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FRAGMENTATION BY DETERGENTS OF THE RESPIRATORY CHAIN OF *MICROCOCCUS LYSODEIKTICUS* MEMBRANES

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SUMMARY

The respiratory chain of *Micrococcus lysodeikticus* membranes can be disrupted by detergents into two blocks, one of which contains malate and NADH dehydrogenases with cytochrome b_{556} and the other cytochromes b_{560}, c_{550} and a_{601}. These blocks maintain their functional activity, exhibiting various degrees of bonding within the membrane. Hydrophobic interactions being weakened, the substrate portion of the chain is solubilized more readily than the terminal one.

INTRODUCTION

Studies on the fragmentation of biological membranes by detergents and solvents, as well as water, salts, chelating agents and urea, give evidence that hydrophobic interactions play a major role in the stabilization of biological membranes[1,2]. This conclusion finds support in the data on a high content of non-polar amino acids in membrane proteins[3,4]. The concept that hydrophobic interactions are important in maintaining the structural organization of biological membranes is as yet too general and needs to be specified in application to various types of membrane and particularly to the bonding of components in the membrane.

Bearing this in mind, we have made on attempt to evaluate the role of hydrophobic interactions in the arrangement of respiratory chain components of *M. lysodeikticus* membranes, regarding them as markers whose bonding in the membrane and capacity for interaction may help assess both the forces stabilizing the respiratory chain as such and its interactions with adjacent components of the membrane.

Direct methods of measuring the value of hydrophobic interactions in the membrane being unavailable, we have tried to assess them indirectly, investigating the detergent fragmentation of the membrane and properties of the fragments obtained. With this purpose, membranes of *M. lysodeikticus* were treated with Tween-80, sodium cholate, sodium deoxycholate, Triton X-100 and sodium dodecylsulphate. The fragmentation of membranes was evaluated by the distribution of fragments during centrifugation as well as by their content, and the interactions within them, of

Abbreviations: TMPD, tetramethyl *p*-phenylenediamine; DCIP, 2,6-dichlorophenolindophenol.

marker proteins, *i.e.* cytochromes α_{601}, b_{556}, b_{560}, c_{550}, malate and NADH dehydrogenases. In addition to these components constituting not more than 10–15 % of total proteins in the membrane, the content of total protein was measured, and lutein was assayed to serve as a marker of the lipid phase.

METHODS

24-h cultures of *M. lysodeikticus*, Flemming strain, were used. Bacteria were grown in the medium containing beef-extract broth in erlenmeyer flasks at 37° with shaking. By the time of harvesting, bacteria were in the stationary growth phase; they were harvested by centrifugation, washed and stored at —4° not longer than 10–12 days. The bacteria were lysed, and all other procedures were performed in 0.04 M Tris–HCl buffer (pH 7.4), containing $1 \cdot 10^{-3}$ M $MgSO_4$. Lysis was carried out at 37° for 30 min in the medium containing 10 ml buffer per g of cell biomass (humidity 75 %), 1 mg lysozyme and 0.5 mg deoxyribonuclease. Membranes were collected from the lysate by centrifugation at 22000 × *g* for 30 min and twice washed from the cytoplasm. This resulted in a mixture of mesosomal and cytoplasmic membranes whose electron micrographs were published previously[1].

The membranes were homogenized in the buffer by a teflon pestle to obtain a homogeneous suspension. To the suspension of membranes containing 2–2.5 mg protein per ml and aliquot was added of 2 % (or 0.8 % of dodecyl sulphate) of the detergent solution prepared in the buffer. The mixture was incubated for 30 min at 25° (2 % of the deoxycholate solution (pH 7.8)), and the suspension was centrifuged at 22000 × *g* for 30 min; the resultant supernatent fraction was centrifuged at 144000 × *g* for 60 min. Thus, the fraction of large particles (22000 × *g*), the fraction of small particles (144000 × *g*) and that of solubilized material (144000 × *g* supernatant) were obtained. In some experiments 144000 × *g* particles were derived directly, the centrifugation at 22000 × *g* being omitted. In all fractions the activity of oxidases and dehydrogenases was determined, and concentrations of cytochromes, lutein and protein were measured.

NADH oxidase and malate oxidase. Malate and NADH oxidation were determined polarographically on a closed platinum electrode in a cell specially designed by SCHOLTZ AND OSTROVSKY[5]. Tris buffer (1.2 ml) contained malate (3 mM) or NADH (0.8 mM, obtained from Calbiochem Co.) and membranes or their fragments amounting to 0.3–1.0 mg protein per ml of the sample. The activity of malate and NADH oxidases is expressed as μmoles O_2 consumed per min per mg protein.

Ascorbate oxidase. The activity of the cytochrome oxidase fragment of the electron transport chain (c---a---O_2) was measured polarographically, using ascorbate (3 mM) as electron donor in the presence of 0.1 mM tetramethyl *p*-phenylenediamine (TMPD)

Malate and NADH dehydrogenases were measured by the reduction of 2,6-dichlorophenolindophenol (DCIP) in a SP-4A recording spectrophotometer. The medium contained DCIP (0.4 mM), malate (0.5 mM) or NADH (0.8 mM) and Tris–Mg^{2+} buffer. The final volume was 4 ml. The protein concentration varied from 0.005 to 0.03 mg protein per ml. The dehydrogenase activity is expressed in μmoles substrate oxidized per min per mg protein.

Cytochrome assay at room temperature was performed in a DSP-2 difference

spectrophotometer. The content of cytochromes a_{601}, $b_{556} + b_{560}$, c_{550} was calculated according to the method of LISENKOVA AND MOKHOVA[6] and MOKHOVA[7]. The wavelengths used for determination of the absorbance (ΔA) of the above cytochromes as well as the coefficients of millimolar extinction were published previously[1]. For chemical reduction of membrane cytochromes, dithionite at a concentration of 1 mg/ml was added. In the presence of dithionite, cytochromes *a* and *c* were completely reduced within 1–2 min, whereas cytochromes *b*, as determined by their total peak, were reduced only within 5–7 min. 0.5 mM menaquinone (menadione, vitamin K_3) accelerated the complete reduction of cytochromes $b_{556} + b_{560}$. Therefore, in all subsequent experiments that required complete chemical reduction the membrane suspension was supplemented with dithionite and menaquinone added in ethanol, the final ethanol concentration being 1 %.

The substrate reduction of cytochromes was recorded using malate (5 mM), NADH (2 mM) or ascorbate (3 mM) in the presence of TPMD (0.1 mM).

Cytochrome assay at liquid nitrogen temperature was conducted in a low-temperature difference spectrophotometer[8]. Procedures of the assay using a low-temperature spectrophotometer were described in detail previously[9].

Protein content was determined according to LOWRY *et al.*[10] and the calibration curve was plotted using crystalline bovine albumin (Koch-Light).

Lutein was measured on a DSP-2 spectrophotometer. Fig. 1. shows that the spectrum of lutein in the membrane suspension has three absorption peaks (480, 455 and 425 nm). However, the lutein content was determined using only the first two peaks (480 and 455 nm), since the value of the 425-nm peak depends on the contribution of the cytochrome γ-band. Lutein was calculated from the following three points of the spectrum: 500, 480 and 470 nm or 470, 455 and 440 nm according to the procedure used for measuring cytochromes in aqueous suspensions[6,7].

The experiments were carried out using sodium cholate, Tween-80, sodium

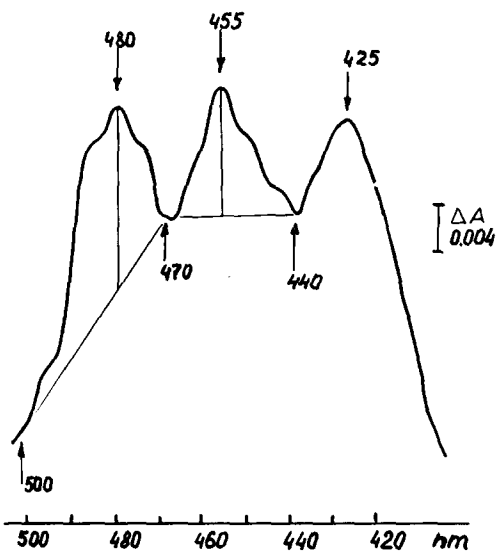

Fig. 1. Lutein spectrum in membrane suspension. Protein concentration 0.2 mg/ml; optical path 1cm; comparison cuvette contained Tris buffer.

TABLE I

DISTRIBUTION OF PROTEIN AND LUTEIN DURING DETERGENT FRAGMENTATION OF MEMBRANES

The table presents average values derived from 3–4 tests with each detergent. The numbers in parentheses show the lower and upper limits of the data scattering. Distribution of protein and lutein is expressed as percentage of protein and lutein in parent membranes.

Fraction	*Detergent*									
	Tween-80 (1 %)		*Sodium cholate* (1 %)		*Sodium deoxycholate* (1 %)		*Triton X-100* (1 %)		*Dodecylsulphate* (0.4%)	
	Protein	*Lutein*	*Protein*	*Lutein*	*Protein*	*Lutein*	*Protein*	*Lutein*	*Protein*	*Lutein*
Particles (22000 × *g*, 30 min)	56 (50–62)	97	40 (23–50)	55	8	0	5	0	0	0
Particles (144000 × *g*, 60 min)	5	2	11 (05–16)	37	10	0	11	1	0	0
Supernatant (144000 × *g*, 60 min)	31 (25–38)	5	50 (41–59)	18	80	100	81	93	100	100

dodecyl sulphate and Triton X-100 obtained from the Schuchardt Co. and deoxycholic acid obtained from the Merck Co.

RESULTS

Summarized data on the fragmentation of *M. lysodeikticus* membranes under the influence of different detergents are presented in Table I which gives numerical values of the distribution of protein and lutein among large particles (22000 × *g*), small particles (144000 × *g*) and supernatant fraction (144000 × *g*).

While the accumulated data were being analyzed, certain peculiarities of the effect of various detergents on the membrane were found. During the procedures employed the detergents tested solubilized the membrane in a different manner. Thus, after treatment of the membrane with Tween-80 the pellets contained 70 % of protein, *i.e.* this detergent solubilized 30 % of the protein whereas cholate solubilized about 50 %. This degree of membrane solubilization in Tween-80 and cholate is maximal, since repeated extraction with Tween-80 as well as 3 % cholate resulted in no additional protein yield. In comparison with Tween-80 and cholate three other detergents affected the membrane in a more aggressive way, solubilizing all (dodecyl sulphate) or nearly all (80 % in deoxycholate, Triton X-100) the membrane protein. Therefore, the detergents tested may be arranged in a sequence where the mildest solubilizing agent is Tween-80; moderate, cholate; strong, Triton X-100 and deoxycholate; and, most effective, dodecyl sulphate.

Solubilization of membranes with detergents of different strengths results in the formation of fragments that differ in the protein: lutein ratio. Thus, Tween-80, mildly affecting the membrane, extracts from it approximately one-third of lutein-free protein. On the other hand, 144000 × *g* particles, remaining after membrane solubilization with Triton X-100 or deoxycholate, contain approx. 20 % of lutein-free protein.

Since lutein may act as a marker of the lipid distribution[11], a difference in the protein: lutein ratio in membrane fragments may be indicative of differences in the binding degree of proteins and lipids within the membrane which can be detected upon treatment with various detergents. Without going into details of the different effects of detergents on the membrane, it should be noted that all detergents split membranes mainly into fragments that cannot be sedimented at 144000 × *g*, the proportion of small particles (144000 × *g* pellet) being insignificant.

In order to give a more detailed characterization of weakly and firmly fixed membrane proteins, we have attempted to elucidate the effect of the above detergents on the respiratory chain enzymes. In this connection, functional disturbances in the respiratory chain of detergent-treated membranes were examined first. Membrane treatment with Triton X-100, cholate or deoxycholate inactivated malate and NADH oxidases, whereas Tween-80 caused practically no changes in the function of these systems (Table II).

TABLE II

EFFECT OF DETERGENTS ON THE RESPIRATORY CHAIN ENZYMES OF MEMBRANES

Specific activity of the enzymes in membranes before the treatment was as follows: oxidases (μmoles O_2 absorbed/min per mg protein), malate 0.21, NADH 0.17, ascorbate, TMPD 0.11; dehydrogenases (μmoles substrate oxidized/min per mg protein), malate 0.23, NADH 0.45.

Enzyme	*Detergent*			
	Tween-80	*Sodium cholate*	*Triton X-100*	*Sodium deoxycholate*
	Activity as percentage of the initial activity			
Malate oxidase	100	9	7	0
NADH oxidase	85	–	14	10
Ascorbate, TMPD oxidase	91	108	100	57
Malate dehydrogenase	75	130	183	8
NADH dehydrogenase	180	360	180	45

Deoxycholate fragmentation of membranes was accompanied by partial or complete inactivation of the dehydrogenase and cytochrome oxidase portions of the chain. However, inhibition of the respiratory chain of membranes incubated with cholate or Triton X-100 was not associated with inactivation of dehydrogenases whose activity (malate and NADH dehydrogenases) increased. It was beyond our purposes to analyze the causes of this activation but such phenomena have been described and interpreted elsewhere[12]. Inhibition of malate and NADH oxidases by Triton X-100 and cholate involved no inactivation of the cytochrome oxidase segment of the chain. This suggests that the above detergents damaged the respiratory chain between the substrate and terminal portions.

The site of the damage of the respiratory chain was found by comparing cytochromes of intact and Triton X-100- or cholate-treated membranes. With each detergent similar results were obtained; therefore, only the data concerning the Triton X-100 effect are presented here (Fig. 2). The quantity and complement of cytochromes coincide in the intact and detergent-treated material which is confirmed by the spectra of dithionite-reduced preparations. Ascorbate spectra and ascorbate oxidase data

(Table II) suggest that electron transport in the cytochrome oxidase segment remains undisturbed. However, the spectrum of cytochrome reduction by malate and NADH in Triton X-100 membranes is significantly changed, and at room temperature instead of the usual complement of *b*-, *c*- and *a* -type cytochromes only one component is reduced which absorbs in the range of *b*-type cytochromes (Fig. 2A). Low-temperature spectrophotometry of Triton X-100 membranes has demonstrated that malate reduces only one of the two *b*-type cytochromes: cytochrome b_{556} (Fig. 2B). The occurrence in intact *M. lysodeikticus* membranes of two cytochromes *b* namely b_{556} and b_{560} in low-temperature spectra has previously been reported[9].

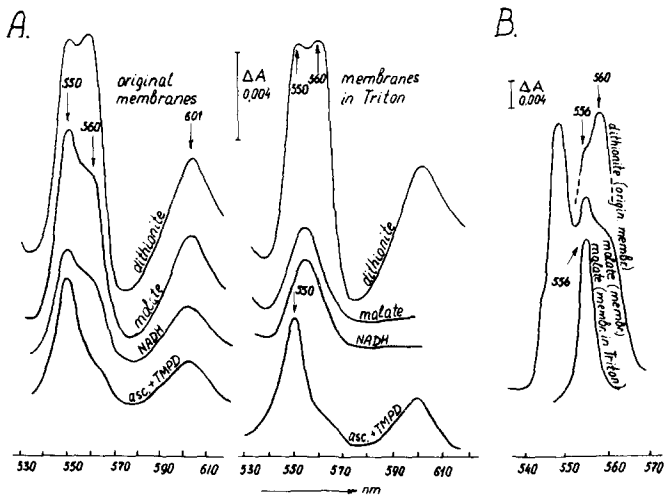

Fig. 2. Cytochromes of membranes before and after Triton X-100 treatment. A at 20°; B at −170°. Protein concentration 1 mg/ml.

Both cytochromes in intact membranes are uniformly reduced by malate (Fig. 2B); this suggests that the contribution of each cytochrome to the total peak ($b_{556} + b_{560}$) in the spectra recorded at 20° is the same. The fact that upon malate reduction the peak b_{556} value in the detergent-treated material constitutes half the total peak of cytochromes *b* in intact membranes (Fig. 2, Table V) suggests that in Triton X-100- or cholate-treated membranes all the cytochrome b_{556} is reduced quantitatively. This implies that the site of malate oxidase inactivation by these detergents lies between cytochromes b_{556} and b_{560}. Similarly, the block in NADH oxidase also reflects an inactivation at a point located on the O_2 side of b_{556}. Deoxycholate also inactivates a given locus of the respiratory chain since NADH reduces only one cytochrome b_{556} in the detergent-treated membranes. Dodecyl sulphate disrupted membrane cytochromes, thus being unsuitable for our purposes. This phenomenon has been described for cytochromes elsewhere[13].

One possible cause underlying the detergent inactivation of the respiratory chain may be spatial uncoupling of its components. To verify this idea, the detergent material was centrifuged at 144000 × *g* for 60 min and the distribution of dehydrogenases and cytochrome oxidase in the pellet and supernatant was analyzed (Table III).

As expected, the particles remaining after Tween-80 treatment of membranes contain all enzymes of the respiratory chain, only NADH dehydrogenase showing par-

TABLE III

DISTRIBUTION OF DEHYDROGENASES AND ASCORBATE OXIDASE DURING DETERGENT FRAGMENTATION OF MEMBRANES

Each detergent was used in 3–4 tests, the table giving the results of one of them. Particles and the supernatant were obtained at 144000 × *g* for 60 min. The enzyme activity of each fraction is expressed as specific activity × mg protein of the fraction. 100 mg protein of membranes were used in the fragmentation. The malate dehydrogenase activity of 100 mg parent membranes was 23 μmoles oxidized substrate per min; the NADH dehydrogenase activity, 45 and ascorbate oxidase, 11. The activity over these values reflects detergent-induced stimulation while the rate below these values shows inhibition.

Enzyme	*Detergent (activity per fraction)*							
	Tween-80		*Sodium cholate*		*Triton X-100*		*Sodium deoxycholate*	
	Particles	*Supernatant*	*Particles*	*Supernatant*	*Particles*	*Supernatant*	*Particles*	*Supernatant*
Malate dehydrogenase	26	1	3	18	0	59	0	1
NADH dehydrogenase	43	12	–	—	0	49	0	12
Ascorbate oxidase	10	0	10	1	6	5	2	3

tial solubilization. On the contrary, cholate splits the respiratory chain very specifically, solubilizing malate and NADH dehydrogenases and leaving active cytochrome oxidase in the particulate pellet. Triton X-100, producing a very strong solubilization of membranes, transfers to the supernatant not only malate and NADH dehydrogenases but also a portion of the membranes' cytochrome oxidase. Nevertheless, about half the cytochrome oxidase remains in the particles which preserve only 10–15 % of the total protein of the membrane. Sodium deoxycholate solubilizes enzymes of the chain similarly to the Triton X-100 effect but the enzyme activity is inhibited completely or partially. Distribution of cytochromes during detergent fragmentation of membranes is indicated in Table IV.

The data show that cytochromes are bound within membranes by relatively strong hydrophobic interactions that cannot be destroyed by every detergent. The

TABLE IV

CYTOCHROME DISTRIBUTION DURING DETERGENT FRAGMENTATION OF MEMBRANES

The content of cytochromes in the fractions is expressed in μmoles × 10^{-3} in the given fraction and calculated from difference spectra of the dithionite-reduced preparation against the oxidized. Other conditions are described in Table II.

Cytochrome	*Detergent*							
	Tween-80		*Sodium cholate*		*Triton X-100*		*Sodium deoxycholate*	
	Particles	*Supernatant*	*Particles*	*Supernatant*	*Particles*	*Supernatant*	*Particles*	*Supernatant*
$b_{556} + b_{560}$	58	0	41	7	18	40	11	38
c_{550}	57	0	37	5	19	19	12	33
a_{601}	22	0	15	3	8	6	6	14

strength of these interactions is indicated by the observation that Tween-80 treatment fails to extract cytochromes from the membranes though it solubilizes approx. 30 % of the proteins. Cholate solubilizes an insignificant portion of the cytochromes but completely extracts dehydrogenase proteins and 50 % of the total protein (Table II).

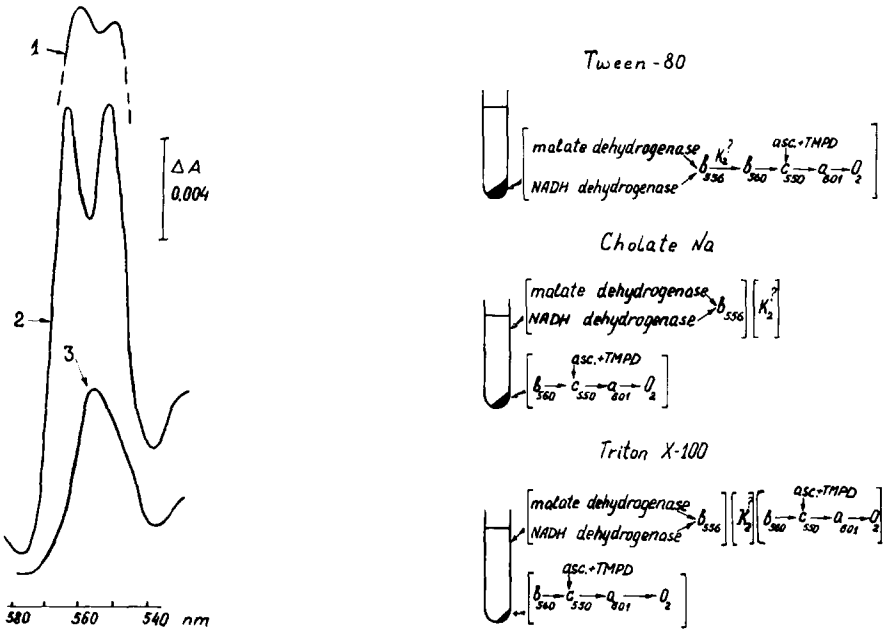

Fig. 3. Cytochromes of membrane fractions after Triton X-100 treatment. 1, membranes in Triton X-100; 2, pellet at 144000 × *g* for 60 min; 3, supernatant at 144000 × *g* for 60 min. Dithionite reduction; protein concentration in the cuvette 0.7 mg/ml.

Fig. 4. Detergent fragmentation of the respiratory chain of *M. lysodeikticus* membranes.

TABLE V

SUBSTRATE REDUCTION OF CYTOCHROMES $b_{556} + b_{560}$ IN MEMBRANE FRAGMENTS

The amount of substrate-reduced cytochromes $b_{556} + b_{560}$ is expressed in μmoles × 10^{-3} per fraction and calculated from the difference spectra (reduction − oxidation) recorded at 20°.

Detergent	*Substrate*	*Cytochromes* $b_{556} + b_{560}$			
		Membranes before treatment (100 mg protein)	*Membranes + detergent*	*144000 × g, 60 min*	
				Particles	*Supernatant*
Tween-80	Malate	—	35	34	0
	NADH	25	27	14	0
Sodium cholate	Malate	23	11	0	10
	NADH	19	9	0	8
Triton X-100	Malate	24	12	0	14
	NADH	16	14	0	16
Sodium deoxycholate	Malate	23	0	0	0
	NADH	10	13	0	6

Deoxycholate and Triton X-100 solubilizes 50–80 % of the membrane cytochromes. Particles retain not more than 20 % of the cytochrome proteins which are not associated with lutein and contain no malate dehydrogenase. These particulate fractions are rich in cytochromes whose specific concentration increases 4–6 times; cytochrome a_{601}, 1.2; cytochrome $b_{556} + b_{560}$, 3.4; cytochrome c_{550}, 3.2 μmoles $\times 10^{-3}$/mg protein. The distribution pattern of cytochromes of Triton X-100 membranes between the pellet and supernatant (144000 $\times$ *g*) gives evidence that during membrane solubilization *b*-type cytochromes behave in a different manner (Fig. 3). The short-wavelength cytochrome *b* undergoes solubilization more readily than the long-wavelength component whose behaviour is more like that of cytochromes *c* and *a*. The relative readiness with which cytochrome b_{556} undergoes solubilization can probably be attributed to its being a constituent of the dehydrogenase complex (Table V). The isolation procedure and certain properties of the malate dehydrogenase–cytochrome b_{556} complex have been described previously[14].

The solubilized complex retains its capacity of reducing cytochrome b_{556} by substrate electrons. During deoxycholate fragmentation malate dehydrogenase activity disappears and no reduction of cytochrome b_{556} occurs.

DISCUSSION

The experimental results obtained from detergent fragmentation of the respiratory chain of *M. lysodeikticus* membranes may be discussed from the point of view of the role of hydrophobic forces in binding respiratory chain components.

Fig. 4 gives a schematic illustration of the distribution of the functionally active segments of the *M. lysodeikticus* respiratory chain during fragmentation with different detergents.

As shown by the Triton X-100 and cholate fragmentation of membranes, the respiratory chain is built into the membrane so that in response to the detergent-induced weakening of hydrophobic interactions it splits off into two fragments one of which carries malate and NADH dehydrogenases and cytochrome b_{556}, and the other has cytochromes b_{560}, *c* and cytochrome oxidase. Obviously it cannot be ruled out that such fragmentation is accompanied by solubilization of some weakly-bound components, *e.g.* vitamin K_2 (ref. 15). Our findings make it doubtful that vitamin K_2 is localized in the site preceding that of cytochrome b_{556}. It seems rather difficult to reconcile the two contradictory observations made during detergent treatment: on the one hand maintenance of the functioning dehydrogenase b_{556} fragment, and on the other, solubilization of vitamin K_2 producing inactivation of the respiratory chain[15].

Thus, forces of hydrophobic binding are different in various segments of the respiratory chain: they are weakest in the locus between cytochromes b_{556} and b_{560}. As judged by the activity maintained, almost complete solubilization of the membrane, caused for instance by Triton X-100 does not result in spatial uncoupling of other components of the chain in the substrate and terminal portions. It is obvious that cytochromes b_{560}, c_{550} and a_{601} are bound by strong hydrophobic interactions that keep them together and cannot be loosened by detergents in our experiments. This is also true for the complex of malate and NADH dehydrogenases with cytochrome b_{556}.

It seems very important that after the Triton X-100 and deoxycholate treatment, which causes solubilization of approx. 80 % of the membrane protein, the parti-

cles retain about 50 % of cytochrome oxidase, its specific activity being sharply increased. Very likely cytochromes remain in this fragment of the respiratory chain owing to protein–protein hydrophobic interactions.

Thus, the functional criterion (relationship of enzymes) suggests that the respiratory chain of *M. lysodeikticus* is composed of two main blocks that can be readily separated in the site between cytochromes b_{556} and b_{560}, if hydrophobic interactions are weakened under the influence of a detergent. A major role in the stabilization of these blocks is played by protein–protein interactions. The data so far accumulated are insufficient to determine the size of the blocks, their protein and lipid composition. Further investigation of these segments of the respiratory chain should involve purification of the preparations required to identify the composition of the smallest functioning fragment of the respiratory chain.

The substrate and terminal portions of the respiratory chain differ not only in the composition of their components but also in the degree of binding in the membrane. As follows from detergent-induced solubilization, the substrate block involving dehydrogenases and cytochrome b_{556} is fixed less firmly than the terminal block involving cytochromes b_{560}, c_{550} and a_{601}. These data cannot yet be interpreted explicitly since different binding of the above enzyme complexes within the membrane may be associated with peculiar properties of the proteins involved or its molecular environment. Neither of the two assumptions can be given preference at this stage of research. Experiments with the membrane fragmentation by Tween-80 yield some data on the relationships between proteins of the respiratory chain and other proteins of the membrane. The treatment removes about 30 % of the protein containing no respiratory chain components. This indicates that at least one-third of the volume of the membrane is empty with respect to the respiratory chain; proteins of these empty sites are not in contact with the respiratory chain and are bound with membranes less strongly than proteins of the respiratory chain.

Even though the respiratory chain of *M. lysodeikticus* is constructed from two blocks that differ in their enzymic composition and binding strengths within the membrane, it does not yet mean that the whole membrane is made of blocks. However, it may be supposed that the structural organization of the membranes carrying multienzyme systems, *e.g.* the respiratory chain, is to meet the requirements of assembling such systems. This justifies the existence of firm blocks of protein–protein or lipid–protein nature in these membranes. Unlike this, membranes devoid of multi-enzyme systems, for instance mycoplasma membranes, contain no firmly-bound adjacent portions because they are functionally unnecessary. This suggestion is confirmed by detergent fragmentation of membranes of both types. Triton X-100 or deoxycholate fragmentation of membranes of mitochondria, chloroplasts and *Azotobacter vinelandii* results in the isolation of functioning fragments of the electron transfer chain[16–18]. The treatment of mycoplasma or plasma membranes with the same detergents used in similar concentrations produces no lipoprotein complexes, yielding instead a mixture of individual proteins and lipids[19,20].

REFERENCES

1 I. M. SIMAKOVA, M. A. LUKOYANOVA, V. I. BIRYUZOVA AND N. S. GEL'MAN, *Biokhimiya*, 34 (1969) 6.
2 N. S. GEL'MAN, *Usp. Sovrem. Biol.*, 68, I(4) (1969) 3.
3 F. HATCH AND A. BRUCE, *Nature*, 218 (1968) 1166.

4 I. G. ZHUKOVA, D. N. OSTROVSKY, N. S. GEL'MAN AND A. I. OPARIN, *Biokhimiya*, 31, 6 (1969) 1209.
5 H. F. SCHOLTZ AND D. N. OSTROVSKY, *Lab. Delo*, 6 (1965) 375.
6 L. L. LISENKOVA AND E. N. MOKHOVA, *Mikrobiologiya*, 33 (1964) 918.
7 E. N. MOKHOVA, *Biofizika*, 10 (1965) 571.
8 E. N. MOKHOVA, S. D. TAPTYKOVA AND S. G. SHAROYAN, *Vopr. Med. Khim.*, 14 (1968) 437.
9 M. A. LUKOYANOVA AND S. D. TAPTYKOVA, *Biokhimiya*, 33, 4 (1969) 888.
10 O. H. LOWRY, N. J. ROSENBROUGH, A. L. FARR, R. J. RANDALL, *J. Biol. Chem.*, 193 (1953) 265.
11 M. SALTON, J. FREER AND D. ELLAR, *Biochem. Biophys. Res. Commun.*, 33 (1968) 309.
12 W. CUNNINGHAM, F. L. CRANE AND G. SOTTOCASA, *Biochim. Biophys. Acta*, 110 (1965) 265.
13 S. TAKEMORI, J. SEKUZU AND K. OKUNUKI, *Biochim. Biophys. Acta*, 51 (1961) 464.
14 D. N. OSTROVSKY, I. G. ZHUKOVA AND N. S. GEL'MAN, *Biokhimiya*, 33 (1968) 612.
15 M. FUJITA, S. ISHIKAWA AND N. SHIMAZONO, *J. Biochem.*, 59 (1966) 104.
16 F. CRANE, J. STILES, K. PREZBINDOWSKI, F. RUZICKA AND F. SUN, in J. JÄRNEFELT, *Regulatory Functions of Biological Membranes*, Elsevier, Amsterdam, 1968, p. 21.
17 J. SHIBUYA, H. HONDA AND B. MARUO, *J. Biochem.*, 46 (1968) 571.
18 C. JONES AND E. REDFEARN, *Biochim. Biophys. Acta*, 143 (1967) 354.
19 S. RAZIN AND V. BARASH, *FEBS letters*, 3 (1969) 217.
20 W. BONT, P. EMMELOT AND H. VAS DIAS, *Biochim. Biophys. Acta*, 173 (1969) 389.