DIFFERENT SENSITIVITY OF NUCLEAR AND MICROSOMAL NADH-CYTOCHROME *c* REDUCTASE ACTIVITIES TO THENOYLTRIFLUOROACETONE*

Krzysztof STAROŃ and Zbigniew KANIUGA
Institute of Biochemistry, University of Warsaw, Al. Zwirki i Wigury 93, 02-089 Warszawa, Poland

Received 20 May 1974

1. Introduction

NADH-cytochrome *c* reductase activity in liver nuclei is coupled with the enzymes of nuclear membranes [1]. The presence of NADH-reducible cytochrome b_5 in nuclear membranes [2] seems to indicate that like in the endoplasmic reticulum both NADH-cytochrome b_5 reductase and cytochrome b_5 are responsible for the nuclear NADH-cytochrome *c* reductase activity [3,4]. Berezney and Crane [2] have found that both nuclear and microsomal NADH-cytochrome *c* reductase activities are sensitive to trypsin digestion and insensitive to rotenone and antimycin A. Moreover, NADH-cytochrome b_5 reductase activity, measured in the presence of ferricyanide as an electron acceptor, is sensitive to sulfhydryl reagent in nuclear membranes as well as in microsomes.

In the present paper we show a difference between nuclear and microsomal NADH-cytochrome *c* reductase activity with respect to sensitivity to thenoyltrifluoroacetone.

2. Materials and methods

Nuclei and microsomes were isolated from bovine liver according to the method described by Berezney et al. [1]. In nuclear fraction there was less than 10% NADH-cytochrome *c* reductase activity originated from mitochondrial and microsomal contaminations.

NADH-cytochrome *c* reductase activity was determined at 25°C. The assay mixture contained: 250 mM sucrose, 100 mM Tris–HCl buffer (pH 7.5), 0.1 mM NADH, 0.02 mM cytochrome *c*, 1 mM KCN and 1.5 μM rotenone. Thenoyltrifluoroacetone was added directly to the cuvette in a methanol and incubated at 25°C for 5 min. Aqueous solution of Triton X-100 was added to the preparation of nuclei or microsomes and incubated at 25°C for 5 min.

Protein was determined according to the method of Lowry et al. [5].

3. Results and discussion

Schulze et al. [6] have found that thenoyltrifluoroacetone results in a stimulation of microsomal NADH-cytochrome *c* reductase activity. In contrast, nuclear NADH-cytochrome *c* reductase activity is inhibited by thenoyltrifluoroacetone added at concentrations similar to those required for the stimulation of microsomal activity (fig. 1).

The stimulation of NADH-cytochrome *c* reductase activity by thenoyltrifluoroacetone was observed only in intact microsomal membranes. On solubilization of microsomal NADH-cytochrome *c* reductase system by Triton X-100 thenoyltrifluoroacetone has no stimulating effect [7,8]. In contrast, treatment with detergent does not change the sensitivity of nuclear NADH-cytochrome *c* reductase activity to thenoyltrifluoroacetone (table 1), although this concentration of detergent is sufficient to solubilize the nuclear NADH-cytochrome *c* reductase system (table 2).

A different sensitivity to thenoyltrifluoroacetone of nuclear NADH-cytochrome *c* reductase system than

* *Abbreviation:* thenoyltrifluoroacetone – 4,4,4-trifluoro-1-(2-thienyl)-1,3-butadione.

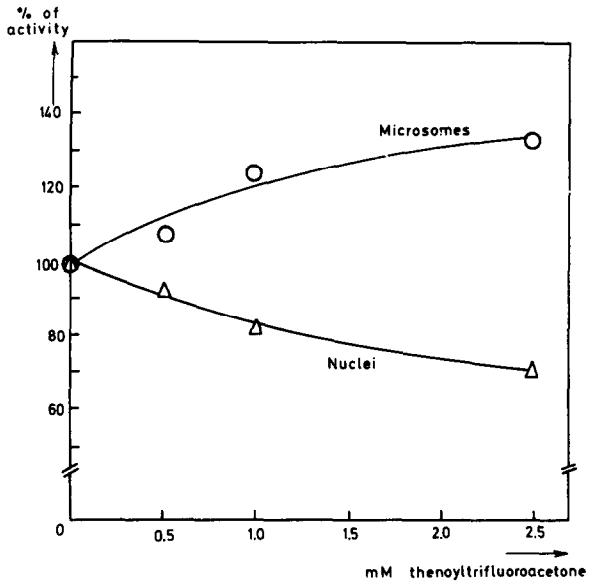

Fig. 1. Effect of thenoyltrifluoroacetone on NADH-cytochrome *c* reductase activity in nuclei (△) and microsomes (○).

that of microsomal one following the treatment with Triton X-100 seems to be likely due to differences in membrane structure rather than in enzymes of these two systems.

Acknowledgements

We are grateful to Miss Barbara Stażka for skilful assistance. This work was supported in part by a grant from the Polish Academy of Sciences.

Table 2
Solubilization of nuclear NADH-cytochrome *c* reductase activity by Triton X-100

Sample	NADH-cytochrome *c* reductase activity		
	Pellet*	Supernatant*	
	nmoles/min	nmoles/min	% of total activity
Control	4 500	150	3.3
Nuclei+ Triton X-100	0	2 600	100

* 10 ml samples of nuclei (83 mg protein in buffer containing: 250 mM sucrose, 50 mM Tris–HCl, pH 7.5, 25 mM KCl and 5 mM $MgCl_2$) were incubated for 5 min at 25°C either with or without 1% Triton X-100 and centrifuged for 20 min at 1800 *g*. Pellets were suspended in 10 ml of the buffer.

References

[1] Berezney, R., Maculay, L. K. and Crane, F. L. (1972) J. Biol. Chem. 247, 5549–5561.
[2] Berezney, R. and Crane, F. L. (1972) J. Biol. Chem. 247, 5562–5568.
[3] Strittmatter, P. and Velick, S. F. (1956) J. Biol. Chem. 221, 253–264.
[4] Strittmatter, P. and Velick, S. F. (1957) J. Biol. Chem. 228, 785–799.
[5] Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275.
[6] Schulze, H. U., Gallenkamp, H. and Staudinger, H. (1970) Hoppe-Seyler's Z. Physiol. Chem. 351, 809–817.
[7] Staroń, K. and Kaniuga, Z. (1972) Hoppe-Seyler's Z. Physiol. Chem. 353, 14–18.
[8] Staroń, K. and Kaniuga, Z. (1974) Acta Biochim. Polon. 21, 55–60.

Table 1
Sensitivity to thenoyltrifluoroacetone of both nuclear and microsomal NADH-cytochrome *c* reductase activities following treatment with Triton X-100

Sample	NADH-cytochrome *c* reductase activity		
	Control	+ 2.5 mM Thenoyltrifluoroacetone	
	nmoles/min/mg protein	nmoles/min/mg protein	% of control
Nuclei	52	38	73
Nuclei + 1% Triton X-100	28	21	73
Microsomes	383	511	133
Microsomes + 1% Triton X-100	194	187	96