NUCLEAR ELECTRON TRANSPORT I

ELECTRON TRANSPORT ENZYMES IN BOVINE LIVER NUCLEI AND NUCLEAR MEMBRANE

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SUMMARY

The electron transport enzymes NADH-cytochrome c reductase, NADH oxidase and cytochrome c oxidase are present in isolated bovine liver nuclei and highly concentrated in a nuclear membrane fraction. Evaluation of contamination indicates that the enzymes are of nuclear origin. Nuclear NADH oxidase is distinguished from mitochondrial NADH oxidase by the effects of exogenous cytochrome c, DNase and histone on enzyme activity. We suggest that the nuclear electron transport enzymes are located in the nuclear membrane.

Rees and Rowland (1) and Penniall et al. (2) have shown the presence of oxidative enzymes in rat liver nuclei. In identifying electron transport activities in nuclei, it is important to find the location of these activities. The possibility of contamination can not be completely eliminated until nuclear electron transport can be shown to be localized in morphologically recognizable structures endogenous to nuclei. Penniall and co-workers (3) have localized nuclear NADH oxidase in a nucleoli fraction. In contrast, Rees et al. (4) have shown intact nucleoli to be depleted of oxidative enzymes. They found the oxidative enzymes NADH-cytochrome c reductase and cytochrome c oxidase highly concentrated in a lipid-rich nuclear fraction.

Because the electron transport chain in mitochondria as well as in bacteria is membrane bound, it is a reasonable assumption that nuclear electron transport may be associated with the nuclear membrane. We have begun to investigate this possibility by means of a large scale preparation procedure for nuclear membrane

isolation (5). In this communication we present evidence for electron transport enzymes in nuclei and their concentration in nuclear membrane.

METHODS

A detailed procedure for preparation of nuclei, nuclear membrane, mitochondria and microsomes will be presented elsewhere (5). Briefly the procedure which we use follows. Crude nuclei fractions were isolated in a sucrose buffer (0.25 M sucrose, 0.05 M Tris-HCl, pH = 7.5, 0.025 M KCl, 0.005 M $MgCl_2$) (6). Nuclei fractions were purified by centrifuging through 2.3 M sucrose buffer. The mitochondria were obtained by centrifuging the supernatant from the crude nuclei fraction at 4,000 x g for 15 minutes. Microsomes were isolated from this supernatant by first centrifuging at 27,000 x g for 20 minutes and then at 105,000 x g for 60 minutes. Nuclear membranes were prepared by digestion with DNase (100 μg/mg protein) for 14 hours at 2° C followed by resuspension in 0.5 M $MgCl_2$ sucrose buffer. The membranes were collected by centrifugation at 27,000 x g for 15 minutes. For comparison with nuclear membrane, mitochondria and microsomes were similarly treated with DNase and $MgCl_2$. Cytochrome c reductase assays were performed at 30° C according to Ernster et al. (7). Succinoxidase and NADH oxidase were measured polarographically in an assay system containing 66.7 μmoles phosphate in a total volume of 1.8 ml at pH = 7.5 in the presence or absence of cytochrome c. The assays were initiated with 2 mgs of succinate or NADH. Cytochrome c oxidase was assayed according to Sun and Crane (8).

RESULTS

An extensive ultrastructural and biochemical analysis of purity of the nuclei and nuclear membrane fractions will be presented elsewhere (5). In measuring contamination we use succinoxidase and succinate-cytochrome c reductase as a marker enzyme for mitochondria and NADPH-cytochrome c reductase as an indicator of microsomal membranes. Analysis of the activities for these enzymes reported in Table 1 reveals that an average of 1.1% and 3.7% mitochondrial

Table 1

Electron Transport Enzymes in Nuclei, Nuclear Membrane,

Mitochondria, Microsomes, and DNase-$MgCl_2$ Treated Fractions

Fraction	NADH-cyt c reductase activity	NADPH-cyt c reductase activity	Succinate-cyt c reductase activity	Succin-oxidase activity	NADH oxidase activity	cyt c oxidase activity
Nuclei	0.242	0.00089	0.00341	0.00211	0.0451	0.0488
Nuclear Membrane	2.10	0.0052	0.00385	0.00462	0.261	0.274
Mitochondria	-----	-------	0.259	0.264	0.328	0.531
DNase-$MgCl_2$ Mitochondria	-----	-------	0.102	0.128	0.576	1.35
Microsomes	4.15	0.0825	--------	-------	------	------
DNase-$MgCl_2$ Microsomes	4.28	0.0812	--------	-------	------	------

Assays were performed as described in METHODS. NADH, NADPH, and succinate-cyt c reductase activities are given in μmoles cyt c reduced/min/mg protein. NADH, succinate, and cyt c oxidase are measured in μmoles O_2/min/mg protein. NADH oxidase was measured in the presence of 4 mg cyt c for mitochondria and 8 mg for nuclei and nuclear membrane. Succinate oxidase assays contained 2 mg cyt c.

protein and 1.1% and 6.4% microsomal protein is found in the nuclei and nuclear membrane fractions respectively. The low mitochondrial contamination is further substantiated by the absence of succinate-dichloroindophenol reductase activity in both nuclei and nuclear membrane fractions. The contributions of the mitochondrial and microsomal contamination to the enzyme activities reported in Table 1 for nuclei and nuclear membrane can be calculated from the percentage contaminations reported above. In the nuclei fraction 92.0% of NADH oxidase activity, 91.6% of cytochrome c oxidase activity and 81.2% of NADH-cytochrome c reductase activity is not accounted for by contamination and is therefore endogenous to nuclei. Similarly, 91.8% of NADH oxidase activity, 82.2% of cytochrome c oxidase activity and 87.0% of NADH-cytochrome c reductase activity is endogenous to nuclear membrane.

Table 1 also indicates that these enzymes are localized in the nuclear membrane. From a comparison of the activities reported in Table 1, NADH-cytochrome c reductase is 8.7 times higher in nuclear membrane as compared to nuclei. Likewise NADH oxidase is 6.1 times higher and cytochrome c oxidase is 5.6 times higher. The nuclear NADH-cytochrome c reductase is amytal, rotenone, antimycin A, and piericidin insensitive as its microsomal counterpart.

Nuclear NADH oxidase can be distinguished from mitochondrial NADH oxidase by the absolute dependency of the nuclear enzyme on exogenous cytochrome c for activity. As indicated in Table 2, both nuclei and nuclear membrane show this dependency which is in agreement with the findings of Penniall et al. (2,3). In contrast the mitochondrial fractions have considerable activity in the absence of added cytochrome c, although the addition of cytochrome c greatly stimulates activity (Table 2). The nuclear and mitochondrial oxidases also differ in the amount of cytochrome c/mg of protein fraction needed for maximal activity. Four separate experiments show that the nuclear fractions require approximately 10 times more cytochrome c than the mitochondrial fractions.

DNase inhibits 40%-50% of NADH oxidase (Table 2) and cytochrome c oxidase in nuclei but does not inhibit the small amount of succinoxidase which repre-

Table 2

Effect of Cytochrome c, DNase and Histone on

Nuclear and Mitochondrial NADH Oxidases

	Mitochondria	Nuclei	DNase-$MgCl_2$ Mitochondria	Nuclear Membrane
Maximal stimulation due to exogenous cytochrome c	17.1 fold	cyt c dependent	18.2 fold	cyt c dependent
% inhibition due to DNase treatment*	5.9	42.8	---	---
% inhibition due to addition of 0.560 mg histone/mg protein fraction	100	26.4	100	28.4

*Fractions were incubated with 5 μgs DNase/mg protein for one hour at 30° C. The reactions were stopped by centrifuging the fractions. The DNase treated fractions were washed twice by resuspension and centrifugation and then immediately assayed.

sents mitochondrial contamination. DNase, on the other hand, does not inhibit NADH, (Table 2), succinate, or cytochrome c oxidase in mitochondria.

Finally, nuclear NADH oxidase is more resistant to histone inhibition than the mitochondrial oxidase. Addition of histone (calf thymus, type III, Sigma) at a ratio of 0.560 mg histone/mg protein fraction completely inhibits the mitochondrial oxidase but only 26.4% and 28.4% of the oxidase in the nuclei and nuclear membrane respectively.

Besides substantiating previous reports of NADH oxidase and cytochrome c oxidase in liver nuclei, these results indicate that nuclei may have its own unique electron transport system localized on the nuclear membrane. Further work is in progress to determine whether one or both membranes of the nuclear envelope is involved.

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