RESPIRATION IN ISOLATED RAT LIVER NUCLEAR ENVELOPES

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1. Introduction

Isolated cell nuclei possess a low level of respiration which cannot be ascribed to the presence of cytoplasmic contaminants [1–4]. The finding of oxidative enzymatic systems in different membraneous structures [5] enables one to suggest that respiration of cell nuclei may also be associated with the nuclear membranes [5–7].

A method recently developed for the isolation of nuclear envelopes [8–10] permitted to demonstrate that isolated nuclear membranes had a high content of cytochrome oxidase (E.C. 1.9.3.1), NADH-cytochrome-*c*-reductase (E.C. 1.6.2.1), NADPH-cytochrome-*c*-reductase (E.C. 1.6.2.3), and glutamate dehydrogenase (E.C. 1.4.1.2) [7–10].

In this communication we describe direct measurements of respiration in intact rat liver nuclei and isolated nuclear membranes. It is demonstrated that the specific respiratory activity of the nuclear envelope fraction was 4–6 times higher than that of unfractionated intact nuclei.

2. Methods and results

The nuclei were isolated from the livers of male albino rats by a two-step sucrose method [11]. The nuclear envelopes were obtained from isolated cell nuclei disintegrated by incubation with hypotonic phosphate buffer [8–10]. The purity of the isolated nuclear envelopes and nuclei was checked by light and electron microscopy.

The respiration of isolated nuclei and nuclear envelopes was measured by a polarographic method using Clark's electrode. The protein content of the nuclear envelopes and nuclei was determined by the biuret reaction.

The rate of respiration for rat liver nuclei and nuclear envelopes is presented in table 1. The endogenous respiration of nuclei was low (3.0–4.9 μl O_2/mg protein/hr). This value is in agreement with the published data for calf and rat thymus nuclei [4,12,13]. Under similar conditions the respiration of isolated nuclear envelopes was considerably more intensive (17.6–26.2 μl O_2/mg protein/hr). Thus, the specific respiratory activity of the nuclear envelopes was 4–6 times as high as that of whole nuclei.

The uptake of oxygen by isolated nuclei as well as by nuclear envelopes was enhanced by the addition of NADH (5 μmoles per reaction mixture). NADPH also stimulated nuclear respiration but to a lesser extent. The addition of NAD^+ increased this effect of NADPH. Glutamate and succinate did not affect the respiration. Cytochrome *c* (12 mμmoles per reaction mixture) sometimes stimulated the respiration but its effect was poorly reproducible which might be due to different losses of cytochrome *c* from nuclei in the course of their purification. The addition of ADP to the nuclei or nuclear envelopes did not stimulate respiration. That means that under our conditions the exogenous ADP did not appear to be an acceptor of phosphate in

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Table 1
Respiration of isolated rat liver nuclei and nuclear envelopes.

Exp. No.	Additions	Q_{O_2} (μl O_2/mg protein/hr)	
		Nuclei	Nuclear envelopes
1	None	4.3	18.2
	Cyt.*c*		18.2
	Cyt.*c* + succinate		19.7
	Cyt.*c* + NADH	13.7	
	Cyt.*c* + succinate + NADH		33.9
	Cyt.*c* + succinate + NADH + amytal		27.2
	Cyt.*c* + succinate + NADH + amital + azide		12.4
	Cyt.*c* + succinate + NADH + amytal + azide + cyanide		0
2	None	4.3	17.6
	Cyt.*c*	8.7	20.7
	Cyt.*c* + NADH	14.4	40.7
	Cyt.*c* + NADH + ADP	14.4	40.7
	Cyt.*c* + NADH + ADP + cyanide		0
3	None	4.9	26.2
	NADH		61.2
	NADH + Cyt.*c*	13.2	96.2
	NADH + Cyt.*c* + cyanide	0	0
4	None	4.3	19.2
	Cyt.*c*	7.8	19.2
	Cyt.*c* + NADPH	8.8	
	Cyt.*c* + NADPH + NAD^+	11.0	
	Cyt.*c* + NADPH + NAD^+ + glutamate	11.0	
	Cyt.*c* + NADPH + NAD^+ + glutamate + succinate	11.0	
	Cyt.*c* + NADH		82.2
	Cyt.*c* + NADH + antimycin A		0
5	None	4.1	21.8
	Succinate	4.1	21.8
	Succinate + cyt.*c*	5.0	
	Succinate + NADH		28.2
	Succinate + NADH + ADP		28.2
6	None	3.0	19.6
	Cyt.*c*	3.0	
	Cyt.*c* + succinate	3.0	
	Cyt.*c* + succinate + cyanide	0	
	Glutamate		19.6
	Glutamate + NADH		33.2

The basic medium contained in μmoles (unless otherwise stated): KCl 210, EDTA 7.5, $MgCl_2$ 18, K-phosphate buffer pH 7.2, 30, Tris-chloride buffer pH 7.4, 24, Nuclei (or nuclear envelopes) 0.5–1.0 mg protein in a volume of 3.25 ml. Incubation at 30°C. Additions: Cytochrome *c* 12 mμmoles; NADH, NADPH, NAD, succinate, glutamate 5 μmoles; ADP 150 μmoles; cyanide 30 μmoles; antimycin A 4 μg, amytal 30 μmoles, azide 3 μmoles.

oxidative phosphorylation. The respiration was entirely abolished by cyanide or antimycin A, well known inhibitors of the electron transport in the mitochondrial respiratory chain.

3. Discussion

The results obtained in this study demonstrate that rat liver nuclei and nuclear envelopes possess respiration which is stimulated by NADH and NADPH and inhibited by cyanide and antimycin A. This can be interpreted as an indication for the existence of a respiratory chain which resembles in some respects that of mitochondria. The sensitivity of nuclear respiration to cyanide indicates that cytochrome oxidase previously found in rat liver nuclear membranes [7,10] might function as a terminal carrier in the respiratory chain of the nuclear envelopes. The absence of a succinate effect on the respiration is in good agreement with our previous observations demonstrating the absence of succinate dehydrogenase in intact nuclei and nuclear membranes [7]. It was however surprising that glutamate also did not stimulate oxygen uptake although glutamate dehydrogenase activity was found in these preparations [7].

Our results show that the maximal respiratory specific activity (in the presence of NADH and cytochrome *c*) of the nuclear envelopes is about five times higher than that of the whole nuclei. This can be regarded as an indication that nuclear respiratory enzymes may be localized predominantly (if not exclusively) in the nuclear envelope. Our results demonstrate also that nuclear oxidation is not restricted to lymphoid tissue nuclei as has been proposed in the literature [3,6,14].

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