STUDIES ON THE FUNCTION OF CELL MEMBRANE
7TH REPORT: INFLUENCE OF CCl_4 ADMINISTRATION ON SOLUBILIZA-
TION OF THE ENZYMES IN ELECTRON TRANSPORT SYSTEM
IN RAT LIVER PLASMA MEMBRANE AND MICROSOME

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Abstract—In liver microsomes of normal rats, both NADH-ferricyanide dehydrogenase and NADH-cytochrome c reductase were almost completely solubilized by addition of 0.5% DOC. Solubilization patterns were little affected by CCl_4 injection. In membranes, solubilization of dehydrogenase and reductase was less than that in microsomes. CCl_4 administration lowered the solubility of the dehydrogenase in membranes and increased three-fold the ratio of the reductase to the dehydrogenase remaining in the non-solubilized fraction. The reductase in membranes from CCl_4 -treated rat liver was found to be more resistant to solubilization by DOC. The possibility of accumulation of NADH-cytochrome c reductase in plasma membranes after CCl_4 administration was discussed.

It has been already reported that the elevation of NADH-cytochrome c reductase activity in rat liver plasma membrane following CCl_4 administration, accompanying a distinct sex difference (1), is not due to contamination by mitochondria, microsomes, or an enzyme such as DT-diaphorase present in the supernatant during cell fractionation (2); is not a product of enzyme induction; but is the result of a migration of flavoprotein (NADH dehydrogenase) and hemoprotein (cytochrome b_5) from microsome to plasma membrane (3).

An electron microscopic investigation demonstrated an ultrastructural alteration of the membrane following CCl_4 administration, e.g., disappearance of the hexagonal sub-unit pattern and elementary particles, occurring in 3-6 hr (4), at which time an elevation in NADH-cytochrome c reductase activity was revealed.

Accordingly, one of the causes of the elevation may be due to the accumulation of the migrated NADH dehydrogenase and cytochrome b_5 in the membranes resulting from a varied binding ability in plasma membrane which may or may not be mediated by the elimination and/or the addition of some constituents.

The present experiments were undertaken to detect whether or not there is any difference in the enzyme binding ability in both plasma membrane and microsome of rat liver cells and to observe changes following CCl_4 administration.

MATERIALS AND METHODS

Female albino rats of Donryu S strain weighing 150–250 g were used. Animals were sacrificed by decapitation 6 hr after i.p. injection of CCl_4 in a dose of 0.2 ml/kg. Control rats were given i.p. 0.8 ml/kg of olive oil, the dose of which produced no effects on liver functions in our experiments.

Fractionation of liver plasma membranes and microsomes, determination of NADH-cytochrome c reductase and NADH-ferricyanide dehydrogenase (NADH dehydrogenase) activities and protein assay were carried out using previously described methods (1).

Treatment and solubilization of the plasma membranes or microsomes with deoxycholate (DOC) were conducted using procedures shown in Figs. 1 and 2, respectively.

FIG. 1. Procedure for treatment of membrane or microsome with deoxycholate.

FIG. 2. Procedure for solubilization of membrane or microsome with deoxycholate.

RESULTS

1. *Influence of DOC on NADH-cytochrome c reductase and NADH dehydrogenase activities in both liver plasma membrane and microsome isolated from normal and CCl_4 -administered rats*

a) *Experiments in plasma membranes (Fig. 3)*

Although addition of DOC below a concentration of 0.1% did not significantly affect NADH-cytochrome c reductase activity in liver plasma membranes of non-treated rats, a concentration of 0.2–0.3% produced a decrease in the reductase activity by approx. 50%. A concentration of DOC over 0.3% no longer caused an additional depression.

In CCl_4 -administered rats, the inhibitory effect of DOC on the reductase activity was somewhat milder, although the activity in plasma membranes had already elevated approx. 4 times that of control.

FIG. 3. Effects of deoxycholate on NADH-cytochrome c reductase and NADH-ferricyanide dehydrogenase activities in plasma membranes from rat livers. (Each point represents mean $\pm$ s.d.)

FIG. 4. Effects of deoxycholate on NADH-cytochrome c reductase and NADH-ferricyanide dehydrogenase activities in microsomes from rat livers. (Each point represents mean $\pm$ s.d.)

Although NADH dehydrogenase activity in the membrane of both normal and CCl_4 -treated rats, unlike the reductase, increased to some extent by addition of DOC, no significant difference in activity was observed.

b) Experiments in microsomes (Fig. 4)

Addition of DOC showed no significant effect on the reductase and dehydrogenase activities in microsomes regardless of CCl_4 treatment in contrast with those in the plasma membranes.

2. Solubilization with DOC of NADH-cytochrome *c* reductase and NADH dehydrogenase in both liver plasma membranes and microsomes isolated from normal and CCl₄-administered rats

a) Solubilization of NADH dehydrogenase

Although NADH dehydrogenase in plasma membranes was hardly solubilized with 0.1% DOC, 0.5% of the detergent solubilized more than 50% of membrane protein and

FIG. 5. NADH-ferricyanide dehydrogenase activity in supernatant and sediment fractions of plasma membranes treated with DOC (normal rat liver).

(Protein : %, S.A. : red. ferri. μ moles/mg prot./min, T.A. : S.A. $\times$ Prot. (%))

FIG. 6. NADH-ferricyanide dehydrogenase activity in supernatant and sediment fractions of plasma membranes treated with DOC (CCl₄-treated rat liver).

Protein : %, S.A. : red. ferri. μ moles/mg prot./min, T.A. : S.A. $\times$ prot. (%)

FIG. 7. NADH-ferricyanide dehydrogenase activity in supernatant and sediment fractions of microsomes treated with DOC (normal rat liver).

(Protein : %, S.A. : red. ferri. μ moles/mg prot./min, T.A. : S.A. $\times$ prot. (%))

FIG. 8. NADH-ferricyanide dehydrogenase activity in supernatant and sediment fractions of microsomes treated with DOC (CCl_4 -treated rat liver).

(Protein : %, S.A. : red. ferri. μ moles/mg prot./min, T.A. : S.A. $\times$ prot. (%))

the dehydrogenase. Approximately 50% of the total dehydrogenase activity remained in the sediment (Fig. 5). A similar tendency was observed in liver plasma membranes of CCl_4 -administered rats (Fig. 6).

The microsome, unlike the plasma membrane, was largely solubilized with 0.5% DOC irrespective of CCl_4 administration. More than 90% of NADH dehydrogenase appeared in the supernatant fraction (Figs. 7 and 8).

FIG. 9. NADH-cytochrome c reductase activity in supernatant and sediment fractions of plasma membranes treated with DOC (normal rat liver).
(Protein : %, S.A. : red. cyt. c μ moles/mg prot./min, T.A. : S.A. $\times$ prot. (%))

FIG. 10. NADH-cytochrome c reductase activity in supernatant and sediment fractions of plasma membranes treated with DOC (CCl₄-treated rat liver).
(Protein : %, S.A. : red. cyt. c μ moles/mg prot./min, T.A. : S.A. $\times$ prot. (%))

b) Solubilization of NADH-cytochrome c reductase

As shown in Fig. 9, the plasma membrane of normal rat liver was hardly solubilized with 0.1% DOC. 0.5% of the detergent, however, greatly inhibited the reductase activity in both supernatant and sedimental fractions, although the membrane protein was solubilized with 0.5% DOC.

While the specific activity of the reductase in liver plasma membranes of CCl₄-administered rats hardly lowered even after treatment with 0.5% DOC, a large amount of the

FIG. 11. NADH-cytochrome c reductase activity in supernatant and sediment fractions of microsomes treated with DOC (normal rat liver).
(Protein : %, S.A. : red. cyt. c μ moles/mg prot./min, T.A. : S.A. $\times$ prot. (%))

FIG. 12. NADH-cytochrome c reductase activity in supernatant and sediment fractions of microsomes treated with DOC (CCl_4 -treated rat liver).
(Protein : %, S.A. : red. cyt. c μ moles/mg prot./min, T.A. : S.A. $\times$ prot. (%))

reductase remained in the sediment (Fig. 10). These results were quite different from those seen in NADH dehydrogenase solubilization.

The reductase in liver microsomes, however, was markedly solubilized in both normal and CCl_4 -administered rats (Figs. 11 and 12).

From these findings it is evident that plasma membrane protein was solubilized less easily than the microsomal and also solubilization of NADH dehydrogenase in the two fractions showed a similar pattern in both normal and CCl_4 -administered groups, while

TABLE 1. Retention of NADH-ferricyanide dehydrogenase, NADH-cytochrome c reductase and their ratios in the sediment fractions following solubilization of membranes or microsomes with 0.5% deoxycholate.

Fraction	Treatment	Dehydrogenase	Reductase	Reductase
				Dehydrogenase
Membrane	Normal	38.5	10.4	26.0
	CCl ₄	58.6	50.6	86.4
Microsome	Normal	10.9	4.0	36.7
	CCl ₄	10.9	3.3	30.3

All values are expressed as per cent of the total activities recovered in the sediment fractions of 20 mM Tris buffer media.

the NADH-cytochrome c reductase was considerably resistant to solubilization with DOC (Table 1).

DISCUSSION

NADH-ferricyanide dehydrogenase and NADH-cytochrome c reductase in liver microsomes were almost completely solubilized (90%) with 0.5% DOC regardless of CCl₄ administration, as reported by Strittmatter (5) and Mackler (6), while the solubilization of the dehydrogenase in plasma membranes did not exceed 60% in normal rats and 40% in CCl₄-administered animals. Furthermore, in CCl₄-administered groups, the degree of solubilized NADH-cytochrome c reductase in the membrane was less than that in microsomes or in normal plasma membranes, and inhibition of the membrane reductase activity by DOC addition in CCl₄-poisoned rats was weaker than that in the normal. Consequently, it is likely that flavoprotein and cytochrome b₅ in the NADH-cytochrome c reductase system, particularly the latter, are relatively firmly bound to plasma membrane constituents in CCl₄-administered rat liver in comparison with those in the normal.

It has been demonstrated in an 8th report (7) that ability of plasma membranes to bind basic hemoprotein, cytochrome c, is much more potent than that of microsomes and the cytochrome c previously bound to plasma membranes is more resistant to release than it is with the microsome. In addition, such ability of the membrane is enhanced after CCl₄ administration. These results are in close agreement with those obtained in this experiment, although cytochrome b₅ carries a relatively high net negative charge (5).

Summarizing the results obtained in the series of experiments on rat liver plasma membrane, we may state that the elevation of NADH-cytochrome c reductase activity in liver plasma membranes at an earlier stage after CCl₄ administration does not result from enzyme induction, but rather from migration of NADH dehydrogenase and cytochrome b₅, together or separately, to the membrane, combining with it and then accumulating there, as the ability of the cell membrane to bind flavoprotein and its coenzyme hemoprotein has already been enhanced. Furthermore, the accumulated reductase in the membrane may be liberated into the circulating blood plasma in part with cytochrome b₅, when-

ever amounts migrating from microsomes to cell membranes exceed the respective binding capacity of the cell membrane.

On the other hand, Recknagel et al. (8, 9) emphasized that the primary site of hepatotoxic action of CCl_4 is in microsomes. The applied dose of CCl_4 was relatively large, however, and the testing time after CCl_4 administration was later, thus it would be difficult to pursue the primary loci of CCl_4 action under such experimental conditions.

On the contrary, the dose of CCl_4 used herein was relatively small and the time tested was earlier. Biochemical changes by CCl_4 in liver microsomes reported by Recknagel et al. (8, 9), e.g., an elevation in microsomal NADH-cytochrome c reductase activity, was not produced at all in our experiment, while numerous biochemical and morphological changes in the plasma membrane were observed (1-4). Accordingly, this indicates that the change by CCl_4 in the liver cell membrane precedes that in the microsome, although the suggestion of Recknagel et al. cannot be definitely denied.

Finally, our results suggest that administered CCl_4 reaching the liver cell membrane causes biochemical changes and thereafter or at the same time certain changes in the microsome occur, resulting in release of the localized enzymes from the microsomes and migration of the enzymes to the cell membrane.

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