a fluid bilayer in enzymatic function, we have quantitatively related lipid fluidity to protein rotational mobility. The most important step in this effort was to define lipid fluidity in a way that satisfies two requirements: (a) the definition must lend itself to quantitative and unambiguous measurement, and (b) there must be a straightforward theoretical prediction for the relationship between lipid fluidity and protein rotational mobility. Most previous studies have focused on the first requirement at the expense of the second.

That is, lipid fluidity has usually been characterized by the measurement of lipid probe rotational motions, usually using either fluorescent probes or spin labels. Although these motions can be measured directly and unambiguously, they occur with a much smaller amplitude and on a much faster time scale than overall protein rotational motions, and no reliable theory has been developed to describe their relationship to protein motions. In contrast, a quantitative theory has been developed relating the overall brownian rotational diffusion of membrane proteins to the viscosity ($\eta$) of the surrounding lipids (Saffman and Delbrück, 1975). The key result of this theory is that the protein mobility (rate of rotational diffusion) is directly proportional to $T/\eta$, as long as the size and shape of the protein remain constant. This theory suggests that membrane viscosity is obtained more appropriately by measuring the rotational diffusion of membrane proteins, rather than lipid hydrocarbon chain dynamics (Cherry and Godfrey, 1981; Jähnig, 1986).

Thus, it is appropriate to define fluidity to be $T/\eta$, associating fluidity with the lipid viscosity, which describes the potential energy barrier to protein rotational motion. The remaining problem is to define lipid viscosity and to relate it empirically to spectroscopic probe measurements of lipid motions. Since $\eta$ discussed in the theory (Saffman and Delbrück, 1975) describes the motion of a large object (protein) through small solvent molecules (lipids), it is appropriate to define $\eta$ from the same kind of measurement that is used to measure the macroscopic viscosity of bulk liquids. Thus, a spectroscopic measurement with a lipid probe in a locally ordered long chain solvent, which is structurally similar to a phospholipid bilayer, can be used to calibrate the viscosity of membrane lipids provided that the macroscopic viscosity of the solvent is known at various temperatures. That is, if the spectroscopic measurement gives the same result in the membrane as in the solvent, the two can be said to have the same viscosity (fluidity).

This type of calibration has been employed previously with fluorescent probes, but the measurements of viscosity obtained were dependent upon the probe and the reference solvent chosen (Shinitzky et al., 1971; Hare and Lussan, 1977). A possible source of these problems is that most of the fluorescent probes and solvents had molecular structures unlike those of phospholipid hydrocarbon chains, making it unlikely that the same kind of anisotropic motions would occur in the lipid bilayer. The type of measurement usually used in those studies was steady-state fluorescence polarization, which lacks the resolution needed to characterize in detail the type of anisotropic motion occurring.

Therefore, in this study, we have chosen a combination of spectroscopic probes and reference solvents more likely to approximate lipid motions in the biological membrane, i.e. spin-labeled fatty acid analogs in reference solvents composed of long chain triglycerides. Our spectroscopic measurements, EPR spectra, are quite sensitive to the details of anisotropic rotational motion, providing a very rigorous test of whether the motions are the same in the reference solvents as in the membrane. We have developed an empirical measure of lipid fluidity ($T/\eta$), where $T$ and $\eta$ are the temperature and viscosity of the reference solvent in which the spin label spectrum matches that observed in the membrane. We have evaluated the relevance of this measurement to membrane function and protein dynamics in SR by comparing the temperature dependence of lipid fluidity with that of the calcium-dependent ATPase activity and overall protein rotational mobility of the Ca-ATPase, as measured by saturation-transfer EPR (ST-EPR). This study was undertaken as a result of our previous finding that Arrhenius plots of lipid probe mobility and protein mobility are both linear, but have different apparent activation energies (Bigelow et al., 1986). In this study, we ask whether the agreement in activation energy is better if a more appropriate measure of lipid fluidity (rather than probe mobility) is employed.

EXPERIMENTAL PROCEDURES

Membrane Preparations—Vesicles of fragmented SR were prepared from rabbit skeletal white (fast twitch) muscle, essentially as described previously (Fernandez et al., 1980), producing a preparation in which 60% to 65% of the protein is the Ca-ATPase (7.5 nmol of Ca-ATPase/mg) and that contains about 80 phospholipids/Ca-ATPase molecule (i.e. 580 nmol of phospholipid/mg of SR) (Bigelow et al., 1986). All preparation was done at 4 °C. The membrane vesicles were suspended in 0.3 M sucrose, 20 mM MOPS (pH 7.0) and stored in liquid nitrogen. Total SR lipids were extracted by a modification (Hidalgo et al., 1976) of the method of Folch et al. (1957) using nitrogen-saturated solvents to prevent oxidation. This lipid extract, as characterized by fatty acid composition, head group composition, and cholesterol content, is the same as that of the native SR vesicles (Bigelow et al., 1986). The extracted lipid was stored in chloroform/methanol (2:1) at −20 °C. Liposomes were prepared by drying an aliquot of extracted lipid under nitrogen and vortexing in 0.5 M sucrose, 20 mM MOPS (pH 7.0).

Enzymatic Assays—Calcium-dependent ATPase activity was normally measured in a solution containing 0.06 mg of SR protein/ml, 80 mM KCl, 0.5 mM MgCl$_2$, 2 μM A23187, 25 mM MOPS (pH 7.0), and either 0.1 mM CaCl$_2$ or 2 mM EGTA. The pH of the solution varied between 6.9 and 7.1 from 0 to 40 °C. The reaction was started by the addition of 5 mM ATP, and the initial rate of release of inorganic phosphate was measured by the method of Lanztet et al. (1979). ATPase activity measured in the presence of EGTA (basal activity) was subtracted from the ATPase activity measured in the presence of Ca-ATPase (total ATPase activity) in order to obtain the calcium-dependent ATPase activity. The calcium-independent ATPase activity was less than 5% of the total ATPase activity. Under these experimental conditions (i.e. saturating calcium and ATP), substrate binding is never rate-limiting (Inesi, 1985), allowing us to probe the rate-limiting step in the transport process. Alternatively, ADP production was assayed by monitoring absorbance at 340 nm with the enzyme-linked assay of Warren et al. (1974). Activity assayed in the presence of EGTA (basal activity) was subtracted from that assayed in the presence of CaCl$_2$ (total Ca-ATPase activity) in order to obtain calcium-dependent ATPase activity. Protein concentrations were determined by a modification of the biuret method using bovine serum albumin as a standard (Gornall et al., 1949).

Calcium transport was measured spectrophotometrically using the differential absorbance of arsenazo III, a calcium-sensitive dye, as an indicator of extravesicular calcium concentration (Saffman and Scarpa, 1983). Reaction conditions were 0.7 mg of SR protein/ml, 80 mM KCl, 10 mM MgCl$_2$, 10 mM MOPS (pH 7.0), 0.1 mM CaCl$_2$, and 0.1 mM arsenazo III. 330 mM ATP was added to start the reaction. Spectra were recorded sequentially each second with a 1.0-s integrator time on a Hewlett-Packard 8451A diode array spectrophotometer. Time-dependent absorbance changes of arsenazo III at 675 and 685 nm were calculated from stored spectra. Alternatively, a qualitative index of calcium coupling to ATP hydrolysis was obtained by assaying the calcium-dependent ATPase activity in the presence and absence of 2 μM ionophore (see above).

Reference Solvents—Triglycerides are appropriate model systems for lipid bilayers since they form a homogeneous phase whose bulk
viscosity may be directly measured, and their structure is similar to that of membrane phospholipids. Biological triglycerides (oils) meet these criteria; castor oil (Sigma) and olive oil (Eastman) are relatively homogeneous triglycerides containing approximately 80% ricinoleic and oleic acid, respectively (Sober, 1988; Windholz, 1985), and therefore possess a similar degree of lipid chain saturation to that found in SR (Bigelow et al., 1986). Viscosities of these solvents were obtained from standard tables (Mellan, 1979; Weast, 1979).

Spin Labeling—Hydrocarbon chain mobility was measured with fatty acid spin labels, N-oxyl-4,4′-dimethoxyazolidine derivatives of stearic acid, designated 5-, 12-, and 16-SASL, and methyl esters of these derivatives, designated 5-, 12-, and 16-MITSL (Aldrich). The number designation indicates the relative position of the nitroxide on the stearic acid. Spin labels were diluted from a stock solution in dimethylformamide into ethanol before adding to liposomes of extracted SR lipids at a ratio of less than one spin label/200 phospholipids, with the final ethanol concentration less than 1%. The lipid concentration was made sufficiently high (greater than 50 mM) so that the EPR spectrum contained a negligible contribution from unbound (i.e. aqueous) spin labels. In the case of reference solvents, the spin label was added from an ethanol solution to make a final concentration of 0.1 mM spin label and the final ethanol concentration less than 1%.

To measure the rotational motion of the Ca-ATPase protein, SR was labeled with a short chain maleimide spin label, N-(1-oxyl-2,2,6,6-tetramethyl-4-piperidinyl)maleimide (Aldrich) as described previously (Squier and Thomas, 1986b; Bigelow et al., 1986), resulting in a preparation with <0.8 ± 0.8 mOD/mg of SR, (b) unhydrolyzable Ca-ATPase activity, and (c) ST-EPR spectra that report directly the overall rotational mobility of the protein (Squier and Thomas, 1986b; Bigelow et al., 1986; Squier et al., 1988).

EPR Spectroscopy—EPR spectra were obtained with a Varian E-109 spectrometer as described previously (Squier and Thomas, 1986a; Thomas, 1986). Submicronsecond rotational motion of spin labels was detected by conventional EPR (first harmonic absorption in phase, indicated by dots) using a Varian E-109 spectrometer as described previously (Squier and Thomas, 1986a; Squier et al., 1988). All ST-EPR studies were done in the absence of oxygen. Oxygen was removed from reference and experimental samples using gas-permeable sample cells purged with N₂ (Popp and Hyde, 1981). Temperature was controlled to within 0.5°C with a Varian V4540 variable temperature controller. During data acquisition, temperature was monitored with a Bailey digital thermometer (Model BAT-12) using a thermocouple probe (IT-21) positioned outside the sample cell in the center of the cavity.

Spectral Analysis—Conventional EPR spectra of fatty acid spin labels were evaluated by measuring the effective order parameter S, which depends only on the angular amplitude of the motion of the probe, assuming very fast anisotropic motion (<1 ms; where r, is the effective rotational correlation time), such that an increase in rate has no effect on the positions of the spectral features (see below). Values of S greater than 0.3 can be measured using the standard formula relating S to both inner and outer extrema (Gaffney and Lin, 1976; Gaffney, 1976), i.e.

\[ S = \frac{T_r - (T_i' + C)}{T_r + (T_i' + C)} \times 1.66 \]  

where \( C = 1.4 - 0.063(T_i' - T_r) \), \( T_i' \), and \( T_r \) are the measured inner and outer extrema resolved in the EPR spectrum in gauss (see Fig. 1). For values of S less than 0.3, the outer extrema cannot be resolved (see 12-SASL; Fig. 3), resulting in systematic errors in the calculation of S (Marsh, 1981; Bigelow et al., 1986). However, even at these low values of S, the inner extrema are well resolved. Therefore, in those cases where \( T_i' \) values are not resolved, we have used \( T_i' \) to measure S (valid for S > 0.1) using the relationship (Gaffney, 1976) of Equation 2:

\[ S = \frac{T_i - T_r}{T_o - T_r} \]  

where \( T_i \) is the isotropic hyperfine splitting constant in the absence of anisotropic effects, \( T_o \) is the principal value of the hyperfine constant for an axially symmetric system (e.g. a lipid bilayer) (Poole, 1983). Values of \( T_i \) and \( T_o \) correspond to values of \( T_i' \) at \( S = 0 \) (isotropic) and \( S = 1 \) (ordered), respectively. \( T_i \) was determined for each spin label and solvent in two ways, which gave results in good agreement. 1) The separation between the low field and high field zero-crossing points (i.e. \( 2T_i' \)) was measured from a spectrum where S approaches zero, i.e. increasing the sample temperature does not further narrow the spectrum. 2) \( T_i' \) was plotted as a function of S and extrapolated to \( S = 0 \) using Equation 1. Similarly, \( T_i \) was determined by extrapolating this plot to \( S = 1 \). Values of \( T_i \) for 5-SASL in castor oil, olive oil, and vesicles made from extracted SR lipid are 13.4, 14.4, and 13.2 G, respectively. Values of \( T_i \) for 5-SASL in castor oil, olive oil, and vesicles made from extracted SR lipid are 5.5, 4.7, and 6.6 G, respectively. Since Equation 2 can be used over the entire range of spectra obtained in this study, Equation 2 was used to determine the order parameters shown below. For order parameters S > 0.3, where both Equations 1 and 2 are valid, the two methods of measuring S gave good agreement.

The effective rotational correlation times for maleimide spin-labeled SR were determined from ST-EPR spectra using a plot of the \( V_i' \) integral versus correlation time based on reference spectra of known correlation time obtained from isotropically tumbling spin-labeled hemoglobin in aqueous glycerol solutions (Squier and Thomas, 1986a).

Arrhenius Analysis—Apparent activation energies \( E_a \) were determined from the slopes (−\( E_a/R \)) of Arrhenius plots by using linear regression least-squares analysis as described previously (Bigelow et al., 1986). The report of a change in slope of an Arrhenius plot required that the correlation coefficient above and below the break point was significantly larger than the correlation coefficient for a single line (Bigelow et al., 1986).

RESULTS

Comparison of Anisotropic Motion in Biological Membranes with Triglyceride Reference Solvents—The motion of SASL in vesicles made from SR lipids is analogous to that observed in biological triglycerides, as can be seen by the virtually identical EPR spectra obtained in the olive oil in comparison to those obtained in SR (Fig. 1). These spectra show clear evidence for anisotropic (restricted amplitude) rotational motion since the line shapes are qualitatively different from those corresponding to isotropic rotational motion (Thomas, 1986). For both spectra, the inner and outer extrema are at the same field position, indicating that they have the same order parameter (S; see “Experimental Procedures”). Similarly excellent line shape matches can be obtained for other spin labels and temperatures in SR using either olive oil or
castor oil and varying the temperature. Therefore, we define lipid fluidity to be $T/\eta$, where $T$ is the absolute temperature and $\eta$ is the viscosity of a long chain reference solvent in which the fatty acid spin label gives the same conventional EPR spectrum (as characterized by $S$) as observed in the membrane.

**Calibration Plot for Measuring Lipid Fluidity**—The data in Fig. 2 were obtained from EPR spectra of lipid analogs (stearic acid spin labels and their corresponding methyl ester derivatives, i.e. 5- and 12-SASL and 5- and 12-MESL) in castor oil and olive oil (reference solvents). Fig. 2 illustrates the relationship between measurements of lipid hydrocarbon chain dynamics, expressed as spin label order parameters ($S$; see "Experimental Procedures"), and fluidity, expressed as $T/\eta$. $T/\eta$ is plotted instead of $1/\eta$ because the diffusion of a macromolecule in either a bulk solvent or a membrane is predicted to be proportional to $T/\eta$ (Saffman and Delbrück, 1975; Thomas, 1986).

The order parameters for a given $T/\eta$ are about the same for 5- and 12-SASL in triglycerides (oils; see Fig. 2) as opposed to these spin labels in biological membranes, where there is a large difference in the order parameter for these two labels at the same temperature (Figs. 3 and 4). This highlights the major structural difference between phospholipid bilayers and triglycerides. Phospholipids are extremely amphiphilic molecules that generally form lyotropic smectic mesophases in aqueous solution and consequently display a "fluidity gradient" normal to the bilayer due to the lipid polar head group's strong interaction with the aqueous phase (Tanford, 1980). On the other hand, triglycerides are devoid of water and form thermotrophic smectic mesophases resulting from the interdigitation of the triglyceride molecules to form symmetrical layers (Chapman, 1962). In this case, the similar distances of the nitroxide position in 5- and 12-SASL from an end of the stearic acid result in the measurement of a similar order parameter. Likewise, this calibration plot is independent of the charge on the spin label (Fig. 2). Therefore, it is appropriate to use this empirical calibration plot to express measurements of $S$, obtained from spin labels in SR lipids, in terms of $T/\eta$, thus determining the fluidity for the membrane lipids.

**Temperature Dependence of SR Lipid Fluidity**—Lipid chain mobility was probed as a function of temperature at various bilayer depths using stearic acid analogs spin-labeled at several positions along the hydrocarbon chain depths in vesicles of extracted SR lipids (Fig. 3). These vesicles have a lipid composition identical to that in intact SR (Bigelow et al., 1986) and have the advantage of being a homogeneous motional population, allowing us to quantitatively analyze the motional properties. The presence of the Ca-ATPase in intact SR results in a heterogeneous motional population of lipid probes (Thomas et al., 1982), making it difficult to accurately measure the order parameter for either population of probes (Masah and Watts, 1982; Meirovitch et al., 1984). The exchange between these lipid populations is probably fast (i.e. $10^{-7}$ s$^{-1}$) (East et al., 1985) in comparison to the overall rotational motion of the Ca-ATPase (i.e. $\tau$, $\approx 10^4$ s$^{-1}$) (Thomas and Hidalgo, 1978), indicating that the protein's overall rotational motion is modulated by the entire lipid population (with the possible exception of cholesterol, which may be excluded from the lipid adjacent to the protein) (Silvius et al., 1984). Therefore, to a good approximation, we can estimate the average viscosity experienced by the Ca-ATPase by using vesicles made from extracted SR lipid.

Spectra obtained from 5- or 12-SASL in extracted SR lipids correspond to a homogeneous population of spin labels whose line shapes are characteristic of significant orientational order; however, only spectra from 5-SASL resolve the outer extrema over the entire temperature range studied (i.e. 0–40°C), allowing $S$ to be calculated from either Equation 1 or 2 (see "Experimental Procedures"). In the case of 12-SASL, the outer extrema are only resolved from 0 to 27°C, requiring $S$ to be calculated from the inner splitting (i.e. Equation 2) for temperatures above 27°C. In the case of 16-SASL, the spectral anisotropy is almost completely averaged (5% ± 0.1% for most or all of the spectra in Fig. 3, right), indicating little anisotropy (little orientational order), thus preventing the quantitative measurement of $S$ (see "Experimental Procedures"). In the presence of such low order, the precise model describing the rotational motion of stearic acid spin labels is unclear (Bigelow et al., 1986). Therefore, 5- and 12-SASL were used for further studies.

As the nitroxide is placed down the fatty acyl chain toward the center of the bilayer, the spectra display a rotational mobility gradient typical of lipid bilayers, from a relatively restricted polar head region (i.e. 5-SASL) to a more mobile terminal methyl region (i.e. 16-SASL). Spectra from 12-SASL exhibit decreased order (increased fluidity) relative to those

![Fig. 2. Calibration plot of lipid chain dynamics related to viscosity. The order parameter ($S$) is plotted as a function of log ($T/\eta$), where $T$ is the temperature (Kelvin) and $\eta$ is the solvent viscosity (centipoise) for 5 (○), 12 (●) -SASL and 5 (○) and 12 (●) -MESL in both castor oil (closed symbols) and olive oil (open symbols). The line represents the best fit of the data, for $S > 0.1$, by linear regression least-squares analysis. The slope for this fit is $-0.42$, the intercept is 0.56, and the correlation coefficient is 0.99. Thus, the relationship between the order parameter and the viscosity is $S = -0.42 \log (T/\eta) + 0.56$. The symbols represent the standard error of the mean.](image)

![Fig. 3. Effect of temperature on conventional EPR spectra of probes at different bilayer depths. From left-to-right, the spectra correspond to 5-, 12-, and 16-SASL incorporated into vesicles of extracted SR lipids suspended in 0.3 M sucrose, 20 mm MOPS (pH 7.0) at 3, 18, and 36 °C. All spectra were recorded with a 100-G scan range.](image)
Lipid Fluidity and Protein Rotational Mobility in SR

The order parameters plotted in Fig. 4 can be expressed in terms of fluidity, using the calibration plot of \( S \) versus \( T/\eta \) in Fig. 2, and the resulting values of \( T/\eta \) can be plotted in the form of an Arrhenius plot (Fig. 5) since the rate of overall protein rotational motion (measured by ST-EPR) (Squier et al., 1988) is predicted to be proportional to \( T/\eta \) (Saffman and Delbrück, 1975). The slopes, i.e. apparent activation energies, are 11.5 ± 0.3 and 11.0 ± 0.4 kcal/mol/degree for 5- and 12-SASL, respectively. Thus, although the absolute values of SR lipid fluidity depend on the depth of the probe, due to the fluidity gradient, their activation energies are the same. Values of the membrane viscosity measured in this study range from 0.27 poise at 39 °C at the 12-position along the hydrocarbon chain to 8.8 poise at 0 °C at the 5-position along the hydrocarbon chain, in general agreement with independent measurements of membrane viscosity (Almeida et al., 1982; Jähnig, 1986).

Temperature Dependence of Ca-ATPase Rotational Mobility in SR—Protein rotational mobility was measured using ST-EPR, a technique that provides a sensitive measurement of the microsecond rotational mobility characteristic of many membrane proteins; and in the case of the Ca-ATPase, it is primarily sensitive to the overall rotational motion of the Ca-ATPase with respect to the membrane normal (Thomas, 1986; Squier et al., 1988). An Arrhenius plot demonstrates a linear temperature dependence of protein rotational mobility (\( r^{-1} \)), with an apparent activation energy of 11.2 ± 0.4 kcal/mol/degree (Fig. 6). This activation energy is the same as that observed for \( T/\eta \) in SR lipids (Fig. 5 and Table I). Thus, as predicted from theory (Saffman and Delbrück, 1975), the rate of protein rotational motion is proportional to \( T/\eta \) (Fig. 7), indicating that our definition of fluidity (\( T/\eta \)) is appropriate.

**Fig. 4.** Temperature dependence of lipid dynamics. The order parameter \( S \) for 5- (○) and 12- (△) SASL incorporated into aqueous dispersions of extracted SR lipids is plotted as a function of temperature. The lines drawn through the data were obtained from linear regression least-squares analysis, with correlation coefficients of 0.998 and 0.986 for 5- and 12-SASL, respectively.

**Fig. 5.** Arrhenius plot of SR lipid fluidity. Values for fluidity (\( T/\eta \)) were obtained from the order parameters in Fig. 4, analyzed according to the calibration plot in Fig. 2. \( T \) is in Kelvin, and \( \eta \) is in centipoise. Lines represent the best fits to the data from 5- (○)-SASL and 12- (△)-SASL, with correlation coefficients of 0.997 and 0.986, respectively.

**Fig. 6.** Arrhenius plot of overall protein rotational mobility of Ca-ATPase in SR. Rates of motion were plotted as \( r^{-1} \) (inverse of the correlation time). Correlation times were obtained through the comparison of reference spectra corresponding to isotropic motion. The Arrhenius activation energy was computed (see “Experimental Procedures”) to be 10.7 ± 0.5 kcal/mol/degree for this data set, with a correlation coefficient of 0.988. When an attempt was made to fit this plot to two lines, the mean correlation coefficient was 0.973, indicating that the single line fit was superior, confirming previous results (Bigelow et al., 1986). Similar results were obtained with six other data sets with an activation energy for protein rotational motion of 11.2 ± 0.5 kcal/mol/degree (mean ± S.E.).

**Fig. 7.** Relationship between protein rotational mobility and lipid fluidity. Values of protein mobility (\( r^{-1} \)) from Fig. 6 and lipid fluidity (\( T/\eta \) from Fig. 5) are compared. Both protein mobility and \( T/\eta \) are normalized to their respective values at 37 °C. The line drawn through the data was obtained from a linear regression least-squares analysis, with a slope of 1.10, an intercept of 0.03, and a correlation coefficient of 0.993.
In contrast, a definition of fluidity as either the rate or amplitude of lipid chain motions measured by EPR spectral parameters does not yield an activation energy comparable to that of protein motion (Bigelow et al., 1986). Apparent activation energies for lipid chain motions in SR lipids were measured to be 3.0 and 3.6 kcal/mol/degree for 5- and 12-SASL, respectively.

Temperature Dependence of Enzymatic Activity—The functional significance of the modulation of enzyme rotational mobility by lipid fluidity is suggested by the measurement of the activation energies for Ca-ATPase activity in SR (Table I and Fig. 8). Although we have focused our attention on the calcium-dependent ATPase activity, this activity is coupled to calcium transport. The Arrhenius plot of the calcium-dependent ATPase activity in SR (in the presence of KCl, i.e. normal assay conditions; Fig. 8) exhibits a change in slope at 20 °C, with apparent activation energies of 11.8 ± 0.4 and 23 ± 1 kcal/mol/degree, above and below 20 °C, respectively. In either the absence of KCl or the presence of diethyl ether, the rate-limiting step of ATP hydrolysis is temperature-independent, and the apparent activation energies are 22 ± 1 kcal/mol/degree. The observation of Arrhenius activation energies that are modified between two discrete values as a function of temperature, salt, or ether concentration supports the concept that Arrhenius plots of Ca-ATPase activity accurately measure the rate-limiting step of the enzymatic reaction and that the change in slope is the result of a temperature-dependent change in the rate-limiting step.

These perturbations affect the reaction mechanism in different ways: the absence of KCl slows the overall reaction rate, whereas the addition of ether accelerates the reaction rate. Using both conventional and saturation-transfer EPR to probe the effect of these perturbants on the membrane dynamics, we find that KCl has no effect on either the protein or lipid dynamics. On the other hand, diethyl ether fluidizes the membrane (Bigelow and Thomas, 1987), resulting in greater protein rotational mobility. These results suggest that protein mobility contributes an important, albeit not exclusive, effect on the rate-limiting step and that, under assay conditions that preferentially reduce the phosphatase activity of the Ca-ATPase (i.e. minus KCl) or that accelerate protein mobility (plus ether), protein mobility is not rate-limiting.

**DISCUSSION**

**Summary of Results**—We have explored the functional significance of lipid fluidity in SR to the Ca-ATPase reaction mechanism by quantitatively comparing the lipid hydrocarbon chain dynamics, as measured by conventional EPR spectra of stearic acid spin labels in SR lipids, with the overall rotational mobility of the protein, as measured by ST-EPR spectra of maleimide spin-labeled Ca-ATPase. In order to make this comparison, it was necessary to construct an empirical calibration plot (Fig. 2) relating the lipid probe dynamics to lipid fluidity (T/η, where η is the viscosity). The validity of our calibration plot is supported by the linear relationship between T/η and the protein rotational mobility (r−1) (Fig. 7), as predicted by hydrodynamic theory (Saffman and Delbrück, 1975; Hughes et al., 1982; Peters and Cherry, 1982; Wiegol and Heringa, 1985). Thus, protein mobility is modulated directly by lipid fluidity in SR. The apparent activation energy (from an Arrhenius analysis) of lipid fluidity (Fig. 5) is the same as that for protein mobility (Fig. 6). Under near-physiological conditions (above 20 °C in the presence of KCl), the Arrhenius activation energy of the calcium-dependent ATPase activity (Fig. 8 and Table I) is the same as that of both lipid fluidity and protein mobility, suggesting that membrane fluidity (as defined by both lipid and protein mobility) is essential to the rate-limiting step of the Ca-ATPase reaction. Under other assay conditions (achieved by decreasing the temperature below 20 °C, removing KCl, or adding diethyl ether), the Ca-ATPase activation energy is about twice that of fluidity (Fig. 8), suggesting that a different step has become rate-limiting.

**Choice of Reference Solvent**—The appropriate model system for motion in a membrane bilayer is a structure resembling a phospholipid, but whose bulk viscosity can be readily measured. The latter criterion rules out the use of lyotropic crystals (e.g. hydrated phospholipids) as calibration standards since the presence of water prevents the direct measurement of the bulk viscosity. Triglycerides, however, exist as thermotropic liquid crystals, forming a homogeneous phase whose bulk viscosity may be directly measured. Biological triglycerides

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**TABLE I**

| Rate process                        | Eₐ kcal/mol/degree |
|-------------------------------------|--------------------|
| Lipid fluidity                     | 11.5 ± 0.3         |
| 5-SASL                              | 11.0 ± 0.4         |
| 12-SASL                             | 11.2 ± 0.5         |
| Protein mobility                    | 11.8 ± 0.4         |
| Ca-ATPase activity >20 °C           | 23.0 ± 1.0         |
| Ca-ATPase activity <20 °C           | 23.0 ± 1.0         |

**FIG. 8. Arrhenius plot of calcium-dependent ATPase activity.** The lines and break point were determined by linear regression least-squares analyses of the experimental data (Bigelow et al., 1986) for near-physiological conditions (C, 80 mM KCl (pH 7.0), in the absence of KCl (D) or in the presence of activating concentrations of diethyl ether (E) (Bigelow and Thomas, 1987). The apparent activation energies obtained in the absence of KCl, in the presence of diethyl ether, or below 20 °C are identical (Eₐ = 23 ± 1 kcal/mol/degree). The apparent activation energy obtained under physiological conditions above 20 °C is 11.8 ± 0.4 kcal/mol/degree (see Table I).
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possess a similar structure to phospholipids (i.e., unsaturation at the 9-position and ordered acyl chains that form a smectic mesophase (Chapman, 1962; Seelig, 1976); synthetic oils (e.g., paraffin) have much less structural similarity to biological phospholipids, possessing neither the characteristic unsaturation nor the triglyceride backbone). The reference solvents chosen are ordered long chain hydrocarbons, in which the type of molecular motion is similar to that in membranes, as confirmed by the observation that the EPR line shapes of fatty acid spin labels in these solvents have the same characteristic features as those observed with the same spin labels in lipid bilayers. EPR order parameters, characterizing probe motions in these solvents, were plotted against $\log(T/\rho)$, where $T$ is temperature and $1/\rho$ is viscosity. The resulting calibration plots are independent of the solvent used, the position of the nitroxide group along the fatty acid chain, and the charge on the probe, supporting the model independence of using these calibration plots. Castor oil was chosen due to its uniquely wide range of relevant viscosities. Olive oil, being more fluid, was used for comparison purposes in order to demonstrate the solvent independence of the measured spectral parameter.

**Correlation between Protein Mobility and Lipid Fluidity**

Previous studies of the Ca-ATPase and other membrane proteins have, in general, compared protein rotational mobility directly with the mobility of lipid probes without translating the latter into fluidity, as defined in this study. Nevertheless, qualitative correlations between protein and lipid probe mobility have often been demonstrated (reviewed by Shinitsky, 1984; Thomas, 1985, 1986), most dramatically as a large decrease in protein mobility below the first-order phase transition temperature in a recombinant membrane having only one lipid component (Hidalgo et al., 1978; Peters and Cherry, 1982). Some aspects of the theory of membrane protein rotational motion (Saffman and Delbrück, 1975) have been verified quantitatively, e.g., the ratio of translational to rotational diffusion coefficients and the relationship of both coefficients to the size of the protein (Peters and Cherry, 1982; Vaz et al., 1982). The primary technical contribution of this study is to show that our definition of lipid fluidity, based on spectroscopic measurements of the effective viscosity $\eta$, leads to a dependence of protein mobility on fluidity that agrees quantitatively with the prediction of hydrodynamic theory (Saffman and Delbrück, 1975). That is, overall protein rotational mobility with respect to the membrane normal should be proportional to $T/\eta$, as long as the size and shape of the protein remain constant. Using the empirical calibration relating the motional properties of fatty acid spin labels to lipid fluidity presented in this paper, other modifiers of lipid fluidity (e.g., diethyl ether) have also been shown to modulate protein rotational mobility through lipid fluidity (Bigelow and Thomas, 1987).

**Correlation of Calcium-dependent ATPase Activity with Membrane Fluidity**

A number of previous studies have obtained evidence qualitatively correlating lipid probe mobility (related to lipid fluidity) with calcium-dependent ATPase activity in SR. A substantial decrease of lipid probe mobility, relative to that observed under physiological conditions, has consistently produced a decrease in calcium-dependent ATPase activity whether the decrease in lipid fluidity was achieved by substitution of a more solid lipid (Hidalgo et al., 1976, 1978; Hesketh et al., 1976) or by a decrease in temperature (Hidalgo et al., 1976; Hesketh et al., 1976; Bigelow et al., 1986). An apparent exception to this principle was obtained with cholesterol, which decreases lipid fluidity in SR vesicles without affecting enzymatic activity (Warren et al., 1975; Johannson et al., 1981b). However, since at normal lipid-to-protein ratios cholesterol analogs do not exchange into (i.e., are preferentially excluded from) the layer of lipids directly surrounding the protein (Warren et al., 1975; Simmons et al., 1982, 1984; Silvius et al., 1984), the effective viscosity experienced by the Ca-ATPase is unaffected by the presence of cholesterol. However, when the lipid content is reduced so that cholesterol must directly interact with the Ca-ATPase, i.e. at lipid-to-protein ratios less than or equal to 30, cholesterol has significant and complex effects (Simmons et al., 1984). Similarly, an increase in boundary lipid fluidity, caused by the addition of diethyl ether, has been shown to activate both calcium-dependent ATPase and associated transport activity, although there is an optimal fluidity above which the enzymatic activity decreases (Bigelow and Thomas, 1987). Thus, changes in lipid fluidity consistently modulate Ca-ATPase activity as long as they include the boundary lipid. Although East et al. (1984) emphasized the importance of membrane thickness rather than fluidity within a given membrane thickness, they also observed a correlation between hydrocarbon chain mobility and enzymatic activity, supporting our conclusion that there is a direct relationship between hydrocarbon chain mobility and enzymatic activity.

**Inhibitors of Calcium-dependent ATPase Activity That Do Not Affect Fluidity**

However, a number of physical perturbations have been shown to substantially inhibit the Ca-ATPase activity without greatly affecting lipid fluidity, even in the boundary layer, e.g., changes in bilayer thickness (Moore et al., 1981; Caffrey and Feigenson, 1981; Johannson et al., 1981a, 1981b; East et al., 1984), protein-protein cross-linking (Thomas et al., 1982; Squier et al., 1988), addition of nonaqueous solvents (Squier and Thomas, 1988), addition of decavanadate (Lewis and Thomas, 1986), and the addition of millimolar calcium (Hidalgo et al., 1978). Thus, there are clearly other physical constraints affecting the enzymatic activity of the Ca-ATPase besides lipid fluidity. Nevertheless, in the cases above where protein rotational mobility has also been monitored, decreases in protein mobility (usually due to protein aggregation) consistently accompanied the inhibition of the Ca-ATPase (Hidalgo et al., 1978; Lewis and Thomas, 1986; Squier and Thomas, 1988; Squier et al., 1988). Thus, the apparent functional requirement for membrane fluidity in SR can most simply be interpreted as a requirement for enzyme mobility, as proposed previously (Thomas and Hidalgo, 1978; Hidalgo et al., 1978; Moore et al., 1978; Almeida et al., 1982, 1984; Bigelow et al., 1986; Bigelow and Thomas, 1987).

**Possible Role for Protein Mobility in Ca-ATPase Mechanism**

It is beyond the scope of this study to define the role of specific protein motions in the Ca-ATPase mechanism because high resolution structural data for the enzyme do not yet exist and because further measurements of protein dynamics must be made and correlated with specific steps in the reaction cycle. There are many aspects of protein mobility that may prove to be important to the enzymatic mechanism of the Ca-ATPase, from small-scale conformational fluctuations (not detected directly by the probe in this study) to overall protein rotations (which our probe directly detects). Rotational mobility could facilitate specific protein-protein interactions necessary for ion translocation, as previously suggested (Singer, 1974; Dutton et al., 1976), and we have obtained evidence supporting this proposal in SR (Squier et al., 1988). Orientational constraints could limit the rate of productive associations between Ca-ATPase polypeptide chains, resulting in a rate constant for the process of protein association that is slower than the diffusion-limited process.
by several orders of magnitude (Berg and von Hippel, 1985), thus reconciling the time scale of protein rotation (about $10^{-5}$ s at $37 \, ^\circ\, C$) with the time scale of enzyme turnover (about $10^{-1}$ s at $37 \, ^\circ\, C$). Rates of rotational and lateral diffusion are related; both are predicted to be inversely proportional to the viscosity of the surrounding medium (Saffman and Delbrück, 1975; Hughes et al., 1982; Peters and Cherry, 1982). Therefore, the results from this study do not distinguish between the functional significance of rotational versus lateral diffusion.

Conclusions—This and the two previous papers (Squier et al., 1988; Squier and Thomas, 1988), as well as earlier studies (Hidalgo et al., 1978; Thomas and Hidalgo, 1978; Thomas et al., 1982; Squier and Thomas, 1986b; Bigelow et al., 1986; Bigelow and Thomas, 1987), have probed the physical and functional significance of rotational dynamics in SR. When either the mean molecular weight of the spin-labeled Ca-ATPase oligomer or the fluidity of the hydrocarbon chain environment is altered, we find that the relationship between membrane fluidity and protein rotational mobility agrees with that predicted theoretically (Saffman and Delbrück, 1975). Whenever protein mobility has been perturbed, it has been found that there is a direct correlation with enzymatic activity. Under physiological conditions, protein rotational mobility has the same Arrhenius activation energy as enzymatic activity; and conditions that increase protein mobility (e.g., diethyl ether) change the rate-limiting step at physiological temperatures, suggesting that protein mobility is involved in the rate-limiting step (phosphoenzyme decomposition). In fact, conditions that selectively inhibit phosphoenzyme decomposition also decrease protein mobility, emphasizing the importance of protein mobility to the enzymatic reaction mechanism (Squier and Thomas, 1988). The methods developed in this study should be directly applicable to other membrane systems and should provide a useful tool in clarifying the quantitative role of membrane fluidity in the mechanisms of integral membrane enzymes.

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REFERENCES

Almeida, L. M., Vaz, W. L. C., Zachariasse, K. A., and Madeira, V. M. C. (1982) Biochemistry 21, 5972-5977
Almeida, L. M., Vaz, W. L. C., Zachariasse, K. A., and Madeira, V. M. C. (1984) Biochemistry 23, 4714-4720
Benga, G., and Holmes, R. P. (1970) Industrial Solvents Handbook, Noyes Data Corp., Park Ridge, NJ
Blanquet, A., Rees, E. D., and Singer, S. J. (1976) Proc. Natl. Acad. Sci. U. S. A. 73, 1532-1536
East, J. M., and Lee, A. G. (1982) Biochemistry 21, 4144-4151
East, J. M., Jones, O. T., Simmonds, A. C., and Lee, A. G. (1984) J. Biol. Chem. 259, 8070-8071
East, J. M., Melville, D., and Lee, A. G. (1985) Biochemistry 24, 2615-2623
Folch, J., Lees, M., and Sloane Stanley, G. H. (1957) J. Biol. Chem. 226, 497-509
Fong, T. M., and McMann, M. G. (1987) Biochemistry 26, 3871-3880
Frnd, R. J., East, J. M., Rooney, E. K., and Lee, A. G. (1986a) Biochemistry 25, 7555-7544
Froué, R. J., East, J. M., Jones, O. T., and Lee, A. G. (1986b) Biochemistry 25, 7544-7552
Gaffney, B. J. (1976) in Spin Labeling (Berliner, L. J., ed) pp. 567-571, Academic Press, New York
Gaffney, B. J., and Lin, D. C. (1976) in The Enzymes of Biological Membranes: Physical Chemical Techniques (Marston, A., ed) Vol. 1, pp. 1-90, Plenum Press, New York
Gornall, A. G., Bardawill, C. J., and David, M. M. (1949) J. Biol. Chem. 177, 751-766
Hare, F., and Lussan, C. (1977) Biochim. Biophys. Acta 467, 262-272
Hesketh, T. R., Smith, G. A., Houslay, M. D., McGill, K. A., Birdsal, N. J. M., Metcalfe, J. C., and Warren, G. B. (1976) Biochemistry 15, 4145-4151
Hidalgo, C. (1985) in Membrane Fluidity in Biology (Boggs, J. M., and Shinitzky, M., eds) Vol. 4, pp. 51-96, Academic Press, New York
Hidalgo, C. (1987) Crit. Rev. Biochem. 21, 319-347
Hidalgo, C., Ikemoto, N., and Gergely, J. (1976) J. Biol. Chem. 251, 4224-4232
Hidalgo, C., Thomas, D. D., and Ikemoto, N. (1978) J. Biol. Chem. 253, 6879-6887
Hughes, B. D., Paulthorpe, B. A., White, L. R., and Sawyer, W. H. (1982) Biochim. Biophys. Acta 676, 262-272
Inesi, G. (1985) Annu. Rev. Physiol. 47, 573-601
Jahng, F. (1986) Eur. Biophys. J. 14, 63-64
Johansson, A., Keightley, C. A., Smith, G. A., Richards, C. D., Hesketh, T. R., and Metcalfe, J. C. (1981a) J. Biol. Chem. 256, 1643-1650
Johansson, A., Smith, G. A., and Metcalfe, J. C. (1981b) Biochim. Biophys. Acta 641, 416-421
Kates, M., and Manson, L. A. (1984) Biomembranes: Membrane Fluidity, Vol. 12, Plenum Press, New York
Lanzetta, P. A., Alvarez, L. J., Reinsch, P. S., and Candia, O. A. (1979) Anal. Biochem. 100, 95-97
Lewis, B. A., and Engelmann, D. M. (1983) J. Mol. Biol. 166, 211-217
Lewis, S. M., and Thomas, D. D. (1986) Biochemistry 25, 4615-4621
Marsh, D. (1981) in Membrane Spectroscopy (Grell, E., ed) Vol. 31, pp. 51-142, Springer-Verlag, Berlin
Marsh, D. (1985) in Progress in Protein-Lipid Interactions (Watts, A., and De Pont, J. H. H., eds) Vol. 1, pp. 143-172, Elsevier/North-Holland Biomedical Press, Amsterdam
Marsh, D., and Watts, A. (1982) in Lipid-Protein Interactions (Jost, P. C., and Griffith, O. H., eds) Vol. 2, Chap. 2, Wiley-Interscience, New York
McElhaney, R. N. (1984) Biochim. Biophys. Acta 779, 1-42
Meirovitch, E., Nayeem, A., and Freed, J. (1984) J. Chem. Phys. 88, 3454-3465
Mellan, I. (1970) Industrial Solvents Handbook, Noyes Data Corp., Park Ridge, NJ
Moore, B. M., Lentz, B. R., and Meissner, E. R. (1984) Biochemistry 23, 130-135
Peters, R., and Cherry, R. J. (1982) Proc. Natl. Acad. Sci. U. S. A. 79, 4317-4321
Poole, C. F. (1983) Electron Spin Resonance: A Comprehensive Treatise on Experimental Technique, John Wiley & Sons, New York
Popp, C. A., and Hyde, J. S. (1981) J. Magn. Reson. 43, 249-258
Roeofoen, B. (1981) Life Sci. 29, 2235-2247
Saffman, P. G., and Delbrück, M. (1975) Proc. Natl. Acad. Sci. U. S. A. 72, 3111-3115
Salama, G., and Scarpa, A. (1983) Biochim. Biophys. Acta 72, 3465-3477
Sandermann, H., Jr. (1978) Biochim. Biophys. Acta 515, 209-237
Seelig, J. (1976) in Spin Labeling (Berliner, L. J., ed) pp. 373-409, Academic Press, New York
Shinitzky, M. (1984) in Physiology of Membrane Fluidity (Shinitzky, M., ed) Vol. 1, pp. 1-51, CRC Press, Inc., Boca Raton, FL

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Shinitzky, M., Dianoux, A-C., Gitler, C., and Weber, G. (1971) Biochemistry 10, 2106–2113
Silvius, J. R., McMillen, D. A., Saley, N. D., Jost, P. C., and Griffith, O. H. (1984) Biochemistry 23, 538–547
Simmonds, A. C., East, J. M., Jones, O. T., Rooney, E. K., McWhirter, J., and Lee, A. G. (1982) Biochim. Biophys. Acta 693, 398–406
Simmonds, A. C., Rooney, E. K., and Lee, A. G. (1984) Biochemistry 23, 1432–1441
Sinensky, M., Pinkerton, F., Sutherland, E., and Simon, F. R. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 4893–4897
Singer, S. J. (1974) Annu. Rev. Biochem. 43, 805–819
Sober, H. A. (ed) (1968) Handbook of Biochemistry, pp. 20–21, The Chemical Rubber Co., Cleveland, OH
Squier, T. C., and Thomas, D. D. (1986a) Biophys. J. 49, 921–935
Squier, T. C., and Thomas, D. D. (1986b) Biophys. J. 49, 937–942
Squier, T. C., and Thomas, D. D. (1988) J. Biol. Chem. 263, 9171–9177
Squier, T. C., Hughes, S. E., and Thomas, D. D. (1988) J. Biol. Chem. 263, 9162–9170
Tanford, C. (1980) The Hydrophobic Effect: Formation of Micelles and Biological Membranes, Wiley-Interscience, New York

Thomas, D. D. (1985) in The Enzymes of Biological Membranes (Martonosi, A. N., ed) Vol. 1, pp. 287–312, Plenum Press, New York
Thomas, D. D. (1986) in Techniques for the Analysis of Membrane Proteins (Cherry, R., and Ragan, I., eds) pp. 239–257, Chapman & Hall, London
Thomas, D. D., and Hidalgo, C. (1978) Proc. Natl. Acad. Sci. U. S. A. 75, 5438–5492
Thomas, D. D., Bigelow, D. J., Squier, T. C., and Hidalgo, C. (1982) Biophys. J. 37, 217–225
Vaz, W. L. C., Criado, M., Madeira, V. M. C., Schuellmann, G., and Jovin, T. M. (1982) Biochemistry 21, 5606–5612
Warren, G. B., Toon, P. A., Birdsall, N. J. M., Lee, A. G., and Metcalfe, J. C. (1974) Proc. Natl. Acad. Sci. U. S. A. 71, 622–626
Warren, G. B., Houslay, M. D., Metcalf, J. C., and Birdsall, N. J. M. (1975) Nature 255, 684–687
Weast, R. C. (ed) (1979) in Handbook of Chemistry and Physics, 60th Ed., The Chemical Rubber Co., Cleveland, OH
Wiegel, F. W., and Heringa, J. R. (1985) Can. J. Phys. 63, 44–45
Windholz, M. (1983) The Merck Index, 10th Ed., Merck and Co., Inc., Rahway, NJ
Yeagle, P. L. (1985) Biochim. Biophys. Acta 822, 267–287