Volume 189, number 2 FEBS 2902 September 1985

Redox-dependent activation of 5′-nucleotidase in rat liver plasma membranes

Alexander M. Tarakhovsky, Victor J. Umansky, Vladimir A. Shlyakhovenko and Konstantin P. Balitsky

R.E. Kavetsky Institute of Oncology Problems, Academy of Sciences of Ukrainian SSR, Vasilkovskaya 45, Kiev 252127, USSR

Received 17 July 1985

Addition of NADH, but not NAD^+ or NADPH, to rat liver plasma membranes resulted in the increase of their 5′-nucleotidase activity. NADH-dependent activation of 5′-nucleotidase was significantly suppressed by atebrine, an inhibitor of NADH dehydrogenase of plasma membranes, and completely abolished by 2,4-dinitrophenol (2×10^{-4} M) and Triton X-100 (2%). Inhibitors of electron transfer in the mitochondrial respiratory chain, rotenone and potassium cyanide, failed to affect 5′-nucleotidase activity in both the presence and absence of NADH. The data obtained give reasons to suggest a redox-dependent mechanism of 5′-nucleotidase activation in rat liver plasma membranes.

Rat liver *Plasma membrane* *Redox chain* *NADH dehydrogenase* *5′-Nucleotidase*

1. INTRODUCTION

It was established in recent years that plasma membranes of certain types of mammalian cells contain an NADH-dependent redox chain, which differs in a number of properties from similar electron transfer chains of mitochondria and microsomes [1,2]. It was also found that this redox chain may play an essential role in hormone action [2–4], cell adhesiveness and proliferation [5] and sensitivity to antitumour antibiotics [6]. Though the mechanism of the above-mentioned phenomena still remains unknown, NADH oxidation by membrane-bound oxidoreductases in plasma membranes is supposed to be accompanied by changes in the functional state of cell surface proteins [2,7].

In this paper we present the results of our research on the effect of exogenously added NADH on 5′-nucleotidase activity in rat liver plasma membranes. Predominant localization of this enzyme in the plasma membrane, and application of a highly sensitive method of determination of 5′-nucleotidase activity, make the protein in question a most suitable experimental model to study the mechanisms of redox-dependent regulation of plasma membrane proteins.

2. MATERIALS AND METHODS

2.1. *Materials*

NADPH, NADH, NAD^+ were received from Reanal, Hungary. Atebrine, 2,4-dinitrophenol, hypoxanthine, inosine and adenosine were from Serva, Heidelberg. AMP, from Sigma, USA. [8-^{14}C]adenosine 5′-monophosphate (10 mCi/mmol) was from 'Isotope', USSR.

2.2. *Methods*

Rat liver plasma membranes were prepared using the Neville procedure [8]. Male albino rats (150–200 g body wt) were used. Directly after isolation, the plasma membranes (100–200 μg protein) were suspended in 50 μl of 100 mM Tris-HCl, pH 7.5, containing 6 M $MgSO_4$ and 50 μl compound to be tested, dissolved previously in the same buffer, were added to reaction mixture and rigorously mixed. Unless otherwise stated, after 20 min of incubation at room temperature the

Published by Elsevier Science Publishers B.V. (Biomedical Division)

reaction tubes were placed on ice and 10 μl aliquots were taken to determine the 5′-nucleotidase activity by a micromodification of the method described [9]. Briefly, the assay was performed in 40 μl reaction mixture containing the following: membranes (1–3 μg protein); 50 mM Tris-HCl buffer, pH 7.5, 3 mM $MgSO_4$, 1 mM [8-^{14}C]AMP (~200000 cpm). The reaction was initiated by addition of membranes and incubation continued for 20 min at 30°C. The reaction was stopped by heating to 80°C for 3 min. 10 μl aliquots of reaction mixture and 10 μl of a solution containing 4 μg/ml each of adenosine, hypoxanthine, inosine and AMP, were spotted on Whatman 3 MM sheets. The nucleosides and nucleotides were separated by ascending chromatography using 0.05% NH_4OH as solvent. The spots corresponding to adenosine plus inosine and hypoxanthine were identified under ultraviolet light, cut off, immersed in a vial, containing 10 ml toluene scintillation liquid, and counted. The 5′-nucleotidase activity was expressed as nmol adenosine, inosine and hypoxanthine/min per mg plasma membrane protein.

The protein concentration was measured by the Lowry method using bovine serum albumin as standard.

3. RESULTS AND DISCUSSION

As shown, preincubation of rat liver plasma membranes with NADH, but not with NAD^+ or NADPH, results in a significant increase of their 5′-nucleotidase activity (table 1). The growth of NADH concentration from 0.01 mM to 20 mM increased 5′-nucleotidase activity, the maximum being 10 mM (fig.1). The kinetics of enzyme activation in the presence of 10 mM NADH is shown in fig.2. A marked increase of 5′-nucleotidase activity was observed after 1 min of incubation, with its maximum at 10 min and without any change for the next 20 min.

NADH-dependent activation of 5′-nucleotidase was found only in the case of preincubation of membranes with NADH. If NADH was added to the reaction mixture simultaneously with AMP, the activation effect was not detected (table 1). One of the possible reasons for this phenomenon seems to be a change of conformation of

Table 1

NADH-dependent activation of 5′-nucleotidase in rat liver plasma membranes

Solution	5′-Nucleotidase activity (%)[a]
Buffer solution + membranes	100
+ 10 mM NADH	252
+ 20 mM NAD	110
+ 20 mM NADPH	113
+ 3 mM atebrine	110
+ 2% Triton X-100	113
+ 2×10^{-4} M 2,4-dinitrophenol	112
+ 3 mM KCN	105
+ 10 μg/ml rotenone	96
Buffer solution + membranes + 10 mM NADH	252
+ 1 mM AMP	92
+ 3 mM atebrine	145
+ 2% Triton X-100	113
+ 2×10^{-4} M 2,4-dinitrophenol	105
+ 3 mM KCN	230
+ 10 μg/ml rotenone	260

[a] Mean values of the data, obtained in 3–7 individual experiments are given. One experiment corresponds to estimation of 5′-nucleotidase activity in the batch of plasma membranes, derived from the liver of 2–3 rats. The 5′-nucleotidase activity equal to 990 ± 50 nmol adenosine + inosine + hypoxanthine/min per mg was taken as 100%. For all details refer to section 2

5′-nucleotidase during interaction with its substrate, AMP, which in turn may lead to masking of the center(s) of either direct or indirect NADH action.

Further experiments suggested that NADH-dependent activation of 5′-nucleotidase in rat liver plasma membranes occurs indirectly in a redox-dependent manner. This was indicated by the fact that the addition of NADH to the partially purified preparation of rat liver 5′-nucleotidase (not shown) or to the membranes solubilised by Triton X-100 (table 1) did not lead to any changes in enzyme activity. On the other hand, NADH-dependent activation of 5′-nucleotidase was significantly suppressed by atebrine, the inhibitor of NADH dehydrogenase of rat liver plasma membranes [1] (table 1). Inhibitors of the NADH-dependent mitochondrial redox chain such as

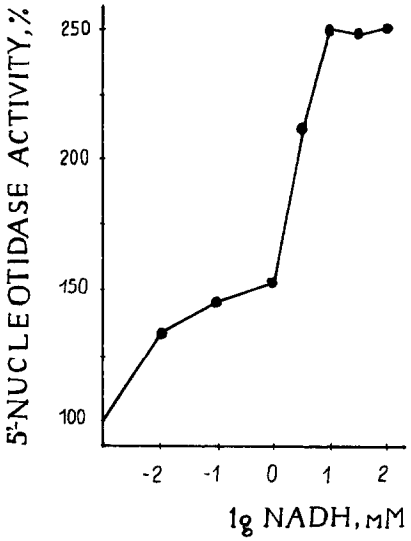

Fig.1. Dependence of 5'-nucleotidase activity in rat liver plasma membranes on NADH concentration. Mean values of the data obtained in 2–7 individual experiments are given. Time of membrane-NADH co-incubation, 20 min.

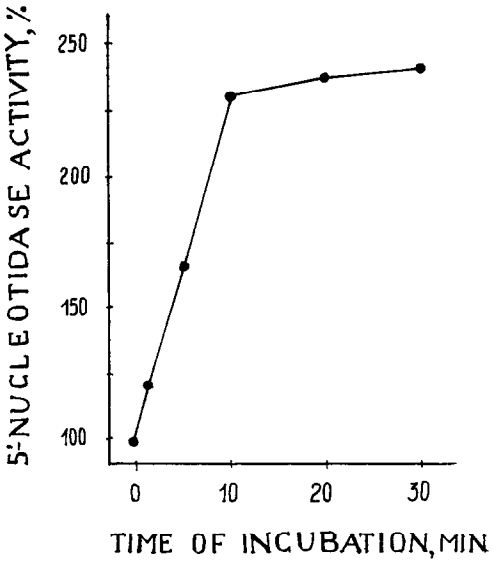

Fig.2. Dependence of 5'-nucleotidase activity in rat liver plasma membranes on the time of their co-incubation with NADH. NADH concentration, 10 mM. 5'-Nucleotidase activity of membranes put on ice immediately after NADH addition was taken as 100%.

potassium cyanide and rotenone did not alter 5'-nucleotidase activity in both the presence or absence of NADH (table 1).

Surprisingly, we found that simultaneous co-incubation of membranes with NADH and 2,4-dinitrophenol (2.0×10^{-4} M) completely abolished NADH-dependent activation of 5'-nucleotidase, while 2,4-dinitrophenol alone in the mentioned concentration did not alter 5'-nucleotidase activity (table 1). Recently Howland et al. [10] have shown an inhibitory effect of different uncouplers of oxidative phosphorylation on the NADH dehydrogenase activity in erythrocyte plasma membranes. We also found that 2,4-dinitrophenol is able to inhibit NADH cytochrome *c* reductase activity in rat liver plasma membranes (not shown). Thus we suggest that abolishment of NADH-dependent activation of 5'-nucleotidase by 2,4-dinitrophenol may be due to its inhibitory effect on NADH dehydrogenase activity.

On the basis of the above findings we suggest that there is a redox-dependent mechanism of 5'-nucleotidase activation in rat liver plasma membranes. The mechanism might work, through a conformational change induced by the redox change in the protein or in a region of membrane, as seen for example for the ADP/ATP carrier in the mitochondrial membrane [11].

REFERENCES

[1] Crane, F.L. and Löw, H. (1976) FEBS Lett. 68, 153–156.
[2] Goldenberg, H. (1982) Biochim. Biophys. Acta 694, 203–223.
[3] Löw, H. and Crane, F.L. (1976) FEBS Lett. 68, 157–159.
[4] Goldenberg, H., Crane, F.L. and Morre, D.J. (1978) Biochem. Biophys. Res. Commun. 83, 234–240.
[5] Ellem, K.A.O. and Kay, G.F. (1983) Biochem. Biophys. Res. Commun. 112, 183–190.
[6] Sun, I.L., Crane, F.L., Chou, J.L., Löw, H. and Grebig, C. (1983) Biochem. Biophys. Res. Commun. 116, 210–216.
[7] Löw, H. and Werner, S. (1976) FEBS Lett. 65, 96–98.
[8] Neville, D.M. (1968) Biochim. Biophys. Acta 154, 540–552.
[9] Slavik, M., Kartner, N. and Riordan, J. (1977) Biochem. Biophys. Res. Commun. 75, 342–349.
[10] Howland, J.L., Orsin, D., Donatelli, M. and Theofrastous, J.P. (1984) Biochim. Biophys. Acta 778, 1984–1992.
[11] Michejda, I. and Vignais, P.V. (1981) FEBS Lett. 132, 129–132.