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LIPID-DEPENDENT INTERACTION OF D-β-HYDROXYBUTYRATE DEHYDROGENASE WITH CELLULAR MEMBRANES

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Summary

A mechanism of selective localization of membrane-bound enzymes was examined by studying the interaction between D-β-hydroxybutyrate dehydrogenase (EC 1.1.1.30) and native cellular membranes in which the lipid components were altered.

(1) The catalytic activity of the purified lipid-free enzyme could be restored by the re-interaction with microsomal and mitochondrial membranes, whereas with erythrocyte membranes or liposomes from lipids of erythrocyte membranes this activity could not be restored (Miyahara, M., Utsumi, K. and Deamer, D.W. (1981) Biochim. Biophys. Acta 641, 222—231). In the erythrocyte lipid components, only lysophosphatidylcholine markedly inhibited the enzyme reactivation.

(2) The inhibitory effect of lysophosphatidylcholine was confirmed in microsomes in which the lysophosphatidylcholine contents had been increased, by phospholipase A_2 treatment, to the levels in erythrocyte membranes.

(3) Selective digestion by phospholipase C of phosphatidylcholine in the microsomes was accompanied by a lowering of the level of reactivation in the membranes.

(4) The presence of lipophilic alkyl compounds such as cetylamine and cetyltrimethylammonium bromide, which contain the ammonium group, in the membranes also inhibited the enzyme reactivation. However, negatively charged and neutral alkyl compounds were less suppressive.

The results above suggested that the interaction of D-β-hydroxybutyrate dehydrogenase with native cellular membranes is dependent on the amounts of phosphatidylcholine and lysophosphatidylcholine exposed on the membrane surface. It was also suggested that the presence of the ammonium group of

non-diacyl compounds is unfavorable for the effective interaction of the enzyme.

Introduction

The effective interaction (or association) of an enzyme with its specific cellular membranes is of interest because the interaction may reflect a mechanism by which the polypeptide synthesized in the cytosol is transferred to and selectively interacts with its respective target membranes. In an attempt to elucidate this mechanism, the interaction between an isolated membrane-bound enzyme and liposomes or native cellular membranes has been extensively studied (for a review, see Refs. 1 and 2). Some purified mitochondrial membrane proteins such as cytochrome oxidase and oligomycin-sensitive ATPase effectively incorporate into liposomes from mitochondrial lipids, and the incorporation of these proteins is either increased or decreased depending on the proteins and phospholipids inserted previously into the acceptor membranes [3,4]. In addition, some glycolytic enzymes such as phosphofructokinase, aldolase and glyceraldehyde-3-phosphate dehydrogenase associate with band 3 protein in the erythrocyte membranes [5,6]. This shows that the pre-existing membrane proteins in the acceptor membranes are responsible for receiving exogenously added proteins. McIntyre et al. [7] recently reported that D-β-hydroxybutyrate apodehydrogenase purified from beef heart mitochondria was inserted into and activated in a controlled manner by sarcoplasmic reticulum membranes of varying phospholipid and protein ratios. They concluded that the insertion of the enzyme in the membrane is a simple index of the amount of phosphatidylcholine available on the surface of the membranes. We have recently purified a similar enzyme from rat liver mitochondria [8]. The lipid-free enzyme shows little catalytic activity, but activity is restored when the enzyme interacts with different kinds of native cellular membrane. The enzyme, however, did not interact with erythrocyte plasma membranes [9]. Although in the previous paper [9] it was suggested that the selective interaction of the enzyme with cellular membranes is controlled largely by lipid components in the membranes, the details of its interaction process remain obscure. The present paper reports some controlled properties of the enzyme-cellular membrane interaction.

Materials and Methods

Materials. Mitochondria were isolated from the livers of male Wister strain rats weighing 250—300 g by using the method of Hogeboom [10]. The isolated mitochondria were suspended in 0.25 M sucrose containing 2 mM Tris-HCl, pH 7.4, at a concentration of 30—40 mg protein per ml and stored at −80°C. D-β-Hydroxybutyrate apodehydrogenase was purified from the stored mitochondria without using detergent or phospholipase treatment [8]. The enzyme used in the present experiments was purified about 220-fold in specific activity. The enzyme activity was determined by measuring the absorbance changes at 340 nm in a medium containing 50 mM Tris-HCl, pH 8.1, 3 mM NAD^+, 0.5 mM

dithiothreitol, 0.5 mM EDTA and appropriate amounts of liposomes or native cellular membranes. Rat liver microsomes were prepared by using the method of Okuyama and Lands [11], suspended in the above-mentioned buffered sucrose solution at a concentration of 20—30 mg protein per ml and stored at —80°C under N_2. Rat erythrocyte plasma membranes were prepared from blood collected from the orbital sinus in about 0.1 vol. of cold 3.8% sodium citrate. The ghosts prepared by using the method of Dodge et al. [12] were suspended in the above-mentioned isotonic sucrose solution and stored similarly, as described.

Preparation of liposomes. The lipids extracted and purified from native cellular membranes by using the method of Folch et al. [13] were stored in a small known volume of $CHCl_3/CH_3OH$ (2 : 1, v/v) at —80°C. For the preparation of liposomes, an aliquot of the stored lipid solution was exposed to a stream of N_2 to remove the solvent and further degassed overnight at room temperature in vacuo. To 20 mg of lipids, 1 ml of 20 mM Tris-HCl, pH 8.1, containing 1 mM EDTA was added, vortex mixed for 5 min, and further sonicated for 2 h in the cold with a bath-type sonifier (Branson B-22) under N_2. The sonicated lipid suspension was centrifuged at 105 000 × *g* for 40 min and the supernatant fraction was used as liposomes. Egg phosphatidylcholine was prepared by using the method of Hanahan et al. [14] with silicic acid column chromatography. The preparation of the phosphatidylcholine liposomes was performed as described above. For modification of liposomes by different kinds of lipids, a small amount (less than 1/10 vol. of liposome suspension) of their respective alcoholic solutions was directly added to 0.3 ml liposomal suspension by using the method of Batzri and Korn [15].

Modification of microsomes by phospholipase treatment. Microsomes (120 mg protein) were suspended in a medium containing 50 mM Tris-HCl, pH 7.4, 10 mM $CaCl_2$ and 0.25 mM $MgCl_2$ at 37°C in a final volume of 55 ml. The warmed microsome suspension at 37°C was then further incubated by adding phospholipase A_2 or phospholipase C at a final concentration of 1 I.U. to 12—14 mg microsomal proteins. At specified times of incubation, a 10 ml aliquot of the incubation mixture was transferred to a centrifuge tube and hydrolysis stopped by adding 12 mM EDTA (final concentration) at 0°C. The microsomes were washed twice with an isotonic sucrose solution containing 1 mM EDTA and 2 mM Tris-maleate, pH 7.4, by repeated centrifugation at 105 000 × *g* for 60 min. Membranes prepared in this manner were finally suspended with a small known volume of a similar sucrose solution but without EDTA, and used for the activation of D-β-hydroxybutyrate apodehydrogenase.

Analysis of membrane components. Lipids of native and modified microsomes and liposomes were extracted, purified by using the method of Kates [16], dried under a stream of N_2, and dissolved in a small known volume of $CHCl_3/CH_3OH$ (2 : 1, v/v). Lipids were analysed by thin-layer chromatography using basic silica gel plates (0.5 mm thickness) and developed with $CHCl_3/CH_3OH/CH_3CO_2H/H_2O$ (50 : 25 : 7 : 3, v/v) by using the method of Skipski et al. [17]. The spots visualized by exposure to iodine vapor were individually scraped into test tubes and the lipid phosphorus was determined by using the method of Lowry et al. [18]. Neutral lipids were also separated by thin-layer chromatography using commercial plates (Kieselgel, No. 5772, Merck). The

lipids were developed with petroleum ether/ether/acetic acid (82 : 18 : 1, v/v) and the relative amounts were determined by scanning the spots colorized by 5% phosphomolybdate spray at 700 nm using a Chromatoscanner (Shimadzu CS-900). Proteins were determined by using the method of Ross and Schatz [19] using bovine serum albumin as standard.

Phospholipase A_2 (from *Crotalus adamanteus* venom), phospholipase C (from *Chlostridium welchii*), NAD^+, dithiothreitol, DL-hydroxybutyrate, sphingomyelin (from bovine erythrocytes), lysophosphatidylcholine (from egg yolk) and cholesterol were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Other chemicals were analytical grade commercial products from Nakarai Chemical Co., Kyoto, Japan.

Results

The enzyme, purified in a lipid-free form from rat liver mitochondria, interacts with liver microsomal and mitochondrial membranes, and its catalytic activity is restored. On the other hand, it does not interact with the plasma membranes of rat erythrocytes and hence the activity cannot be recovered [9]. A similar efficiency of the reactivation was also proved by using liposomes prepared from lipids of the respective cellular membranes. This indicates that the membrane lipids are mainly responsible for the interaction of the enzyme approaching exogenously the membranes.

As reported elsewhere, lipid components of red cell plasma membranes are distinct from other subcellular membranes. They contain much larger amounts

TABLE I

EFFECT OF SOME LIPID COMPONENTS ON THE REACTIVATION OF D-β-HYDROXYBUTYRATE APODEHYDROGENASE

Egg lecithin liposomes (470 ng phosphorus) were modified with shingomyelin, cholesterol and lysophosphatidylcholine, respectively, at the concentrations shown below. To 1.5 ml of modified liposome suspension containing 50 mM Tris-HCl, pH 8.1, 3 mM NAD^+, 0.5 mM EDTA and 0.5 mM dithiothreitol at 37°C, 10 μl of the enzyme (20 μg protein) were added and incubated at 37°C for 15 min. The enzyme activity was determined by measuring the absorbance changes at 340 nm after adding 20 mM DL-β-hydroxybutyrate (final concentration) at 37°C. When the enzyme was incubated in the absence of liposomes and in the presence of non-modified liposomes, 0.200 and 2.85 μmol NADH/min per mg apoenzyme protein, respectively, were produced. PL, phospholipids.

Lipids used for modification	Concentration (mole ratio of lipid : liposome PL)	Per cent of activation
None		100
Sphingomyelin	0.05	100
	0.10	100
	0.20	100
	0.30	100
Cholesterol	0.04	100
	0.08	100
	0.16	100
	0.50	100
Lysophosphatidylcholine	0.006	78
	0.017	38
	0.033	21
	0.048	10

TABLE II

CHANGES IN LIPID COMPONENTS OF RAT LIVER MICROSOMAL MEMBRANES AFTER TREATMENT WITH PHOSPHOLIPASE A_2

0.2 ml aliquots of the treated microsomes (1.472, 1.432, 1.337 and 1.314 mg protein for 0, 5, 10 and 20 min incubated microsomes, respectively) were subjected to lipid analysis. Lys, lysophosphatidylcholine; SM, sphingomyelin; PC, phosphatidylcholine; PI, phosphatidylinositol; PE, phosphatidylethanolamine; CHOL, cholesterol; FA, free fatty acids; TG, triglycerides.

Incubation time (min)	Phospholipids (μmol/0.2 ml microsomes)					Neutral lipids (arbitrary units/spot)		
	Lys	SM	PC	PI	PE	CHOL	FA	TG
0	0.003	0.018	0.548	0.084	0.190	26.0	2.3	10.5
5	0.018	0.018	0.527	0.093	0.181	32.0	19.0	12.3
10	0.028	0.023	0.520	0.087	0.168	32.0	46.0	13.2
20	0.048	0.020	0.497	0.086	0.159	32.8	58.0	11.2

of cholesterol, sphingomyelin and lysophosphatidylcholine [20]. Egg lecithin liposomes containing various amounts of erythrocyte lipid components were prepared and the interaction of the purified apodehydrogenase with these liposomes was examined by measuring the enzyme reactivation (Table I). Modification of liposomes by lysophosphatidylcholine markedly suppressed the enzyme reactivation. The amounts which induced complete inhibition were similar to those seen in the erythrocyte membranes. However, sphingomyelin, which also contains the phosphatidylcholine polar moiety, caused no suppression. Cholesterol also did not interfere with the enzyme reactivation. These results could not be explained simply by a previous report by Vidal et al. [21] who reported

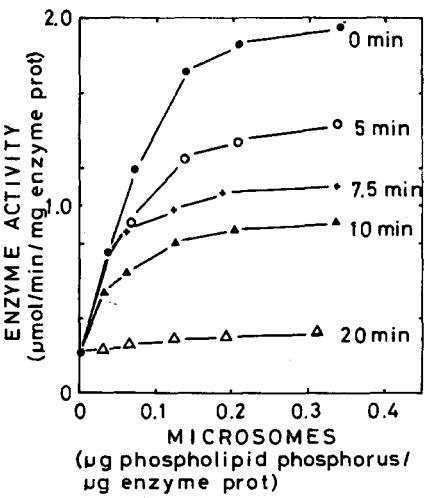
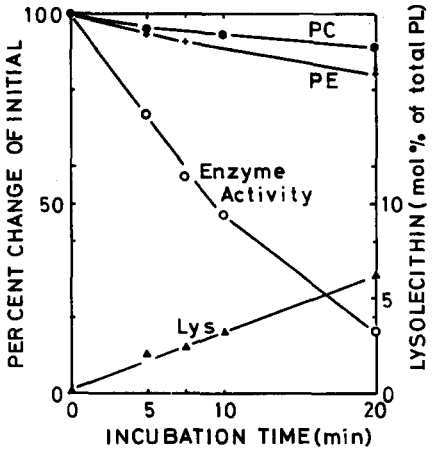


Fig. 1. Changes in reactivation level of microsomal membranes after treatment with phospholipase A_2. The treatment of the microsomes with phospholipase A_2 was as described in Table II and in the text. The apodehydrogenase (19.34 μg protein) was incubated with various amounts of the treated microsomes, and the reactivation of the enzyme was determined as shown in Table I. The time of phospholipase A_2 treatment is given next to each curve.



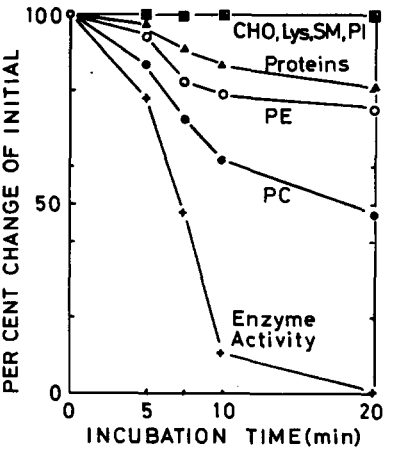


Fig. 2. The relationship between lysophosphatidylcholine contents and the loss of enzyme reactivation after treatment with phospholipase A_2. The microsomes were treated with phospholipase A_2 and the data were obtained from Table II and Fig. 1. For details refer to Table II and Fig. 1. The enzyme activity was compared to the values obtained with microsomes (0.335 μg phospholipid phosphorus/μg apoenzyme protein) at 0 min incubation and the equivalent amounts for other microsomes.

Fig. 3. Relationship between the loss of apodehydrogenase reactivation and the disappearance of lecithin in the microsomal membranes. The changes in the lipid components and the ability of the enzyme to be reactivated after phospholipase C treatment were determined as shown in Table II and Fig. 1, and the expression of the data is as shown in Fig. 2.

that the enzyme interaction was a simple index of the amounts of phosphatidylcholine per unit area of the membrane surface.

The inhibitory effect of lysophosphatidylcholine was also examined using microsomes in which the lysophosphatidylcholine contents were altered enzymatically. Rat liver microsomes were incubated with phospholipase A_2 and the changes in lipid contents in the recovered microsomes were first estimated (Table II). Phospholipase treatment caused gradual decreases in phosphatidylcholine and phosphatidylethanolamine content, followed by marked increases in lysophosphatidylcholine and free fatty acid levels. Changes in other phospholipid components and neutral lipids during incubation were slight. When apodehydrogenase was allowed to interact with these microsomes, the restoration of enzyme activity was found to be dependent on the incubation time (Fig. 1). In the absence of membranes, the enzyme displayed little activity and was reactivated maximally by microsomes of 0.35 μg phospholipid phosphorus/μg enzyme protein. However, the membranes treated with phospholipase A_2 lost their reactivation capacity with prolonged time of incubation. The membranes treated for 20 min displayed little reactivation of the added apoenzyme. These suppressive effects were characterized by the progressive decline in V (maximally restored activity of the enzyme), indicating non-competitive inhibition of the reactivation. It was also noted that the loss in the reactivation was nearly proportional to the increased amounts of lysophosphatidylcholine in the membranes (Fig. 2). Lysophosphatidylcholine in amounts of 5—6% of total phospholipids, which are the amounts seen in rat erythrocyte membranes, caused little restoration of enzyme activity.

Next, it is of interest, and important, to determine which lipid components are concerned with enzyme reception on the surface of native target membranes. An experiment was performed to remove lipid components individually from the membranes. The microsomal membranes were treated with phospholipase C, which is reported to hydrolyze phosphatidylcholine specifically and does not act on any other lipid components. The relationship between the loss of enzyme reactivation and the lowered contents of phosphatidylcholine in the membranes is presented in Fig. 3. Changes in membrane lipids such as lysophosphatidylcholine, cholesterol, sphingomyelin and phosphatidylinositol after phospholipase C treatment were slight. However, phospholipase C hydrolyzed nearly 50% of the membrane phosphatidylcholine and 20% of the phosphatidylethanolamine during 20 min incubation. The ability to activate the apodehydrogenase in these treated membranes decreased markedly, concomitant with the time lapse of incubation, and its mode of loss in reactivation was similar to the disappearance of phosphatidylcholine from the membranes.

As is well known, this enzyme is one of the most intensively studied lipid-requiring membrane proteins. When the purified apoenzyme is reactivated with

TABLE III

EFFECT OF MODIFICATION OF LIPOSOMES BY VARIOUS DETERGENTS ON THE REACTIVATION OF D-β-HYDROXYBUTYRATE APODEHYDROGENASE

Liposomes from total lipids of rat liver mitochondria were prepared and modified by the respective detergents at the concentrations shown below. Other details are described in Table I. The enzyme activity in the absence and in the presence of non-modified liposomes was 0.220 and 12.75 μmole NADH produced/min per mg apoenzyme protein, respectively. The values of the critical micellar concentration (CMC) were taken from the paper of Helenius and Simons [25] except for a [26] and b [27]. The amounts of detergent used were less than 1/10—1/100 of the critical micellar concentration. SDS, sodium dodecyl sulfate; CTAB, cetyltrimethylammonium bromide; C_{16}-NH_3^+, cetylamine; LBS, laurylbenzenesulfonate.

Modification detergent	Concentration (mole ratio to liposome PL)	CMC (M)	Per cent of activation
None			100
Lysophosphatidylcholine	0.0006	$2—0.2 \cdot 10^{-5}$ (a)	79
	0.016		34
	0.048		13
C_{16}-NH_3^+	0.05	$1.1 \cdot 10^{-3}$ (b)	90
	0.12		66
	0.32		4
$CTAB^+$	0.05	$9.2 \cdot 10^{-4}$	79
	0.12		50
	0.32		4
SDS^-	0.16	$3.1 \cdot 10^{-3}$	100
	0.32		100
	0.48		98
LBS^-	0.16	$1.3 \cdot 10^{-2}$	100
	0.32		100
	0.48		95
Triton X-100	0.16	$2.4 \cdot 10^{-4}$	100
	0.32		100
	0.48		100
Brij 35	0.16	$7.5 \cdot 10^{-5}$	95
	0.32		89
	0.48		80

different lipid vesicles, it shows a specific and absolute requirement for phosphatidylcholine [22]. The results above elucidated that a similar enzyme from rat liver mitochondria also interacts with phosphatidylcholines in cellular membranes and that its catalytic activity is restored. Therefore, the enzyme does not seem to exhibit strict specificity for the interaction with cellular membranes. McIntyre et al. [7] reported that the enzyme interaction with the membranes is a simple index of the amount of phosphatidylcholine molecules available on the membrane surface. However, as shown in Table I and Fig. 2, the presence of lysophosphatidylcholine in the membranes is unfavorable for enzyme reactivation. When the lysophosphatidylcholine liposomes were used as acceptor membranes, the enzyme was only slightly reactivated [23]. This is explained by the fact that lysophosphatidylcholine acts as a potent detergent [23,24].

To determine whether lysophosphatidylcholine, as a potent detergent, simply interferes with the enzyme interaction with the membranes, the effect of lysophosphatidylcholine was compared with that of other detergents (Table III). Lysophosphatidylcholine markedly inhibited the enzyme reactivation as compared to other detergents. It may be possible that this strong suppression is due to its marked fluidizing action on the membrane lipids and this, in turn, may perturb an acceptor structure for the exogenously added enzyme. It was also noted that the positively charged detergents, which contain the ammonium group, were also inhibitory, whereas negatively charged and neutral detergents were ineffective. The results suggest that the increased exposure of the polar ammonium groups of the following unfavorable hydrophobic tail in the membranes is disadvantageous for the enzyme reactivation. Furthermore, the extent of these suppressive effects was not consistent with the critical micellar concentration values of the respective detergents. K_i values of lysophosphatidylcholine and of cetylamine calculated from Dixon plots were 2 and 14 mol% of liposome phospholipids, respectively. Both of these detergents inhibited the enzyme reactivation non-competitively (data not shown).

Discussion

There are two main types of interaction reported for the membrane-bound enzyme and the cellular membranes: one is the direct protein-protein interaction in the membranes, the other is the lipid-protein interaction [1—7]. The enzyme used in the present study shows a specific and absolute requirement for phosphatidylcholine when it is reactivated with phospholipid liposomes. On the other hand, when native cellular membranes are used as the acceptor membranes, the catalytic activity of the enzyme is restored by insertion of the enzyme into mitochondrial and microsomal membranes, but it does not interact with erythrocyte plasma membranes. The interaction of the enzyme with microsomal membranes increases when some proteins are stripped from the membranes [9]. McIntyre et al. [7] reported that the insertion was a simple index of the amounts of phosphatidylcholine available on the membrane surface. However, the enzyme could not interact with erythrocyte membranes (mixture of inside-out and right-side-out membrane fragments) from which almost all membrane proteins were removed [9]. A similar failure of the

enzyme reactivation to occur was also observed with liposomes from lipids of erythrocyte membranes [9]. These results suggested that the membrane lipids are mainly responsible for the reception of exogenously approaching proteins, and that the proteins do not play any direct role in the interaction. Therefore, the present work focussed on the role of membrane lipids in the interaction. As shown in Fig. 1 and Tables I and III, lysophosphatidylcholine, which occurs in relatively large amounts, markedly inhibited the enzyme reactivation. This was also confirmed using microsomal membranes in which the lysophosphatidylcholine contents were increased. It was also shown that phosphatidylcholine molecules exposed on the membrane surface are involved in the acceptance of D-β-hydroxybutyrate apodehydrogenase, and that the removal of some membrane proteins from membranes increased the enzyme interaction [7,9].

The suppressive effect of lysophosphatidylcholine is reported to be due to its strong detergent effect [23,24]. As shown in Tables I and III, the suppression by lysophosphatidylcholine is extraordinarily strong as compared to the other detergents tested. This might suggest that lysophosphatidylcholine has a specific biological effect on the membrane structure and function. The data also showed that the increased exposure of the ammonium groups, of which the hydrophobic groups are different from the usual hydrophobic domains of diacyl membrane phospholipids, markedly suppressed the enzyme reactivation. Alkyl amines, of which the chain length is less than eight carbons, do not insert into the membranes and do not inhibit the enzyme reactivation. This suggests that the agents in a water-soluble form cause no inactivation, but that the agents occurring at the membrane surface are unfavorable for the effective interaction of the enzyme. The effective association or interaction of the enzyme may be dependent on a specific lipid environment on the membrane surface. Webster et al. [28] have suggested that when a certain soluble protein interacts with membranes, an initial ionic interaction may induce conformational changes on the protein that cause more hydrophobic groups to become exposed, and that these groups may then penetrate the membrane interior. The present data indicate that both hydrophilic and hydrophobic domains of phospholipids at the acceptor membranes somehow facilitate the initiation process of the polypeptide interaction or the specific localization to the target membranes.

References

1 Chua, N.-M. and Schmidt, G.W. (1979) J. Cell Biol. 81, 461—483
2 Schatz, G. (1979) FEBS Lett. 103, 203—211
3 Eytan, D.G., Matheson, M.J. and Racker, E. (1976) J. Biol. Chem. 251, 6831—6837
4 Eytan, D.G. and Racker, E. (1977) J. Biol. Chem. 252, 3208—3212
5 Strapazon, E. and Steck, T.L. (1977) Biochemistry 16, 2966—2971
6 Higashi, M., Richards, C.S. and Uyeda, K. (1979) J. Biol. Chem. 254, 9542—9550
7 McIntyre, J.O., Wang, C. and Fleischer, S. (1979) J. Biol. Chem. 254, 5199—5207
8 Miyahara, M., Utsumi, K. and Deamer, D.W. (1980) J. Biol. Chem., submitted
9 Miyahara, M., Utsumi, K. and Deamer, D.W. (1981) Biochim. Biophys. Acta 641, 222—231
10 Hogeboom, G.H. (1955) Methods Enzymol. 1, 16—19
11 Okuyama, H. and Lands, W.E.M. (1969) J. Biol. Chem. 244, 6514—6519
12 Dodge, J.T., Mitchell, C. and Hanahan, D.J. (1963) Arch. Biochem. Biophys. 100, 119—130
13 Forch, J., Lees, M. and Stanley, G.H.S. (1957) J. Biol. Chem. 226, 469—509
14 Hanahan, D.J., Dittmer, J.C. and Warashina, E. (1957) J. Biol. Chem. 228, 685—700

15 Batzri, S. and Korn, E.D. (1973) Biochim. Biophys. Acta 298, 1015—1019
16 Kates, M. (1972) in Techniques of Lipidology (Work, T.S., ed.), pp. 351—352, North-Holland, Amsterdam
17 Skipski, V.P., Peterson, R.F., Sanders, J. and Barclay, M. (1963) J. Lipid Res. 4, 227—228
18 Lowry, O.H., Roberts, N.R., Leiner, K.Y., Wu, M.-L. and Farr, A.L. (1954) J. Biol. Chem. 207, 1—17
19 Ross, E. and Schatz, G. (1973) Anal. Biochem. 54, 304—306
20 Nelson, G.J. (1967) Biochim. Biophys. Acta 144, 221—232
21 Vidal, J.C., Gugleilmucci, E. and Stoppani, A.O.M. (1978) Arch. Biochem. Biophys. 187, 138—152
22 Fleischer, B., Casu, A. and Fleischer, S. (1966) Biochem. Biophys. Res. Commun. 24, 189—194
23 Isaacson, Y.A., Deroo, P.W., Rosenthal, A.F., Bittman, R., McIntyre, J.O., Bock, H.-G., Gazzotti, P. and Fleischer, S. (1979) J. Biol. Chem. 254, 117—126
24 Rydström, J. (1976) Biochim. Biophys. Acta 455, 24—35
25 Helenius, A. and Simons, K. (1975) Biochim. Biophys. Acta 415, 29—72
26 Weltzien, H.U. (1979) Biochim. Biophys. Acta 559, 259—287
27 Klevens, H.B. (1953) J. Am. Oil Chem. Soc. 30, 74—80
28 Webster, K.A., Patel, H.V., Freeman, K.B. and Papahadjopoulos, D. (1979) Biochem. J. 178, 147—158