Biochimica et Biophysica Acta 965 (1988) 90–92
Elsevier

BBA 20215

BBA Report

The use of glucose dehydrogenase to monitor the integrity of microsomes

Clark Bublitz and Shirley Steavenson

Department of Biochemistry, Biophysics and Genetics, University of Colorado Health Sciences Center, Denver, CO (U.S.A.)

(Received 14 December 1987)

Key words: Microsomal membrane; Glucose dehydrogenase; Mannose-6-phosphatase

Various concentrations of Tergitol NP-10 stimulate mannose-6-phosphatase and glucose dehydrogenase to the same extent in untreated rat liver microsomes. Thus, the latency of glucose dehydrogenase may be used as an alternative to mannose phosphatase as a measure of the integrity of the microsomal membrane. The advantage of using glucose dehydrogenase rather than mannose phosphatase to monitor microsomal integrity is that NADH is more easily measured than P_i.

The latency of mannose-6-phosphatase has been widely used as a convenient measure of the integrity or intactness of microsomes since its introduction for this purpose several years ago by Arion et al. [1]. These workers found various concentrations of taurocholate to alter the microsomal permeability to EDTA and the activity of mannose phosphatase at low substrate concentration to exactly the same extent. Previously, Widnell and co-workers had shown that EDTA does not penetrate into untreated microsomes [2,3]. In principle, the latency of any cisternal enzyme which uses a substrate that does not penetrate the microsomal membrane may be used as a measure of microsomal intactness. The choice of enzyme is largely dependent on the ease with which its activity is measured. The enzyme glucose dehydrogenase or hexose-6-phosphate dehydrogenase (EC 1.1.1.47) is a latent enzyme located in the cisternae of microsomes or the lumen of the hepatic endoplasmic reticulum [4,5]. It also is present in the microsomes of most mammalian tissues [6,7]. Its latency is due largely to the inability of NAD to penetrate the microsomal membrane [4]; recently Nordlie et al. have presented kinetic data which indicate a transport function for free hexoses [8]. The enzyme may be assayed readily by following the glucose-dependent reduction of NAD either fluorometrically or spectrophotometrically. Although this dehydrogenase has a broad substrate specificity, the glucose : NAD oxidoreductase reaction seems best suited to determine latency because this activity is absent in the cytoplasm [9].

Glucose, NAD, Tergitol NP-10, bovine serum albumin, and mannose 6-phosphate were obtained from Sigma. Rat liver microsomes washed once or thrice with 0.15 M Tris-HCl, pH 8.0, were prepared essentially according to the method of Dallner [10]. The washed microsomes were suspended in 1.0 ml 0.25 M sucrose per 25 g liver homogenized.

All reactions were carried out at room temperature (23° C) unless otherwise specified. The system for mannose phosphatase is basically that of Arion et al. [1]. The test system contained in a volume of 0.4 ml: 40 μmol imidazole-HCl, pH 7.0; 20 μmol sodium sulfate; 0.4 μmol mannose phosphate; microsomes; and Tergitol NP-10 as noted.

Correspondence: C. Bublitz, Department of Biochemistry, Biophysics and Genetics, University of Colorado Health Sciences Center, 4200 E. 9th Ave., B-126, Denver, CO 80262, U.S.A.

The complete system except mannose phosphate was incubated at room temperature for 5 min. Then mannose phosphate was added. Four minutes later, the reaction was stopped by the addition of 60 μl 70% (w/v) perchloric acid and 1.54 ml water. After the precipitated protein was removed by centrifugation, detergent was extracted from the supernatant solutions with 2 ml ethylene dichloride. Phosphate was determined in 1.0 ml aliquots of the aqueous phases by the method of Marinetti [11]. The test system for glucose dehydrogenase contained in a volume of 0.4 ml: 40 μmol imidazole-HCl, pH 7.0, or 40 μmol Tris-HCl, pH 8.0; 20 μmol sodium sulfate; 0.4 μmol NAD; 200 μmol glucose; microsomes; and Tergitol NP-10 as noted. Sodium sulfate was added to the system for glucose dehydrogenase because Horne and Nordlie found that this anion stimulated the glucose dehydrogenase reaction [20]. After an incubation of the complete system except NAD at room temperature for 5 min, NAD was added. The reaction was stopped after 20 min by the addition of 1.2 ml ethanol and 20 μl 10% (w/v) sodium sulfate. After the solution was clarified by centrifugation, the concentration of NADH was measured spectrophotometrically. Corrections were made using samples without glucose. The reduction of NAD in this assay is linear with respect to time and concentration of microsomes. Corrections are made for samples without added glucose. This assay is a modification of the method of Glock and McLean [12]. Calculations of the concentration of NADH were made using the molar absorption coefficient of $6.2 \cdot 10^3\ M^{-1} \cdot m^{-1}$ at 340 nm [13]. The reactions are linear with respect to time and concentration of disrupted microsomes. Microsomes were measured by their protein content determined by the method of Lowry et al. [14] with serum albumin as standard. Latency, the percentage of activity of fully disrupted microsomes that is unexpressed in untreated microsomes, is calculated as: latency = 100[1 − (activity in untreated microsomes)/(activity in disrupted microsomes)] [15].

The use of the latency of mannose phosphatase to monitor microsomal intactness stemmed from measurements of EDTA penetration into microsomes which were followed by the solubilization of P_i from lead phosphate precipitates formed on the cisternal side of the membrane in microsomes incubated with lead and glucose 6-phosphate [2,16,17]. Subsequently, Arion et al. [1] found an exact quantitative correspondence between the activation of mannose phosphatase and the induction of the penetration of EDTA at various degrees of disruption of the membrane. This key observation provided definitive proof that the latency of mannose phosphatase is due to the impermeability of mannose phosphate to the membrane and is the experimental underpinning for the use of the latency of mannose phosphatase to monitor the intactness of the microsomal membrane. In order to be certain that the latency of glucose dehydrogenase also is a valid measure of microsomal intactness, it is necessary to show that a precise correspondence exists between the activation of glucose dehydrogenase and either the activation of mannose phosphatase or the induction of the penetration of EDTA at various degrees of disruption of the membrane under the same experimental conditions. The data in Table I show that when measured under the same conditions, except for substrates, the activations of glucose dehydrogenase and mannose phosphatase are the same at various stages of disruption of the membrane caused by different concentrations of Tergitol NP-10. Furthermore, these data indicate that the higher

TABLE I

EFFECTS OF VARIOUS CONCENTRATIONS OF DETERGENT ON MANNOSE PHOSPHATASE AND GLUCOSE DEHYDROGENASE IN INTACT MICROSOMES

The buffer was 0.1 M imidazole-HCl, pH 7.0. Once-washed microsomes (0.49 mg) were used. The maximum activities were: mannose phosphatase, 38.8 nmol P_i formed/4 min; glucose dehydrogenase, 83.9 nmol NADH formed/20 min.

Tergitol NP-10 (μg)	Percent of maximum response	
	mannose phosphatase	glucose dehydrogenase
0	4	6
28	20	19
56	41	44
84	80	88
112	100	100
140	87	95
210	93	95

TABLE II

LATENCIES OF RAT LIVER MICROSOMES AT VARIOUS CONCENTRATIONS OF MICROSOMES AS DETERMINED WITH GLUCOSE DEHYDROGENASE

Thrice-washed microsomes which were stored for 20 h at 2° C were used. The buffer was 0.1 M Tris-HCl, pH 8.0.

Microsomes (μg)	NADH formed (nmol)		Latency (%)
	with 0.2 mg Tergitol NP-10	without Tergitol NP-10	
52	16.9	1.6	91
175	47.4	6.0	87
524	135.4	11.5	92

concentrations of Tergitol NP-10 used in Table I do not significantly inhibit either enzyme.

The experiment described in Table I was done at pH 7.0 because that pH is a suitable compromise to measure the activities of both enzymes [18,19]. However, glucose dehydrogenase is more active in the range pH 7.5–8.0. Therefore the latency of microsomes was tested at pH 8.0 over a range of concentrations of microsomes which were slightly damaged by storage overnight at 2° C (Table II). The data show that the latencies are essentially identical (90 ± 3%) over a 10-fold range in the concentration of microsomes. The results in Tables I and II show that direct measurements of glucose dehydrogenase without corrections for the oxidation of NADH adequately measure the latency of glucose dehydrogenase which is an index of the intactness of the microsomal membrane. Under the conditions used in Table II, the rate of oxidation of added 0.25 mM NADH is 18% that of glucose dehydrogenase.

This study was supported by grant No. PCM-04120 from the National Science Foundation.

References

1 Arion, W.J., Ballas, L.M., Lange, A.J. and Wallin, B.K. (1976) J. Biol. Chem. 251, 4901–4907.
2 Gold, G. and Widnell, C.C. (1976) J. Biol. Chem. 251, 1035–1051.
3 Little, J.S. and Widnell, C.C. (1972) Fed. Proc. 31, 811.
4 Takahashi, T. and Hori, S.H. (1978) Biochim. Biophys. Acta 524, 262–276.
5 Tanahashi, K. and Hori, S.H. (1980) J. Histochem. Cytochem. 28, 1175–1182.
6 Shaw, C.R. (1966) Science 153, 1013–1015.
7 Mandula, B., Srivastava, S.K. and Beutler, E. (1970) Arch. Biochem. Biophys. 141, 155–161.
8 Nordlie, R.C., Stepanik, P.A. and Traxinger, R.R. (1986) Biochim. Biophys. Acta 881, 300–304.
9 Beutler, E. and Morrison, M. (1967) J. Biol. Chem. 242, 5289–5293.
10 Dallner, G. (1974) Methods Enzymol. 31, 191–201.
11 Marinetti, G.V. (1962) J. Lipid Res. 3, 1–20.
12 Glock, G.E. and McLean, P. (1955) Biochem. J. 242, 5289–5293.
13 Horecker, B.L. and Kornberg, A. (1948) J. Biol. Chem. 175, 385–390.
14 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
15 Arion, W.J., Wallin, B.K., Lange, A.J. and Ballas, L.M. (1975) Mol. Cell. Biochem. 6, 75–83.
16 Tice, L.W. and Barrnett, R.J. (1962) J. Histochem. Cytochem. 10, 754–762.
17 Leskes, A., Siekevitz, P. and Palade, G.E. (1971) J. Cell Biol. 49, 264–287.
18 Arion, W.J., Wallin, B.K., Carlson, P.W. and Lange, A.J. (1972) J. Biol. Chem. 247, 2558–2565.
19 Hino, Y. and Minikami, S. (1982) J. Biol. Chem. 257, 2563–2568.
20 Horne, R.N. and Nordlie, R.C. (1971) Biochim. Biophys. Acta 242, 1–13.