THE BINDING OF NADPH-CYTOCHROME c REDUCTASE TO RAT LIVER MICROSOMES

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SUMMARY

Microsomal NADPH-cytochrome c reductase isolated with Triton X-100 is capable to bind to the microsomal membrane. The presented data indicate that the rates of free NADPH oxidation, the reduction of artificial electron acceptors and benzpyrene hydroxylation are increased in microsomes containing extra bound NADPH-cytochrome c reductase. In contrast, the reductase isolated with trypsin is not capable to bind to microsomes.

It has been reported that NADH-cytochrome b_5 reductase and cytochrome b_5, enzymes of microsomal NADH-specific electron transfer chain, can bind additionally to the intact microsomal membrane (1,2). The investigators concluded that these enzymes are randomly distributed and very mobile in the microsomal membrane. Miwa and Cho (3) have demonstrated the ability of NADPH-cytochrome c reductase to bind to microsomes. However, further characterization of the molecular arrangement and mobility of NADPH-cytochrome c reductase is needed. Electron transfer from NADPH to cytochrome P-450 involving this enzyme is another unsolved problem.

MATERIAL AND METHODS

Microsomes were isolated from rat liver by the method of differential centrifugation (4). The activity of NADPH-cytochrome c reductase was measured as previously described

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(4). Cytochrome P-450 content, the hydroxylation of benzpyrene, the activity of NADPH-cytochrome P-450 reductase were determined as described elsewere (5,6,7). NADPH-cytochrome c reductase was isolated with the non-ionic detergent Triton X-100 (DFp) by the modified method of Ichihara et al. (8). Isolation of this enzyme with trypsin (TFp) was performed by the procedure of Iyanagy and Mason (9). The activity of the purified DFp was 1680 and that of the purified TFp was 2400 (expressed in nmoles cytochrome c reduced per min per mg of protein).

The binding of NADPH-cytochrome c reductase to microsomes was performed in 0.05 M Tris-HCl buffer, pH 7.8, containing 0.2 mM EDTA (buffer A). Incubation was carried out for 20 min at 37°C. The incubation system contained 5 mg of microsomal protein and various amounts of the purified enzyme. After incubation, ice-cold buffer A was added to 3 ml of the incubation mixture to give a final volume of 10 ml. The mixture was centrifuged at 105,000 xg for 1 hour. The unbound reductase was removed by washing the microsomes by centrifugation 3 times with buffer A. The last centrifugation yielded a supernatant with no detectable NADPH- cytochrome c reductase activity.

Protein was determined by the method of Lowry et al. (10).

RESULTS AND DISCUSSION

The results obtained demonstrate that DFp is capable of binding to microsomes, whereas TFp does not bind to microsomes (Table I). It was inferred that NADPH-cytochrome c reductase belongs to amphipathic protein molecules and that trypsin, presumably, removes the hydrophobic moiety of the enzyme needed for binding to membrane phospholipids. At a 35-fold excess of DFp, the maximum of extra bound NADPH-cytochrome c reductase constitutes 120% of the total activity of the endogenous enzyme. This is considerably less than reported by Miwa and Cho (3), namely 300% of total endogenous NADPH-cytochrome c reductase at a 55-fold excess of the purified enzyme. Such a discrepancy may be due to the presence of non-specific bound reductase in microsomes unremoved by a single centrifugation.

In the microsomes with extra bound NADPH-cytochrome c reductase, NADPH-oxidase activity rised proportionally to

TABLE I

The effect of binding of NADPH-cytochrome c reductase on the microsomal reactions.

Activities measured	Amount of NADPH-cytochrome c reductase added					
	none	DFp 1500	DFp 4500	DFp 10500	DFp[1] 4500	TFp 6000
cytochrome c[2] reductase	70	91	128	155	68	71
adrenaline[3] oxidase	12.0	15.4	19.1	24.6	12.0	11.8
NADPH oxidation[4]						
+0.5 mM EDTA	5.4	7.3	11.4	13.7	5.1	5.3
+0.1 mM metirapon	5.3	7.1	11.3	13.7	5.2	5.2
cytochrome[5] P-450 reduction	2.1	2.1	2.2	2.2	1.7	-
benzpyrene[6] hydroxylation	0.187	0.262	0.382	0.407	0.164	0.193
cytochrome P-450[7]	0.71	0.68	0.70	0.71	0.72	0.73

[1]- DFp was inactivated by heat at 80°C, 5 min; [2]- nmoles of cytochrome c reduced per min per mg of protein; [3]- nmoles of adrenochrome per min per mg of protein; [4]- nmoles of NADPH consumed per min per mg of protein; [5]- nmoles of cytochrome P-450 reduced per min per mg of protein; [6]- nmoles of 3-hydroxybenzpyrene per min per nmoles of cytochrome P-450; [7]- nmoles per mg of protein

NADPH-cytochrome c reductase activity (Table I). NADPH-oxidase was measured in the presence of EDTA, which excluded reaction of lipid peroxidation. The participation of cytochrome P-450 in the NADPH oxidation can also be ruled out, because metirapon, an inhibitor of cytochrome P-450, had no inhibitory effect on the oxidation of NADPH. It may be suggested that the capacity of NADPH-cytochrome c reductase for autooxidation determines the activity of NADPH-oxidase.

TABLE II

Some hydroxylation parameters and cytochrome P-450 reduction in microsomes with bound DFp

	NADPH-cytochrome c reductase in microsomes	Benzpyrene hydroxylation		NADPH-cytochrome P-450 reductase activity	
		V_{max}	K_m[1]	fast phase	slow phase[2]
control	70	0.62	6.0	2.1	(0.04)15min
4500 units of DFp added	128	0.88	3.2	2.1	(0.07)9min

[1]- K_m in uM; [2]- in brackets - reduction rate of cytochrome P-450 in slow phase (nmoles per min per mg of protein), min - time needed for the "complete" reduction of cytochrome P-450

In the microsomes with extra bound DFp cytochrome P-450 content did not change but that of NADPH-cytochrome c reductase activity increased twofold; the reduction rate of cytochrome P-450 did not change in the fast phase and increased in the slow phase (Table II). Obviously, endogenous NADPH-cytochrome c reductase may form a structural complex with cytochrome P-450. However, being randomly distributed in the microsomal membrane, the extra bound NADPH-cytochrome c reductase does not form such complex with cytochrome P-450. Thus, it may be concluded that the mobility of NADPH-cytochrome c reductase is low in the membrane, in contrast to NADH-cytochrome b_5 reductase and cytochrome b_5 (11). It seems plausible that translational mobility of NADPH-cytochrome c reductase determines the rate of the slow phase of cytochrome P-450 reduction.

In the microsomes with extra bound DFp, V_{max} for

benzpyrene hydroxylation increases, while the K_m value for this reaction decreases. Since the reduction rate of cytochrome P-450 did not change in the fast phase of the reaction, we suppose that the increase in the V_{max} value and the decrease in the K_m value were due to the acceleration of the slow phase of the reduction of cytochrome P-450 which was not tightly complexed with bound DFp. The V_{max} and K_m values of the control microsomes characterize the reduction rate of cytochrome P-450, provided that the electron transfer by NADPH-cytochrome c reductase to cytochrome P-450 limits benzpyrene hydroxylation rate (12). With increasing DFp activity in the experimental microsomes the transfer of electrons to cytochrome P-450 was accelerated in the slow phase. In this case, the K_m and V_{max} values reflect, possibly, the functional activity of cytochrome P-450 itself.

We propose that NADPH-cytochrome c reductase transfers electrons to cytochrome P-450 by a mechanism of lateral mobility in the slow phase, while in the fast phase the electrons transfer relates to the mode of the association of the enzymes in microsomal membrane.

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