Biochimica et Biophysica Acta, 641 (1981) 222—231

BBA 79132

SELECTIVE INTERACTION OF D-β-HYDROXYBUTYRATE DEHYDROGENASE WITH INTRACELLULAR MEMBRANES

MASANOBU MIYAHARA [a], KOZO UTSUMI [a] and DAVID W. DEAMER [b]

[a] *Department of Medical Biology, Kochi Medical School, Nankoku, Kochi 781-51 (Japan) and* [b] *Department of Zoology, University of California, Davis, CA 95616 (U.S.A.)*

(Received June 6th, 1980)
(Revised manuscript received October 24th, 1980)

Key words: Enzyme-membrane interaction; Hydroxybutyrate dehydrogenase; Lipid environment; (Rat liver)

Summary

We are investigating the properties of pre-existing membrane structures that may contribute to localization of newly formed polypeptides on target membranes. To this end, D-β-hydroxybutyrate dehydrogenase (EC 1.1.1.30) was purified from inner membranes of rat liver mitochondria and interacted with three different cellular membranes, as well as with liposomes prepared from membrane lipid extracts.

(1) The purified lipid-free enzyme displayed little catalytic activity. Its activity was restored by interaction with rat liver mitochondrial inner membranes or microsomal membranes, but not with rat erythrocyte plasma membrane vesicles.

(2) Plasma membranes from which membrane proteins had been partially removed did not reactivate the enzyme, but microsomal membranes treated in a similar manner displayed an increased efficiency of reactivation.

(3) The selective reactivation found in the three membrane species was confirmed in liposomes prepared with total lipid extracts of the native membranes.

The results suggest that the interaction of exogeneously added enzyme with the membranes is primarily dependent on lipid components or some specific lipid environment on the acceptor membranes.

Introduction

Interaction of purified enzymes with cellular membranes is a useful experimental tool for investigating the mechanism by which polypeptides synthesized

Abbreviation: SDS, sodium dodecyl sulfate.

in the cytosol selectively interact with target membranes. It has been reported that certain subunits of cytochrome peroxidase and F_1-ATPase accumulate as large polypeptides in the cytosol, and are then imported into mitochondria after post-translational processing [1,2]. However, cytochrome *c* is imported directly into mitochondria across both membranes [3]. To assume specific incorporatuon of a newly synthesized protein into the target membrane, the polypeptide presumably binds to a membrane receptor(s) and then undergoes post-translational transport into the membrane interior. It remains unclear as to how target membranes discriminate between various polypeptides that may be avaialble for binding and inward transport.

A useful model system to study such processes is the interaction of an isolated membrane enzyme with liposomes or native cellular membranes. Several mitochondrial membrane proteins such as cytochrome oxidase and the hydrophobic proteins of oligomycin-sensitive ATPase have been incorporated into liposomes composed of mitochondrial lipids [4]. The efficiency of incorporation depended both on the protein and phospholipid species previously inserted into the acceptor membranes.

D-β-Hydroxybutyrate dehydrogenase has also been purified from inner mitochondrial membranes [5,6]. The lipid-free form is inactive, but can be reactivated with phosphatidylcholine and certain cellular membranes [7—9]. McIntyre et al. [9] have concluded that the insertion of the enzyme into a membrane is proportional to the amount of phospholipid available on the membrane surface. In related work, Higashi et al. [10] reported that phosphofructokinase competes with aldolase or glyceraldehyde-3-phosphate dehydrogenase for binding sites on band 3 protein of erythrocyte membranes. This suggests that the presence of certain proteins at a membrane may also modulate binding specificity.

In this study, we have investigated the interaction of D-β-hydroxybutyrate dehydrogenase with three different native membranes and with liposomes prepared from total lipid extracts of each membrane species. Since the isolated lipid-free enzyme shows little catalytic activity and requires the lipid to be reactivated [5—9], we could determine specificity of its interaction with intact and experimentally modified membranes containing different lipid and protein components.

Materials and Methods

Materials. Mitochondria were isolated from the livers of male Wistar strain rats weighing 250—300 g by using the method of Hogeboom [11]. 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 dehydrogenase was purified from the mitochondria without using detergent or phospholipase A_2 as described in detail elsewhere [12]. Briefly, mitochondria were first sonicated to form submitochondrial particles, followed by incubation for 30 min at 0°C in a medium containing 2 mM NAD^+, 0.1 M KH_2PO_4 and 5 mM dithiothreitol, pH 10.25. The proteins released by this treatment were separated by centrifugation at 178 000 × *g* for 60 min and recovered by adding an equal volume of saturated $(NH_4)_2SO_4$ to the superna-

tant. The pellet obtained after the centrifugation step was suspended in buffered sucrose solution and the membranes, termed post-submitochondrial membranes, were used for later experiments. The membrane fraction showed little enzyme activity. Further purification of the enzyme from the $(NH_4)_2SO_4$ precipitate was performed by using the method of Nielsen and Fleischer [13]. The enzyme used in the present experiments was purified about 220-fold and showed 15—18 μmol NADH production/min per mg enzyme protein at 37°C when the enzyme was reactivated with liposomes from mitochondrial lipids. Rat liver microsomes were prepared according to the method of Okuyama and Lands [14], suspended in the buffered sucrose solution at a concentration of 20—30 mg protein per ml and stored at −80°C under N_2 gas. Rat erythrocyte plasma membranes were prepared from blood collected from the orbital sinus in about 0.1 vol. of 3.8% sodium citrate. The plasma membranes were isolated by using the method of Dodge et al. [15], suspended at a concentration of 25 mg protein per ml in 10 mM sodium/potassium phosphate buffer, pH 7.4, and stored as described above. Peripheral proteins were removed from erythorcyte membranes according to a modification of the method of Strapazon and Steck [16]. To 1 vol. of the membrane suspension, 10 vol. of 10 mM potassium phosphate buffer, pH 7.4, were added and the membranes were fragmented by sonication (Branson-185) at 60 W for 3 min at 0°C. The fragmented membrane suspension was adjusted to pH 9.0, 9.5, 10.0 and 10.25, respectively, and incubated at 0—4°C for 30 min. The membrane suspension was then centrifuged at 105 000 × *g* for 60 min, and the membranes were collected by centrifugation after one more washing with 0.25 M sucrose containing 5 mM Tris-HCl, pH 7.4.

Preparation of liposomes. The lipids extracted and purified from subcellular membranes by using the method of Folch et al. [17] were stored in a small known volume of $CHCl_3/CH_3OH$ (2 : 1, v/v) at −20°C. For preparation of liposomes, an aliquot of the lipid solution was separated and the solvent was removed under an N_2 gas stream, followed by overnight storage 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 and mixed with a vortex mixer for 5 min, and then sonicated for 2 h at 0—4°C with a bath-type sonifier (Branson B-220) under N_2. The sonicated sample was centrifuged at 105 000 × *g* for 40 min and the supernatant fraction was used as liposomes.

Analysis of membrane components. Lipid extracts were purified according to the method of Kates [18], dried under a stream of N_2 gas, 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 plates (0.5 mm thickness) and developed with chloroform/methanol/acetic acid/water (50 : 25 : 7 : 3, v/v) by using the method of Skipski et al. [19]. The spots visualized by exposing to iodine vapor were individually scraped into test tubes, and lipid phosphorus was determined by using the method of Lowry et al. [20]. Cholesterol was developed and separated by thin-layer chromatography with petroleum ether/diethyl ether/acetic acid (82 : 18 : 1, v/v) and the relative amounts were determined by scanning spots developed by 5% phosphomolybdate spray at 700 nm using a chromatoscanner (Shimadzu CS-900). Membrane proteins were analysed by SDS-polyacrylamide gele electrophoresis according to the method of Melnick et al. [21]. Densitometry of stained gels was carried out using a Gil-

ford spectrophotometer equipped with a linear transport accessory. Proteins were determined by a biuret method [22] or by using the method of Ross and Schatz [23], using bovine serum albumin as standard.

NAD^+, NADH, dithiothreitol, DL-β-hydroxybutyrate, sphingomyelin, EDTA, antimycin A and Trisma base were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Other chemicals were analytical grade (Nakarai Chem., Kyoto, Japan).

Results

D-β-Hydroxybutyrate dehydrogenase purified from beef heart mitiochondria in a lipid-free form shows little catalytic activity, but it is reactivated when it interacts with phosphatidylcholine-containing liposomes and certain native cellular membranes [5—9]. The present enzyme purified from rat liver mitochondria was incubated in increasing amounts of subcellular membranes and the interaction was monitored by the restoration of catalytic activity. Table I shows that the ractivation was most efficient with the mitochondrial inner membranes (post-submitochondrial membranes) from which the enzyme had been isolated. Liver microsomal membranes were also effective. The enzyme

TABLE I

SELECTIVITY OF REACTIVATION OF THE PURIFIED D-β-HYDROXYBUTYRATE DEHYDROGENASE WITH SUBCELLULAR MEMBRANES

The enzyme (20 μg proteins) was added to 0.15 ml of cold incubation medium containing 3 mM NAD^+, 0.5 mM dithiothreitol, 0.5 mM EDTA, 50 mM Tris-HCl, pH 8.1, and varied amounts of membranes as presented in the table, shaken briefly by hand, made up to 1.5 ml with the above incubation medium (37°C) and incubated at 37°C for 15 min. The reactivation was monitored by absorbance changes at 340 nm by adding 25 μl of 1.2 M DL-β-hydroxybutyrate (Final 20 mM) at 37°C. The enzyme activity was expressed by the specific activity of the added enzyme using an extinction coefficient of 6.22 $mM^{-1} \cdot cm^{-1}$. (a) The membrane fragments prepared by sonicating in a hypotonic solution as described in the text. (b) The membrane vesicles from which D-β-hydroxybutyrate dehydrogenase had been removed by treatment at pH 10.25.

Membranes	Membrane proteins (μg/μg enzyme protein)	Membrane (μg phosphorus/μg enzyme protein)	Specific activity (μmol NADH/min per mg enzyme protein)
None			0.070
(a) Erythrocyte plasma membranes	2.0	0.02	0.073
	4.5	0.05	0.076
	11.8	0.13	0.092
	18.9	0.20	0.092
Microsomal membranes	3.0	0.03	0.360
	6.0	0.05	0.562
	12.0	0.11	0.865
	18.0	0.16	1.020
	40.0	0.35	1.150
(b) Mitochondrial inner membranes	1.5	0.02	0.453
	3.3	0.04	0.799
	6.7	0.07	0.995
	9.3	0.10	1.106
	18.3	0.20	1.275

was not reactivated by erythrocyte plasma membrane fragments. Similar results were reported by McIntyre et al. [9] who prepared the enzyme from beef heart mitochondria and studied its interaction with sealed human erythrocyte ghosts.

There are two possible explanations for the remarkable inability of erythrocyte membranes to reactivate the enzyme: the enzyme may not bind, or it may bind and fail to be reactivated. To solve this question, erythrocyte plasma membranes (a mixture of inside-out and right-side-out membrane vesicles) were incubated at 37°C in the presebce or absence of the purified enzyme, collected by centrifugation and protein components were analysed by SDS-polyacrylamide gel electrophoresis (Fig. 1). As shown in the figure, no peak corresponding to the enzyme could be found in the polypeptide pattern of the membranes incubated with the enzyme, indicating that the enzyme was unable to bind to the membranes. This is consistent with a previous report by Gazzotti et al. [5]

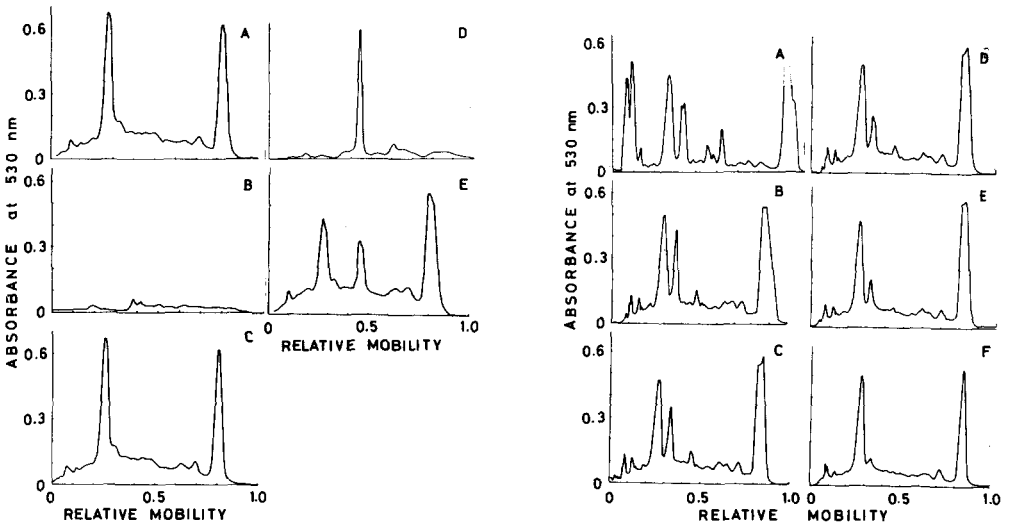


Fig. 1. No interaction of the purified D-β-hydroxybutyrate dehydrogenase with plasma membranes of rat erythrocyte. The purified enzyme (40 μg proteins) was added to 0.3 ml of cold incubation medium, as described in Table I, containing the plasma membrane fragments (300 μg proteins) after treating at pH 10.25 (see Figs. 2 and 3). The enzyme membrane suspension was shaken briefly by hand, made up to 3 ml with the above incubation medium (37°C) and incubated at 37°C for 30 min. After the incubation the membranes were collected by centrifugation at 105 000 × *g* for 60 min, and suspended with a small known amounts of 0.25 M sucrose containing 2 mM Tris-HCl, pH 7.4. For SDS-polyacrylamide gel electrophoresis, 17.5 μl of the membrane fraction (35 μg or equivalent to 35 μg proteins) were applied to the gel tube. For more details refer to the text. Relative mobility is expressed by the relative migration to the tracking dye. A, the plasma membranes after incubating in the presence of the enzyme; B, the precipitate fraction of the enzyme after incubating in the absence of membranes; C, the plasma membranes after incubating in the absence of the enzyme; D, the purified enzyme (4.1 μg proteins) used for the interaction experiments which was directly applied to the gel tube without incubation and centrifugation; E, the mixture of the membranes (31 μg proteins) and the enzyme (4.1 μg proteins) directly applied to the gel tube.

Fig. 2. Changes of membrane proteins in rat erythrocyte plasma membranes after treating at various pH values. Rat erythrocyte ghosts were sonicated, treated at various pH values and collected by centrifugation at 105 000 × *g* for 60 min. An aliquot (35 μg or equivalent to 35 μg proteins) of the collected membranes were applied to the gel tube. For more details see Fig. 1 and the text. A, rat erythrocyte ghosts (40 μg proteins), before sonication; B—F, the membranes after treating at pH 7.4, 9.0, 9.5, 10.0 and 10.25, respectively.

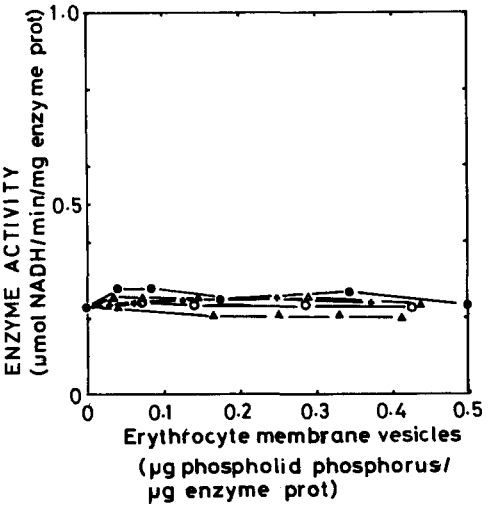


Fig. 3. No reactivation of the purified D-β-hydroxybutyrate dehydrogenase by erythrocyte plasma membranes from which membrane proteins were removed. The purified enzyme (20 μg proteins) was added to varied amounts of the modified membrane vesicles, and the raactivation was monitored as described in Table I. ●———●, the membranes treated at pH 7.4, 0.1 μg phosphorus = 7.24 μg proteins; +———+, the membranes treated at pH 9.0, 0.1 μg phosphorus = 7.04 μg proteins; ○———○, the membranes treated at pH 9.5, 0.1 μg phosphorus = 5.79 μg proteins; △———△, the membranes treated at pH 10.0, 0.1 μg phosphorus = 5.79 μg proteins; ▲———▲, the membranes treated at pH 10.25, 0.1 μg phosphorus = 5.88 μg proteins.

who demonstrated that the enzyme from beef heart mitochondria bonds only to liposomes which reactivate the enzyme. McIntyre et al. [9] also concluded that insertion of the enzyme is necessary for reactivation.

We next investigated which membrane components mediated the specificity of interaction. McIntyre et al. [9] reported that interaction of the purified enzyme is related to the phospholipid avialable on the membrane surface. Furthermore, removal of peripheral proteins from the plasma membranes increased the insertion efficiency. This led us to investigate possibility that certain proteins located at the erythrocyte membrane surface may inhibit interaction.

TABLE II

CHANGES IN MEMBRANE COMPONENTS OF RAT ERYTHROCYTE PLASMA MEMBRANES AFTER TREATING AT VARIOUS pH VALUES

Sonicated membrane fragments (4 mg proteins) were treated at various pH values as shown in the table and collected by centrifugation at 105 000 × *g* for 60 min. The changes in membrane components were determined as described in the text. The values express per cent changes from respective components of pH 7.4-treated and recovered membranes.

Treatment (pH)	% content in proteins	% content in phospholipids	% content in cholesterol
7.4	100	100	100
9.0	95.0	98.1	89.3
9.5	85.8	85.4	83.4
10.0	65.2	82.0	78.6
10.25	58.7	72.3	74.1

TABLE III

PHOSPHOLIPID CONTENTS IN ERYTHROCYTE MEMBRANES TREATED AT VARIOUS pH VALUES

Lipids from the treated membranes as in Table II were analysed. Lys, lysophosphatidylcholine; SM, sphingomyelin; PC, phosphatidylcholine; PI, phosphatidylinositol; PE, phosphatidylethanolamine.

Membranes treated at (pH)	Phospholipids(mol%)			
	Lys + SM	PC	PI	PE
7.4	7.0	30.0	21.6	41.1
9.0	9.4	33.4	19.9	37.2
9.5	8.9	31.3	20.8	38.9
10.0	11.5	35.3	17.5	35.7
10.25	11.0	32.7	18.3	38.0

Membrane proteins were selectively removed from the erythrocyte membranes according to a modification of the method of Strapazon and Steck [16], as described in Materials and Method. Fig. 2 shows SDS-polyacrylamide gel electrophoretic patterns of polypeptides in treated erythrocyte membranes. Bands 1 and 2 were removed by sonication (Fig. 2B), and increasing pH caused gradual removal of band 4 and other membrane proteins (Fig. 2C—E). At pH 10.25, most membrane proteins were removed with the exception of band 3 and small amounts of hemoglobin (Fig. 2F). However, the enzyme was reactivated neither by any of the protein-deficient membranes, nor by unsealed ghosts (Fig. 3). This suggests that peripheral proteins are not responsible for the lack of an enzyme-membrane interaction.

It was possible that alteration of membrane lipids might occur in high pH ranges and thereby affect our results. However, as shown in Tables II and III, the relative content of cholesterol and phospholipid species in the membranes did not show any marked variation after treatment at high pH.

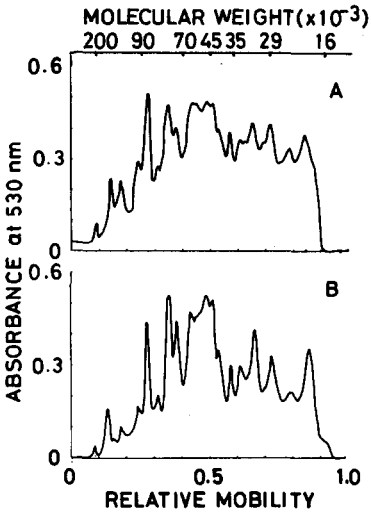
As a further check, microsomal membranes were treated in a similar manner to the erythrocyte membranes and reactivation experiments were performed. Table IV shows that total protein in the microsomal membranes decreased markedly, but that phospholipid content was only slightly altered. In Fig. 4

TABLE IV

CHANGES IN PROTEINS AND PHOSPHOLIPIDS IN RAT LIVER MICROSOMAL MEMBRANES AFTER TREATING AT VARIOUS pH VALUES

Microsomal membranes (4 mg proteins) were treated as described in Table II. The values show the relative contents of the pH 7.4-treated and recovered membranes.

Treatment pH	% content in proteins	% content in phospholipids
7.4	100	100
9.0	66.9	102
9.5	61.7	101
10.0	55.4	100
10.3	49.8	97.8



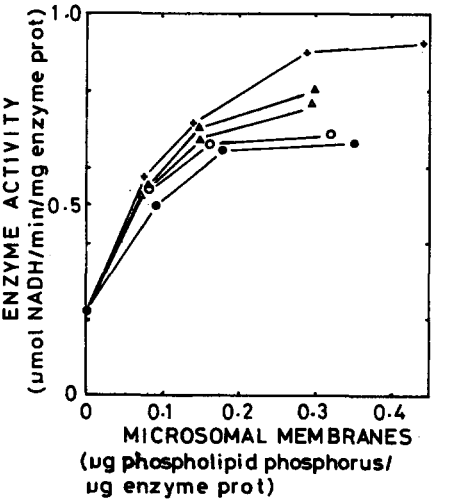


Fig. 4. Changes of membrane proteins in rat liver microsomal membranes after treating at pH 10.3. The microsomal membranes were treated in a manner similar to that in Fig. 2. For more details refer to Fig. 2 and the text. Molecular weight plotted in the figure was extrapolated by positions of polypeptides of erythrocyte membranes migrated in the simultaneous electrophoresis. The charged amounts were 17.5 µl (35 µg proteins for A and equivalent to 35 µg proteins for B). A, the membranes treated at pH 7.4; B, the membranes treated at pH 10.3.

Fig. 5. Increased efficiency of reactivation of D-β-hydroxybutyrate dehydrogenase by microsomal membranes from which membrane proteins were partially removed by treating at various pH values. The purified enzyme (20 µg proteins) was added to varied amounts of modified membranes and the reactivation was monitored as described in Table I. ●———●, the membranes treated at pH 7.4, 0.1 µg phosphorus = 4.71 µg proteins; ○———○, the membranes treated at pH 9.0, 0.1 µg phosphorus = 4.18 µg proteins; △———△, the membranes treated at pH 9.5, 0.1 µg phosphorus = 3.91 µg proteins; ▲———▲, the membranes treated at pH 10.0, 0.1 µg phosphorus = 3.56 µg proteins; +———+, the membranes treated at pH 10.3, 0.1 µg phosphorus = 3.42 µg proteins.

TABLE V

SELECTIVITY OF REACTIVATION OF THE PURIFIED D-β-HYDROXYBUTYRATE DEHYDROGENASE BY LIPOSOMES FROM LIPID EXTRACTS OF DIFFERENT SUBCELLULAR MEMBRANES

The purified enzyme (20 µg proteins) was added to varied amounts of liposomes prepared by total lipid extracts from different subcellular membranes and the estimation of enzyme reactivation was carried out as described in Table I.

Lipid origin	Liposomes (µg phospholipid phosphorus/µg enzyme protein)	Specific activity (µmol NADH/min per mg enzyme protein)
Erythrocyte plasma membranes	0	0.200
	0.5	0.083
	1.0	0.033
	2.0	0.015
Microsomal membranes	0.5	1.917
	1.0	3.083
	2.0	4.500
	3.0	5.667
Mitochondrial inner membranes	0.5	5.000
	1.0	7.583
	2.0	12.833
	3.0	14.000

the polypeptide patterns from membranes treated at pH 10.3 were compared with those of control membranes. No specific disappearance of polypeptides was observed. Reactivation of the enzyme by these membranes is shown in Fig. 5. In contrast to the erythrocyte membranes, restoration of enzyme acrivity was more efficient with protein-depleted microsomal membranes. These results suggest that the interaction of exogenously added D-β-hydroxybutyrate dehydrogenase with intracellular membranes does not depend directly on proteins exposed at the membrane surface, but rather on the specific lipid composition or arrangement in the membrane structure. This was confirmed by preparing liposomes from total lipids of individual subcellular membranes and measuring the efficiency of enzyme reactivation in each system (Table V). The data indicate that liposomes composed of erythrocyte lipids did not reacitivate the enzyme, but the liposomes from lipids of mitochondrial inner membranes and microsomal membranes markedly reacivated the enzyme, with relative efficiencies similar to those of native subcellular membranes. We conclude that the interaction of exogenously added D-β-hydroxybutyrate dehydrogenase is largely dependent on lipids at the acceptor membranes.

Discussion

In this study, our primary concern was to determine which components of pre-existing membranes play significant roles in directing the incorporation and reactivation of a membrane enzyme. A newly synthesized polypeptide may interact either with the lipid environment or with surface proteins of a target membrane, and with the D-β-hydroxybutyrate dehydrogenase system it was possible to determine which of these two components is more important. Clearly, in vivo processing of this enzyme cannot be precisely modeled, since in the cell an inward transport also occurs prior to localization of the enzyme on the inner mitochondrial membrane. Our discussion is therefore limited to the actual steps of incorporation and reactivation.

D-β-Hydroxybutyrate dehydrogenase purified in a lipid-free form is inactive and requires phosphatidylcholine for the restoration of catalytic activity [5]. Optimal reactivation is provided by dioleoyl phosphatidylcholine for the beef heart mitochondial enzyme [5] and didecanoyl phosphatidylcholine for the rat liver mitochondrial enzyme [6]. In the present study, we found that restoration of enzyme activity is also characteristic for the mixture of lipids found in natural membranes. Post-submitochondrial and microsomal membranes reactivated the enzyme efficiently but erythrocyte plasma membranes neither accepted or activated the enzyme. We also found that the purified enzyme was not accepted by rat erythrocyte plasma membranes from which most of the peripheral proteins had been removed, whereas microsomal membranes treated in a similar manner increased the efficiency of reactivation. These results suggest that protein at a membrane surface may have a minor effect on the interaction mechanism, but that lipid is the controlling factor.

These studies were repeated in experiments with liposomes prepared from lipid extracts of the membranes with similar results. We conclude that the lipid environment of erythrocyte membranes differs from that of microsomal and mitochondrial inner membranes, and that the interaction of the exoge-

nously added enzyme with the membranes is dependent on a specific lipid composition or on the arrangement of lipid molecules in individual intracellular membranes. When the lipid composition was compared in these three membranes, lysophosphatidylcholine, sphingomyelin and cholesterol were relatively high in erythrocyte membranes. We found that sphingomyelon and cholesterol had little effect, but that lysophosphatidylcholine inhibited reactivation [24].

Although lipid apparently dominates the reactivation process, protein composition also plays a role. For instance, McIntyre et al. [9] studied the interaction of the beef heart mitochondrial enzyme with reconstituted sarcoplasmic reticulum membranes in which the lipid content was varied by deoxycholate treatment and subsequent supplements of phospholipids and found that reactivation efficiency increased as protein content decreased. In the present paper, membrane proteins were directly removed without altering membrane lipids. The efficiency of reactivation by microsomal membranes increased as membrane protein was depleted.

A potentially significant result is that enzyme reactivation by liposomes was about 10-fold more efficient than in native membranes. Singer [25] has recently reported a decreased insertion of amphiles into biological membranes compared with liposomes, and suggested that a substantial internal pressure exists in biological membranes which tends to exclude amphipathic compounds. Similar forces may inhibit insertion of hydrophobic moieties of polypeptides, and it will be interesting to determine whether these contribute to the specific interaction of D-β-hydroxybutyrate dehydrogenase with native membranes.

References

1 Maccecchini, M.-L., Rudin, Y., Blobel, G. and Schatz, G. (1979) Proc. Natl. Acad. Sci. U.S.A. 76, 343—347
2 Maccecchini, M.-L., Rudin, Y. and Schatz, G. (1979) J. Biol. Chem. 254, 7468—7471
3 Zitomer, R.S. and Hall, B.D. (1976) J. Biol. Chem. 251, 6320—6326
4 Eytan, D.G. and Racker, E. (1977) J. Biol. Chem. 252, 3208—3212
5 Gazzotti, P., Bock, H.-G. and Fleischer, S. (1975) J. Biol. Chem. 250, 5782—5790
6 Vidal, J.C., Guglielmucci, E. and Stoppani, A.O.M. (1978) Arch. Biochem. Biophys. 187, 138—152
7 Miyahara, M. and Deamer, D.W. (1977) Proc. 3rd Japan Bioenergetics Congress, pp. 23—25, Osaka Univ., Japan
8 McIntyre, J.O., Wang, C.-T. and Fleischer, S. (1977) Fed. Proc. 36, 707a
9 McIntyre, J.O., Wang, C.-T. and Fleischer, S. (1979) J. Biol. Chem. 254, 5199—5207
10 Higashi, H., Richards, C.S. and Uyeda, K. (1979) J. Biol. Chem. 254, 9542—9550
11 Hogeboom, G.H. (1955) Methods Enzymol. 1, 16—19
12 Miyahara, M., Utsumi, K. and Deamer, D.W. (1980) Arch. Biochem. Biophys., submitted
13 Nielsen, N.C. and Fleischer, S. (1973) J. Biol. Chem. 248, 2549—2555
14 Okyyama, H. and Lands, W.E.M. (1969) J. Biol. Chem. 244, 6514—6519
15 Dodge, J.T., Mitchell, C. and Hanahan, D.J. (1963) Arch. Biochem. Biophys. 100, 119—130
16 Strapazon, E. and Steck, T.L. (1977) Biochemistry 13, 2966—2971
17 Folch, J., Lees, M. and Stanley, G.H.S. (1957) J. Biol. Chem. 226, 469—509
18 Kates, M. (1972) in Techniques of Lipidology (Work, T.S., ed.), pp. 351—352, North-Holland, Amsterdam
19 Skipski, V.P., Peterson, R.F., Sanders, J. and Barclay, M. (1963) J. Lipid Res. 4, 227—228
20 Lowry, O.H., Roberts, N.R., Leiner, K.Y., Wu, M.-L. and Farr, A.L. (1954) J. Biol. Chem. 207, 1—17
21 Melnick, R.L., Tinberg, H.M., Maquire, J. and Packer, L. (1973) Biochim. Biophys. Acta 311, 230—241
22 Gornall, A.G., Bardawill, C.S. and David, M.M. (1949) J. Biol. Chem. 177, 751—766
23 Ross, E. and Schatz, G. (1973) Anal. Biochem. 54, 304—306
24 Miyahara, M., Nishihara, Y., Moromizato, Y. and Utsumi, K. (1981) Biochim. Biophys. Acta 641, 232—241
25 Singer, S.J. (1979) Proc. Natl. Acad. Sci. U.S.A. 76, 5202—5206