Effect of Lipids on Enzymatic Activity of Pig Heart Mitochondrial Malate Dehydrogenase Monomolecular Films

A. W. MALICK * and N. D. WEINER ‡x

Abstract □ The effect of various lipids on the enzymatic activity of pig heart mitochondrial malate dehydrogenase monomolecular films was studied using the subphase exchange technique described previously. Surface pressure–surface area (π–A) curves of mixed films of the enzyme with dipalmitoyllecithin, egg lecithin, cholesterol, and phospholipids extracted from pig hearts showed that the enzyme interacted with all of the lipids and that the enzyme remained in the film at pressures well above the collapse pressure of malate dehydrogenase in the absence of lipid. The surface enzyme activity was dependent on surface pressure for each lipid; in all cases, the lipids greatly broadened the range of surface pressures where surface enzyme activity was observed. The π–A and enzyme activity data showed good correlation. Although the simple model system employed does not simulate the complexity of the biological membrane, it gives some evidence for the role of lipids in the stability of membrane-bound enzymes.

Keyphrases □ Lipids—effect on activity of pig heart mitochondrial malate dehydrogenase monomolecular films □ Enzyme activity—pig heart mitochondrial malate dehydrogenase, monomolecular films, effect of lipids □ Malate dehydrogenase, pig heart mitochondrial—monomolecular films, effect of lipids on activity □ Films, monomolecular—pig heart mitochondrial malate dehydrogenase, effect of lipids on activity

The importance of lipids for the integrity and function of biomembranes is firmly established. New attempts are being made to ascertain more accurately the contribution of lipids to the properties of biological interfaces. Since cellular proteins are mainly situated at interfaces, the association among proteins and lipids at these interfaces is important to understand (1, 2).

Some lipids appear to be crucial for specialized processes, such as enzyme association that affects the orientation and, in turn, the activity of enzyme molecules. Several enzymes associated with membranes and enzymatic reactions at the biological interface are important, and it is of interest to study the catalytic activity of membrane-bound enzymes and complexes of these enzymes with lipids at an interface.

BACKGROUND

Membrane-bound enzymes are believed to interact with membrane lipids, and these interactions may affect the enzyme activity. A lipid requirement for the catalytic activity of a number of enzymes, *e.g.*, some dehydrogenases, was observed (3, 4). Enzyme activity was lost or reduced when the lipid was removed from the enzyme system. Either stimulation or reactivation by lipids of several enzymes was reported (5–11), and the lipid requirement usually exhibited some specificity (3, 4, 12). Both lipid specificity and lipid concentration apparently are involved in the activity of lipid-requiring enzymes. To perform its enzymatic function, an enzyme also must possess the correct conformation.

Film studies at an air–water interface of enzymes and enzyme–lipid complexes can investigate the behavior of membrane-bound enzymes. The principal inducement to the study of such systems is their close analogy to the membrane of normal cells. Therefore, they have been investigated as working models for cell membranes from the point of view of both membrane structure and function (13).

Previously (14), a technique for studying the catalytic activity of enzymes spread as a film at an air–water interface by exchanging the subphase under the film to remove unspread enzyme molecules was reported. In these studies, pig heart mitochondrial malate dehydrogenase, a vital member of the tricarboxylic acid cycle, was used. Mitochondrial malate dehydrogenase was chosen as a model because it is a membrane-bound enzyme that forms stable monolayers at the air–water interface and offers a convenient assay for catalytic activity. Heart muscle has been used as the primary source for the isolation and purification of this enzyme (15).

Mitochondrial malate dehydrogenase is very unstable in aqueous solutions, and this instability has been ascribed to the removal of the enzyme by severe extraction procedures from its bound states, which suggests that the enzyme is firmly bound in the mitochondrion. Phospholipids constitute about 20% of the total weight of the mitochondrion and are present in particulate fractions isolated from pig heart mitochondria (16).

Whole heart micelles, mitochondrial lipids, and fatty acids reportedly increase the stability of the malate dehydrogenase. An increase in activity and stability of this enzyme was reported in the presence of mitochondrial lipids and a series of saturated and unsaturated fatty acids (16). However, at high concentrations of cephalin or egg lecithin, the activity and stability of the enzyme decreased (17). It was reported that lysolecithin stabilized the enzyme, phosphatidylcholine and phosphatidylethanolamine did not significantly alter the enzyme activity, and cardiolipin and phosphatidylserine strongly inhibited the enzyme (18). In all of the studies cited, enzyme activity was determined in the bulk phase.

One study (19) reported that the enzyme adsorbed onto lipid monolayers from a subphase solution and that enzyme adsorption was greater in the case of negatively charged lipids as compared to neutral or positively charged lipids. The catalytic activity of such complexes when removed from the surface was studied, and the surface enzyme activity was only a fraction of that in the bulk (19).

The purpose of this phase of the study was to investigate both the film behavior and the catalytic activity of pig heart mitochondrial malate dehydrogenase–lipid complexes at the air–water interface using both synthetic lipids and lipids of biological origin. An important aspect was to study enzyme activity with lipids at varying film pressures. This aspect is of interest because it allows a change in the conformations, orientations, and interactions of the enzyme molecules.

EXPERIMENTAL

Materials—Malate dehydrogenase[1] (porcine heart) was supplied as a suspension in 70% saturated ammonium sulfate solution. Oxalacetic acid[2] and reduced nicotinamide adenine dinucleotide[1] (NADH), 90%, were used to measure enzyme activity. The lipids egg lecithin[3], dipalmitoyllecithin[2], and cholesterol were chromatographically pure. The mixed pig heart phospholipids were extracted by a procedure outlined by Kates (20).

The inorganic chemicals, all reagent grade, were checked for surface impurities. The water was first deionized by passing distilled water through a mixed resin bed of a demineralizer[4] and then distilled twice from an all-glass still. All buffer solutions were passed through a column of activated charcoal. Organic solvents were spectrograde and were checked for surface impurities.

Apparatus and Monolayer Methods—The apparatus and procedure for subphase exchange and for determining the π–A isotherms for protein monolayers spread at the air–water interface were described previously (1, 14). In mixed films of malate dehydrogenase with lipids, the lipid was spread first; then, after 15 min, the protein was spread by the Trurnit (21) method. After another 25 min, compression of the mixed film was initiated. For lipid films alone, compression was started 40 min after

[1] P. L. Biochemicals, Milwaukee, Wis.
[2] Schwarz/Mann, Orangeburg, N.Y.
[3] Extracted in this laboratory.
[4] Bantam.

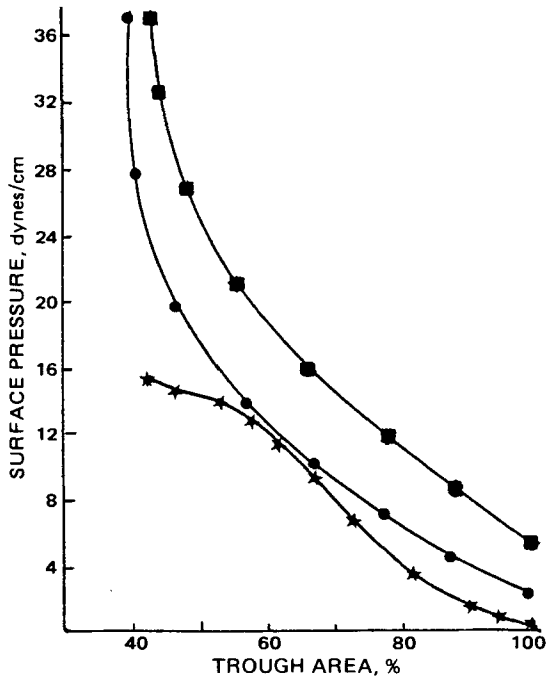

Figure 1—*The π–A curves of films spread on pH 7.4 phosphate buffer. Key: ●, 2.95 × 10^{-2} mg of dipalmitoyllecithin; ■, 8.1 × 10^{-2} mg of malate dehydrogenase and 2.95 × 10^{-2} mg of dipalmitoyllecithin mixed film; and ★, 8.1 × 10^{-2} mg of malate dehydrogenase.*

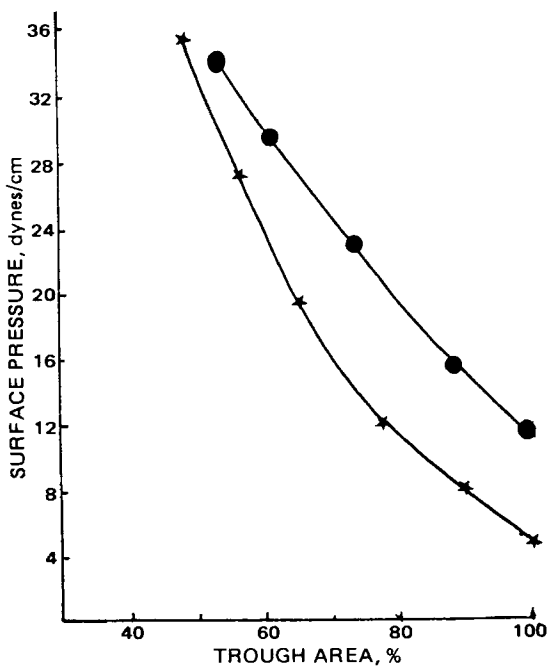

Figure 2—*The π–A curves of films spread on pH 7.4 phosphate buffer. Key: ★, 3.2 × 10^{-2} mg of egg lecithin; and ●, mixed film of 8.1 × 10^{-2} mg of malate dehydrogenase and 3.2 × 10^{-2} mg of egg lecithin.*

spreading. Hexane–ethanol (95:5 v/v) was the solvent used for spreading dipalmitoyllecithin, hexane was used for cholesterol and egg lecithin, and benzene–chloroform (97:3 v/v) was used for pig heart phospholipid extract.

The procedure for determining catalytic activity of the surface-spread enzyme was given previously (14).

To determine the amount of enzyme adsorbed at the air–water interface after subphase exchange, films were removed by negative pressure using a narrow glass tube and then analyzed for protein content by a modification of the Lowry (22) technique.

RESULTS AND DISCUSSION

π–A Behavior of Lipid–Enzyme Mixed Films—The π–A behavior of mixed monolayers of malate dehydrogenase with dipalmitoyllecithin (a saturated phospholipid), egg lecithin (an unsaturated phospholipid), cholesterol, and pig heart phospholipids (a phospholipid of the same source as that of the enzyme) was studied. In all cases, the films were stable and gave reproducible π–A curves.

Penetration and possible interaction between lipids and proteins in monolayers can be detected by an increase in surface pressure if the area is kept constant or by an increase in area if the surface pressure is kept constant. In the presence of a previously spread lipid, the protein can penetrate the film in either a partially unfolded or completely unfolded state, depending on the available space. The protein applied would have its hydrocarbon tails out of the subphase, interacting with the side chains of the lipid, while its polar groups would anchor it to the surface. Thus, protein incorporation into the lipid monolayer indicates the existence of a hydrophobic site capable of association with hydrocarbon chains of the lipid.

Hydrophobic associations of the protein with the phospholipid facilitated by initial electrostatic binding were proposed (23). A similar binding-mediated penetration of cholesterol monolayers by proteins also was proposed (24). It was suggested (25) that the polar portions of the surfactant molecule may interact with their counterparts in the protein film through permanent dipole attraction, dipole-induced dipole interaction, and electrostatic attraction. The nature of this adsorption may disrupt hydrogen bonding, which partially stabilizes the tertiary protein structure and makes the protein molecule more amenable to unfolding.

Adsorption of pig heart mitochondrial malate dehydrogenase from an enzyme subphase solution onto lipid monolayers at high surface pressures was reported (19).

Figure 1 shows the π–A plots of dipalmitoyllecithin, malate dehydrogenase, and dipalmitoyllecithin–malate dehydrogenase mixed films. In all cases, the subphase was exchanged. The dipalmitoyllecithin concentration was chosen so that the interaction of malate dehydrogenase could be studied at low and high surface pressures of the mixed film. The surface pressure of the dipalmitoyllecithin film was about 2 dynes/cm prior to protein addition.

Figure 1 shows that the mixed film was expanded at all areas relative to the dipalmitoyllecithin film alone. The increase in surface pressure at a given surface area is taken as a measure of interaction between the lipid monolayer and the protein. The degree of expansion, $\Delta\pi$, of the mixed film decreased as the surface pressure increased, suggesting that some protein may be squeezed out of the mixed film or may move to a nonarea-determining position at high π values. There is no crossover of the π–A plots of the mixed film and the dipalmitoyllecithin film, and film expansion persisted even beyond the point at which the protein film in the absence of lipid became unstable. An expansion of the mixed film over that of the lipid film at π values below the collapse pressure of the protein can be due to simple adsorption of the protein at the interface. However, if the expansion is observed at higher π values, it suggests penetration of some part of the protein into the hydrocarbon portion of the lipid monolayer (23).

The decrease in $\Delta\pi$ of the mixed film at higher surface pressures suggests that there is no true complex formed. The resultant increase in the area of the dipalmitoyllecithin film after the application of the protein may involve protein penetration, as suggested previously (23, 24, 26, 27), when the polar groups of the protein anchor to the polar head groups of the lipid and the hydrocarbon residues of the enzyme align themselves with the fatty acyl chains of the lipid through van der Waals forces. The possible partial removal of the enzyme at higher surface pressures can be accounted for by differences in chain lengths of the hydrophobic tails of the protein and the lipid. With equal chain lengths, stronger adhesion and less possibility of ejection of the protein molecules would be expected.

Figure 2 shows the π–A plots of egg lecithin and a malate dehydrogenase–egg lecithin mixed film. The molar ratio of lipid to protein was the same as for the dipalmitoyllecithin–enzyme mixed film. The mixed film of the protein and egg lecithin was expanded at all areas relative to the egg lecithin film. The value of $\Delta\pi$ of the mixed film at higher π values decreased, as was the case with the dipalmitoyllecithin–malate dehydrogenase mixed film (Fig. 1). Thus, protein penetration of the lipid film appears to be the interaction mechanism between egg lecithin and malate dehydrogenase.

A comparison of Figs. 1 and 2 shows that the expansion of the lipid

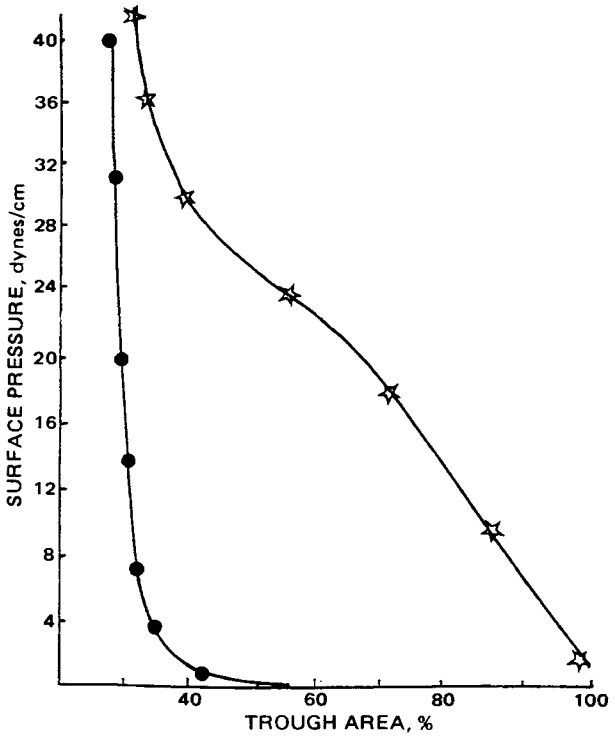

Figure 3—*The π–A curves of films spread on pH 7.4 phosphate buffer. Key: ●, 1.55 × 10^{-2} mg of cholesterol; and ☆, mixed film of 8.1 × 10^{-2} mg of malate dehydrogenase and 1.55 × 10^{-2} mg of cholesterol.*

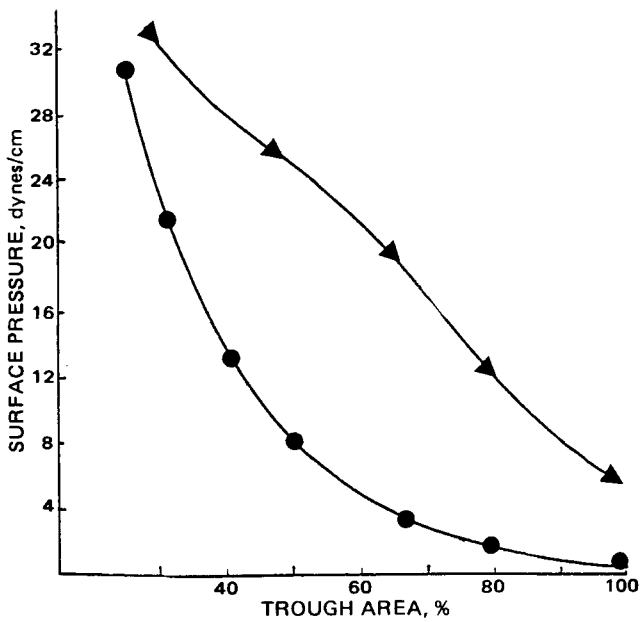

Figure 4—*The π–A curves of films on pH 7.4 phosphate buffer. Key: ●, 2.95 × 10^{-2} mg of pig heart phospholipids; and ▲, mixed film of 8.1 × 10^{-2} mg of malate dehydrogenase and 2.95 × 10^{-2} mg of pig heart phospholipids.*

films by malate dehydrogenase was greater for the egg lecithin film than for the dipalmitoyllecithin film. With the egg lecithin–enzyme mixed film, the expansion seemed to be greatest at intermediate π values.

If the magnitude of expansion of the lipid film by the protein is taken as a criterion of the degree of interaction, these results suggest that malate dehydrogenase interacts more strongly with egg lecithin than dipalmitoyllecithin. The fatty acyl chains of dipalmitoyllecithin are saturated (28), and the molecule of egg lecithin is of the 1-saturated 2-unsaturated type (29). Thus, there is a better packing of molecules in the monomolecular films of dipalmitoyllecithin as compared to egg lecithin, and the greater motional freedom of the acyl side chains of egg lecithin would be expected to result in a greater interaction between the hydrophobic side chains of the protein and the lipid.

Figure 3 shows the π–A plots of cholesterol and a malate dehydrogenase–cholesterol mixed film. The molar ratio of lipid to protein was the same as for the other lipids. The cholesterol film became highly expanded in the presence of malate dehydrogenase, from low π values to about 22 dynes/cm. After this point, an increase in the pressure produced a gradual decrease in the film area. However, beyond 32 dynes/cm, the mixed film became very condensed but not superimposable with the pure cholesterol film.

The large expansion shown suggests a strong interaction of cholesterol and malate dehydrogenase. Reduction of $\Delta\pi$ of the mixed film at higher π values in this case may also be due to the removal of a portion of the enzyme from an area-determining position to a nonarea-determining position or to a portion of the protein being squeezed out of the mixed film.

Colacicco (30) also observed that cholesterol films can pull large quantities of protein into the monolayer. The surface density of anchored protein, as expressed by the $\Delta\pi$ values, is much larger with cholesterol than with the phospholipids studied. How cholesterol interacts with proteins is still quite unclear. Colacicco (30) suggested interspersion of cholesterol within the subunits of protein film. Van Deenen (31) demonstrated that the interaction of the hydrophobic part of the sterol molecule is of critical importance in protein–sterol interactions in monolayers. The presence and orientation of the hydroxyl group in cholesterol also are important for this interaction (30, 31). In mixed monolayers of cholesterol and protein, the hydroxyl group of the cholesterol can interact with the peptide bonds of proteins (32).

Figure 4 shows the π–A plots of films of the total phospholipid extract from the pig heart and the malate dehydrogenase–total phospholipid extract mixed films. On a weight basis, an amount of the total phospholipid extract spread was equivalent to the amount of dipalmitoyllecithin used in the studies of malate dehydrogenase–dipalmitoyllecithin mixed films. The total phospholipid extract from pig heart formed stable films which gave reproducible π–A curves.

Figure 4 shows a large expansion of the total phospholipid extract film by malate dehydrogenase. A comparison of Figs. 1, 2, and 4 shows that the expansion by malate dehydrogenase was greatest for the total phospholipid extract when compared with dipalmitoyllecithin and egg lecithin films. This result suggests a relatively strong interaction between the enzyme and the phospholipids from the same source as the enzyme and could be due to specific interactions between malate dehydrogenase and the phospholipids from the natural source. Penetration of the lipid film, rather than a complex formation between the enzyme and the lipid, seems to be occurring since the degree of expansion is reduced at higher pressures.

Catalytic Activity of Malate Dehydrogenase Films—The catalytic activity of malate dehydrogenase films in the absence of lipids was previously reported (14).

Figure 5 shows the catalytic activity of the enzyme film in the absence of lipid and the catalytic activity of the enzyme film in the presence of dipalmitoyllecithin. The activity of the enzyme–dipalmitoyllecithin film remained relatively constant up to a surface pressure of about 11 dynes/cm. Beyond 11 dynes/cm, catalytic activity decreased with in-

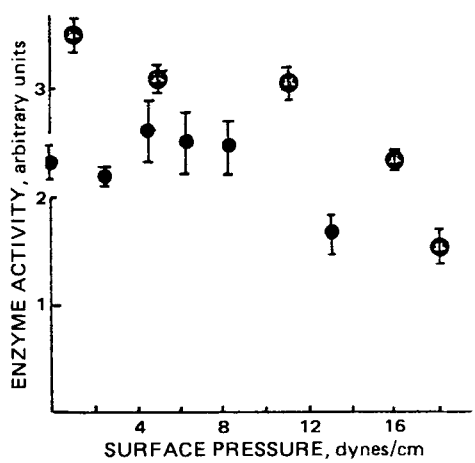

Figure 5—*Catalytic activity of malate dehydrogenase* versus *surface pressure plots. Key: ●, malate dehydrogenase film; and ☆, malate dehydrogenase–dipalmitoyllecithin mixed film.*

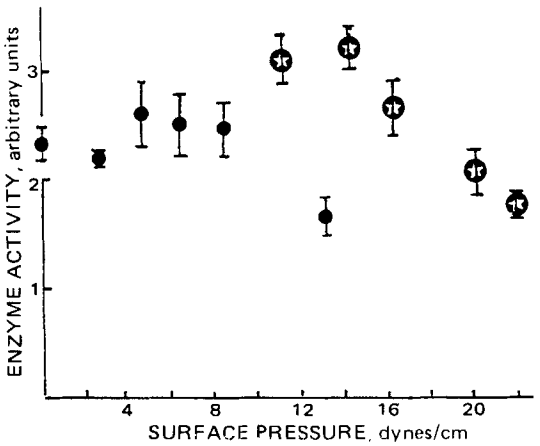

Figure 6—*Catalytic activity of malate dehydrogenase* versus *surface pressure plots. Key:* ●, *malate dehydrogenase film; and* ☆, *malate dehydrogenase–egg lecithin mixed film.*

creasing surface pressures. At high surface pressures, the orientation and conformation of the enzyme–lipid complex must be changing in a way unfavorable for enzyme activity.

Figure 5 shows a stabilizing effect of the lipid, particularly at low surface pressures, where the enzyme activity was significantly increased over that of the enzyme film in the absence of lipid. The stabilizing effect was also apparent at higher surface pressures. At surface pressure values of 16 and 18 dynes/cm, just about all of the surface enzyme activity was lost in malate dehydrogenase films in the absence of dipalmitoyllecithin. Therefore, the dipalmitoyllecithin not only increases the activity of the enzyme but also greatly broadens the pressure range where this activity occurs.

Figure 6 shows the catalytic activity of the enzyme film in the absence of lipid and the catalytic activity of the enzyme film in the presence of egg lecithin. In this mixed film, a dependence of enzyme activity on surface pressure was also observed. At intermediate surface pressures (9–14 dynes/cm), catalytic activity remained relatively constant. Beyond 16 dynes/cm, catalytic activity decreased with increasing surface pressure. Egg lecithin also increased the enzyme activity of malate dehydrogenase and broadened the pressure range over which the enzyme activity was observed as compared to that of the enzyme in the absence of lipid.

A comparison of Figs. 5 and 6 shows that the catalytic activities of the enzyme–dipalmitoyllecithin and the enzyme–egg lecithin films were within the same range of values between surface pressures of 11 and 16 dynes/cm. However, the range of surface pressures over which the enzymatic activity was observed was broader for egg lecithin than for dipalmitoyllecithin. With dipalmitoyllecithin, significant catalytic activity was observed up to a surface pressure of 18 dynes/cm. However, in enzyme–egg lecithin films, catalytic activity was observed up to a surface

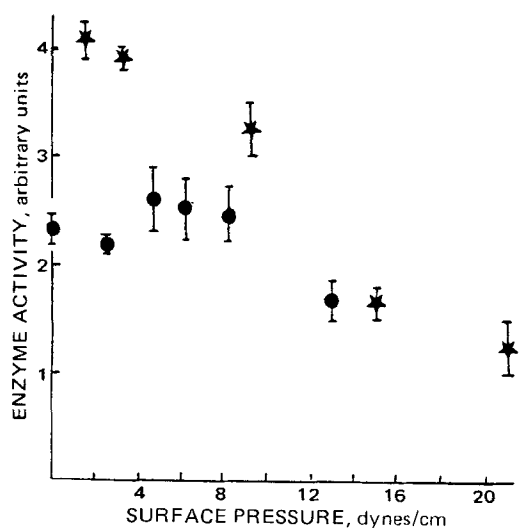

Figure 7—*Catalytic activity of malate dehydrogenase* versus *surface pressure plots. Key:* ●, *malate dehydrogenase film; and* ★, *malate dehydrogenase–cholesterol mixed film.*

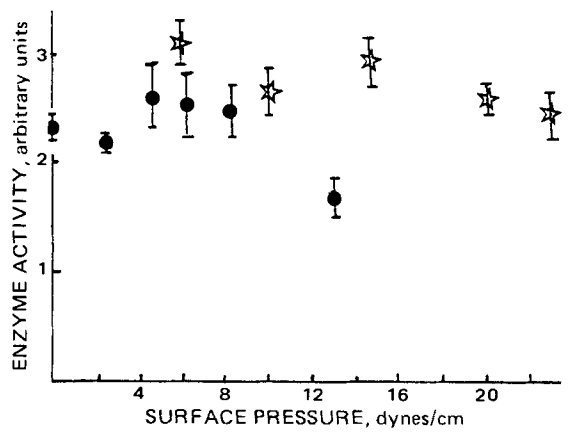

Figure 8—*Catalytic activity of malate dehydrogenase* versus *surface pressure plots. Key:* ●, *malate dehydrogenase film; and* ☆, *malate dehydrogenase–pig heart phospholipids mixed film.*

pressure of about 22 dynes/cm. Therefore, the interaction of the enzyme with the unsaturated phospholipid, egg lecithin, apparently is more favorable for stabilization of the enzyme than the interaction with the saturated phospholipid, dipalmitoyllecithin.

Moreover, the surface area expansion effect was greater with the egg lecithin–enzyme films than with the dipalmitoyllecithin–enzyme films (Figs. 1 and 2), indicating a greater interaction for the former.

Figure 7 shows the catalytic activity of the enzyme film in the absence of lipid and the catalytic activity of the enzyme film in the presence of cholesterol. A comparison of Figs. 5–7 reveals that the maximum catalytic activity observed in any of these systems was with the cholesterol–malate dehydrogenase mixed film at low π values. The maximum activity in arbitrary units observed at any surface pressure was 4.08, 3.5, 3.2, and 2.65 for films of enzyme–cholesterol, enzyme–dipalmitoyllecithin, enzyme–egg lecithin, and enzyme alone, respectively.

Figure 7 shows that catalytic activity of the enzyme–cholesterol film decreased with an increase in surface pressure. Initially, a gradual decrease was observed, but there was a sharp decrease beyond 15 dynes/cm. Comparison with the enzyme activity in the absence of any lipid shows that cholesterol not only stabilized the enzyme film but increased maximum surface enzyme activity significantly at π values up to 9 dynes/cm. Even at a surface pressure as high as 22 dynes/cm, there was significant enzyme activity. A comparison of the surface catalytic activity of enzyme–dipalmitoyllecithin, enzyme–egg lecithin, and enzyme–cholesterol films shows that the activity of the enzyme–cholesterol film was higher at low surface pressure values. The maximum area expansion by the enzyme was observed in the enzyme–cholesterol films.

Figure 8 shows the catalytic activity of the enzyme film in the absence of lipid and the catalytic activity of the enzyme film in the presence of a pig heart phospholipid extract. The enzyme activity of the mixed film was higher at all surface pressures as compared to the film of malate dehydrogenase in the absence of lipids. Considerable enzyme activity was maintained even at pressure as high as 23 dynes/cm. The presence of phospholipid stabilized the enzyme and greatly broadened the surface pressure range over which activity was observed.

A comparison of Figs. 5–7 shows that the activity of the pig heart total phospholipid extract–enzyme mixed film was lower at smaller surface pressure values and greater at higher surface pressure values compared to enzyme–dipalmitoyllecithin, enzyme–egg lecithin, and enzyme–cholesterol mixed films. These results show that the enzyme was stabilized to a greater extent at high surface pressure values by the pig heart phospholipid extract compared to the other lipids studied. This observation is quite meaningful, since the surface pressure exerted by a living membrane is considered to be rather high (33).

Table I—Amount of Protein at the Interface after Subphase Exchange

Film	Found, mg × 10^2
Malate dehydrogenase	2.4
Malate dehydrogenase–cholesterol	2.2
Malate dehydrogenase–dipalmitoyllecithin	2.0
Malate dehydrogenase–egg lecithin	1.9
Malate dehydrogenase–pig heart phospholipids	1.8

It has been suggested that the enzyme may have very specific requirements for optimal stabilization by a phospholipid and that these requirements would be expected to be met by the lipids from the same organ as the enzyme. The greater stabilization of the enzyme at higher surface pressures by the pig heart phospholipid extracts compared to the other lipids studied possibly may result from specific physical interactions between the enzyme and lipid mixture at these higher pressures. An increase in the stability of the enzyme by pig heart lipid micelles also was observed in bulk studies (16).

Amount of Protein Anchored after Subphase Exchange—The results of the protein content analysis for the various malate dehydrogenase films are shown in Table I. The amount of protein remaining at the surface after exchange ranged from 0.018 to 0.024 mg. The amount of protein applied initially to the surface was 0.081 mg. The maximum amount of protein remaining at the interface after subphase exchange occurred with the malate dehydrogenase film in the absence of any lipid. All of the mixed films resulted in a slight reduction in the amount of enzyme remaining at the surface. An obvious explanation for these effects is that protein cannot occupy the same areas of the interface occupied by the lipid molecules and that the area of the interface available to the protein is thereby decreased (34).

Since the amount of protein remaining at the surface in different films studied falls within a narrow range, the differences in enzyme activity of the various enzyme films and the increase in enzyme activity in the presence of the lipids are not results of an increasing amount of enzyme held at the surface but are due to changes in orientation of the enzyme molecules because of surface pressure changes and interaction with lipids.

SUMMARY

The results of this study show that the catalytic activity of the malate dehydrogenase films was dependent both on the lipid present and the surface pressure of the film. The activity of the enzyme was increased in the presence of all of the lipids studied.

Although the simple model system does not simulate the complexity of the biological membrane, it gives some evidence of the lipid requirement for catalytic activity and the role of lipids in the stability of membrane-bound enzymes. Lipids, vis-a-vis membrane-bond enzymes, can be looked upon as serving two functions. First, they restrict and localize the enzyme. Second, they serve a regulatory function, thereby controlling the degree of enzyme activity by changes in the state of compression of the membrane.

REFERENCES

(1) A. W. Malick, A. Felmeister, and N. D. Weiner, *J. Pharm. Sci.*, **62,** 1871 (1973).

(2) L. L. M. van Deenen, in "The Molecular Basis of Membrane Function," D. C. Testeson, Ed., Prentice-Hall, Englewood Cliffs, N.J., 1969, p. 47.

(3) I. Sekuzu, P. Jurtshuk, and D. E. Green, *J. Biol. Chem.*, **238,** 957 (1963).

(4) B. Fleisher, A. Casu, and S. Fleisher, *Biochem. Biophys. Res. Commun.*, **24,** 189 (1966).

(5) H. K. Kimelberg and D. Papahadjopoulos, *Biochim. Biophys. Acta*, **282,** 277 (1972).

(6) G. S. Levey, *Biochem. Biophys. Res. Commun.*, **43,** 108 (1971).

(7) S. Roseman, in "Biomembranes," vol. 2, Plenum, New York, N.Y., 1971, p. 9.

(8) G. S. Gotterer, Ph.D. thesis, Johns Hopkins University, Baltimore, Md., 1964.

(9) P. Cerletti, R. Strom, M. G. Giordano, F. Balestrero, and M. A. Giovenco, *Biochem. Biophys. Res. Commun.*, **14,** 408 (1964).

(10) R. Coleman, *Biochim. Biophys. Acta*, **300,** 1 (1973).

(11) L. Rothfield and D. Romeo, in "Structure and Function of Biological Membranes," L. I. Rothfield, Ed., Academic, New York, N.Y., 1971, p. 252.

(12) M. L. Das and F. L. Crane, *Biochemistry*, **3,** 696 (1964).

(13) J. L. Brash and D. J. Lyman, "The Chemistry of Biosurfaces," vol. I, Dekker, New York, N.Y., 1971, p. 182.

(14) A. W. Malick and N. D. Weiner, *J. Pharm. Sci.*, **66,** 792 (1977).

(15) R. G. Wolfe and J. B. Neiland, *J. Biol. Chem.*, **221,** 61 (1956).

(16) J. W. Callahan and G. W. Kosicki, *Can. J. Biochem.*, **45,** 839 (1967).

(17) S. A. Lodhi, N. D. Weiner, and A. Felmeister, *J. Pharm. Sci.*, **63,** 738 (1974).

(18) G. H. Dodd, *Eur. J. Biochem.*, **33,** 418 (1973).

(19) J. Peters and P. Fromherz, *Biochim. Biophys. Acta*, **394,** 111 (1975).

(20) M. Kates, "Laboratory Techniques in Biochemistry and Molecular Biology," American Elsevier, New York, N.Y., 1972, pp. 349, 350, 394.

(21) H. J. Trurnit, *J. Colloid Sci.*, **15,** 1 (1960).

(22) O. H. Lowry, *J. Biol. Chem.*, **193,** 265 (1951).

(23) D. Papahadjopoulos and H. K. Kimelberg, *Biochim. Biophys. Acta*, **233,** 805 (1971).

(24) G. Colacicco, *Lipids*, **5,** 636 (1970).

(25) M. V. Yudenfreund and P. Becher, in "Monolayers," American Chemical Society Advances in Chemistry Series 144, E. D. Goddard, Ed., American Chemical Society, Washington, D.C., 1975, p. 199.

(26) P. Doty and J. H. Schulman, *Discuss. Faraday Soc.*, **6,** 21 (1949).

(27) R. Matalon and J. H. Schulman, *ibid.*, **6,** 27 (1949).

(28) D. Ghosh, M. A. Williams, and J. Tinoco, *Biochim. Biophys. Acta*, **291,** 351 (1973).

(29) H. Witcoff, "The Phosphatides," Reinhold, New York, N.Y., 1951, pp. 320–322.

(30) G. Colacicco, "Biological Horizons in Surface Sciences," Academic, New York, N.Y., 1973, p. 247.

(31) L. L. M. van Deenen, *Biochim. Biophys. Acta*, **332,** 69 (1974).

(32) D. Papahadjopoulos, M. Cowden, and H. Kimelberg, *ibid.*, **330,** 8 (1973).

(33) J. F. Danielli, "Surface Phenomena in Chemistry and Biology," Pergamon, New York, N.Y., 1959, pp. 246–265.

(34) M. C. Phillips, M. T. A. Evans, and H. Hauser, *Sonderdzuck aus Chemie, Physikalische Chemie und Anwendungstechnik des grenzflachenaktiven Staffe (Zurich)*, **11,** 381 (1972).

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