Vol. 98, No. 3, 1981 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
February 12, 1981 Pages 588-594

Physical Separation of Cytoplasmic and Microsomal 6-Phosphogluconate Dehydrogenases from Rat Liver

Clark Bublitz
University of Colorado Health Sciences Center
Department of Biochemistry, Biophysics and Genetics
4200 East Ninth Avenue
Denver, Colorado 80262

Received December 5,1980

Summary

Washed rat liver microsomes contain a 6-phosphogluconate dehydrogenase (E.C.1.1.1.44) which is partially extracted by a nonionic detergent. This enzyme can be distinguished from the 6-phosphogluconate dehydrogenase in the microsomal supernatant fraction by separations in polyacrylamide gels by electrophoresis or isoelectric focusing. The possible significance of this enzyme in microsomes is discussed.

Hepatic glucose dehydrogenase (E.C. 1.1.1.47), first described in 1931 (1), has no recognized metabolic role. Its role as a glucose dehydrogenase is unlikely in view of the limited intracellular role of unphosphorylated glucose and its very high apparent Km for glucose. Several years ago Beutler and Morrison (2) found the enzyme from mouse liver also oxidizes several hexose-6-phosphates, one of which is glucose-6-phosphate, with NADP or NAD as electron acceptors. They suggested that glucose dehydrogenase is a hexose phosphate dehydrogenase. Consistent with this idea is the observation that the levels of hexose phosphate dehydrogenase due to glucose dehydrogenase in rat and beef liver are about the same while the levels of glucose dehydrogenase in these two livers are very different (3). This difference is due to the different relative substrate specificities of the beef and rat enzymes. The putative function of this enzyme as a hexose phosphate dehydrogenase, or more specifically a glucose-6-phosphate

0006-291X/81/030588-07$01.00/0

dehydrogenase, would be supported by the existence in the microsomes of a 6-phosphogluconolactonase and 6-phosphogluconate dehydrogenase (E.C. 1.1.1.44) which differ structurally from their cytoplasmic counterparts. These enzymes would increase the rate of formation of NADPH in the microsomes over that due to glucose dehydrogenase alone. Physical evidence indicating the existence of a unique 6-phosphogluconate dehydrogenase in the microsomes is described in this paper. Several years ago the presence of a microsomal 6-phosphogluconate dehydrogenase was reported briefly (4) and the existence of this enzyme is implied in the work of Beutler and Morrison (2).

Methods and Materials

The microsomal extract and cytoplasmic or supernatant fraction used as sources of 6-phosphogluconate dehydrogenase were prepared from fresh rat liver essentially according to the method of deDuve et al (5). Diced liver (50 g) was homogenized in 200 mL 0.25 M sucrose in a Potter-Elvehjem teflon-glass homogenizer for 2 min. The homogenate was centrifuged at 17,600 x g for 10 min and the residue which formed was discarded. The clarified supernatant fraction was separated into the cytoplasmic or supernatant fraction and the microsomes (the pellet) by centrifugation at 105,000 x g for 30 min. The microsomes were washed three times in 200 mL 0.25 M sucrose. The washed microsomes were suspended in 2 mL water. The microsomal suspension was extracted by gently shaking it in an ice bath for 30 min with 0.85 mL 0.7 M Tris-glycine, pH 7.5, 2.67 mL 5% (v/v) ICI Americas G-7181 (A polyoxethylene (8) dodecyl alcohol) and 3 mL water. This mixture then was centrifuged at 105,000 x g for 30 min. The supernatant fraction from this centrifugation is the microsomal extract. This extract (0.02 mL) forms 0.65 nmoles NADPH per min dependent upon 6-phosphogluconate. Detection of 6-phosphogluconate dehydrogenase in the residue indicates that the extraction of the enzyme from the microsomes is incomplete. Both the cytoplasmic or microsomal supernatant fraction and the microsomal extract were dialyzed overnight vs. 0.01 M maleate, 1 mM EDTA, pH 6.5 in the cold with external stirring. The isoelectric focusing and electrophoretic patterns were cleaner with dialyzed than undialyzed enzyme preparations. The dialyzed microsomal extract was concentrated to 2 mL by ultrafiltration on an Amicon UM10 membrane before analysis by electrophoresis or isoelectric focusing.

Phosphogluconate dehydrogenase was assayed in a Farrand fluorometer by measuring the emission of light at 460 nm by NADPH following absorption at 340 nm. The assays were made in a system (1.5 mL) containing either 100 umoles glycylglycine, pH 8.3, 30 umoles $MgCl_2$, 0.4 umoles NADP, 4 umoles phosphogluconate and enzyme or 100 umoles Tris, pH 7.3, 0.4 umoles NADP, 4 umoles phosphogluconate and enzyme. Both assays gave similar values for the cytoplasmic and microsomal enzymes. The enzyme was located on gels subjected either to electrophoresis or isoelectric focusing by soaking the gels at room temperature in the dark in a solution (5 mL) containing: 1.5 mg nitroblue tetrazolium, 0.15 mg phenazine methosulfate, 1.25 mmoles Tris, pH 7.3, 4 umoles NADP, 20 umoles phosphogluconate. The gels were washed with water. The enzyme was located by comparing the staining pattern produced by the complete staining solution given above with one with all components except phosphogluconate. The intensity of the stain depended not only upon the level of enzyme but also upon the amount of lipid or detergent present. The gels increased about 10% in length during the staining process.

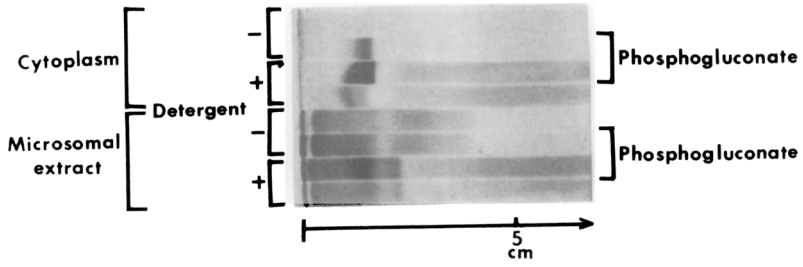

Fig. 1. The effect of ICI Americas G-7181 on the electrophoresis of cytoplasmic and microsomal 6-phosphogluconate dehydrogenases. The procedure is described in the text. Additions to the gels and the enzyme stain are given in the figure.

Electrophoresis was carried out in 10.5 cm. columns of polyacrylamide gel. The gels were prepared from 2.0 mL 0.7 M Tris-glycine, pH 7.5, 4.5 mL polyacrylamide (22.2 g acrylamide and 0.6 g methylenebisacrylamide per 100 mL), 0.04 mL TEMED, 12.86 mL H_2O, 0.6 mL 1.2% ammonium persulfate. Gels with detergent contained also 0.1% ICI Americas G-7181.The electrode buffer was 0.07 M Tris-glycine, pH 7.5. The gels were run at 250 V for 30 min before addition of enzyme. The enzyme preparations added to the gels contained 0.07 M Tris-glycine, pH 7.5, 0.2 saturated sucrose and, where indicated, 0.1% ICI Americas G-7181. These gels were stained for 2 hrs at room temperature.

Isoelectric focusing was carried out in polyacrylamide columns (9 cm) in the apparatus of Righetti and Drysdate (6). The gels were prepared by mixing 1.2 mL acrylamide (20 g acrylamide and 0.8 g methylenebisacrylamide per 100 mL), 0.06 mL 10% (v/v) ICI Americas G-7181, 4.33 mL water, 6.7 uL TEMED, 0.5 mL 40% ampholytes, pH 3.5 - 10, 133 uL 2% ammonium persulfate. The polymerized gels were overlayed with 2% ampholytes and subjected to 1 mA/column for 30 min. Enzymes in 0.1% ICI Americas G-7181, 0.2 saturated sucrose, 2% ampholytes were added. The gels were subjected to 150 V for 12 hrs and then 400 V for 30 min. The electrode solutions were 0.1 M acetic acid and 0.1 M NH_4OH. The pH profile was determined by cutting a replicate gel into segments of 1 cm and shaking these segments for 30 min in 0.5 mL water. The pH of these extracts was then recorded. The gels were stained for dehydrogenase for 15 min at room temperature.

Nitroblue tetrazolium, phenazine methosulfate, 6-phosphogluconate and NADP were purchased from Sigma. The 40% ampholytes pH 3.5-10 were obtained from LKB. ICI Americas G-7181 was the gift of Dr. G.J. Stockberger.

Results and Discussion

The data in Fig. 1 show that in the presence of the nonionic detergent cytoplasmic phosphogluconate dehydrogenase has a slightly higher electrophoretic mobility than the microsomal enzyme. This difference, though small, is reproducible. More striking are the differences in electrophoretic behavior of the two enzymes when detergent is omitted from the gels. The mobility of the cytoplasmic enzyme is unaffected by the detergent. However, the mobility of the microsomal enzyme depends upon the addition of detergent to the gel; this enzyme remains at the

origin in gels without detergent. Even when detergent was added to the gel, some of the microsomal enzyme remained at the origin in the experiment described in Fig. 1. Thus, the microsomal enzyme is insoluble in the absence of detergent. Fig. 1 also shows that detergent intensifies the cytochemical staining reaction in several of the gels. Furthermore, the detergent sometimes elicits a staining reaction. This is most clearly seen by examining the patterns produced by the staining mixtures without phosphogluconate when the dialyzed cytoplasm is electrophoresed. Only the sample containing detergent is stained. Other experiments indicate that there is also a requirement for either NAD or NADP for the development of this stain independent of substrate. These results thus suggest that lipids may have an important role in the development of the "nothing dehydrogenase" stain observed in many histochemical studies (7).

The data in Fig. 2 show that the microsomal and cytoplasmic dehydrogenase focus at different pH's when detergent is added. When the two enzymes are added together, the pattern is essentially the sum of the two individual components. The estimated pI's are 4.5 for the microsomal enzyme and 5.1 for the cytoplasmic dehydrogenase. The pH gradient is in Fig. 3.

The identification of a microsomal 6-phosphogluconate dehydrogenase distinct from its cytoplasmic counterpart supports the idea that microsomal glucose dehydrogenase functions as a glucose-6-phosphate dehydrogenase. The sequential action of these two enzymes could form NADPH twice as fast as glucose-6-phosphate dehydrogenase alone. The presence of both dehydrogenases in the microsomes suggests that they are functioning together and that other enzymes of the pentose phosphate pathway may be found in the microsomes. However, a more precise localization of these enzymes in the microsomal fraction is needed before the idea that these dehydrogenases are functioning sequentially can be accepted. The NADPH produced by these two hydrogenases probably is used in a biosynthetic reduction in the microsomes though there is a possibility that it may function in a translocation process along or through one of the membranes making up the microsomes. It is also possible that these dehydrogen ases have a metabolic functions other than direct participation in the pentose

phosphate pathway. There is good evidence that certain enzymes which catalyze reactions of other metabolic pathways have such special functions. One such example

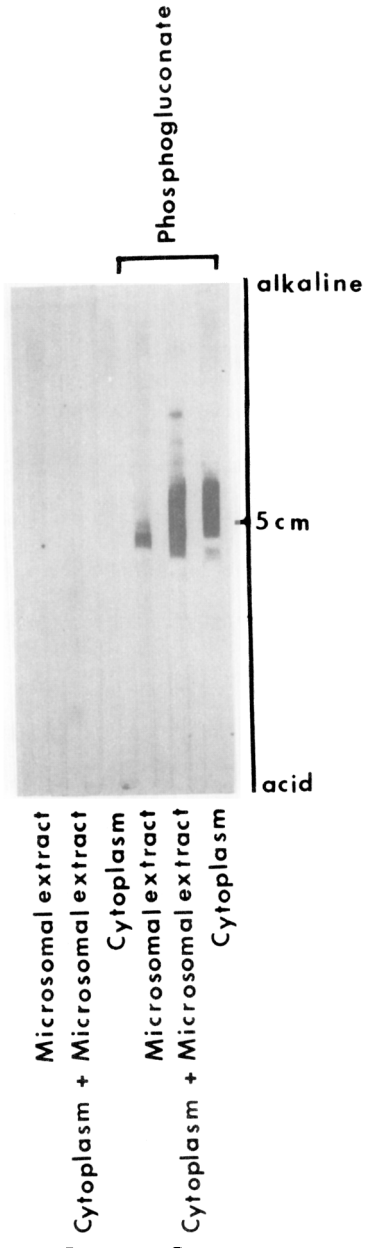

Fig. 2. Isoelectric focusing pattern of cytoplasmic and microsomal 6-phosphogluconate dehydrogenases. The procedure is described in the text. Additions to the gels and enzyme stains are given in the figure.

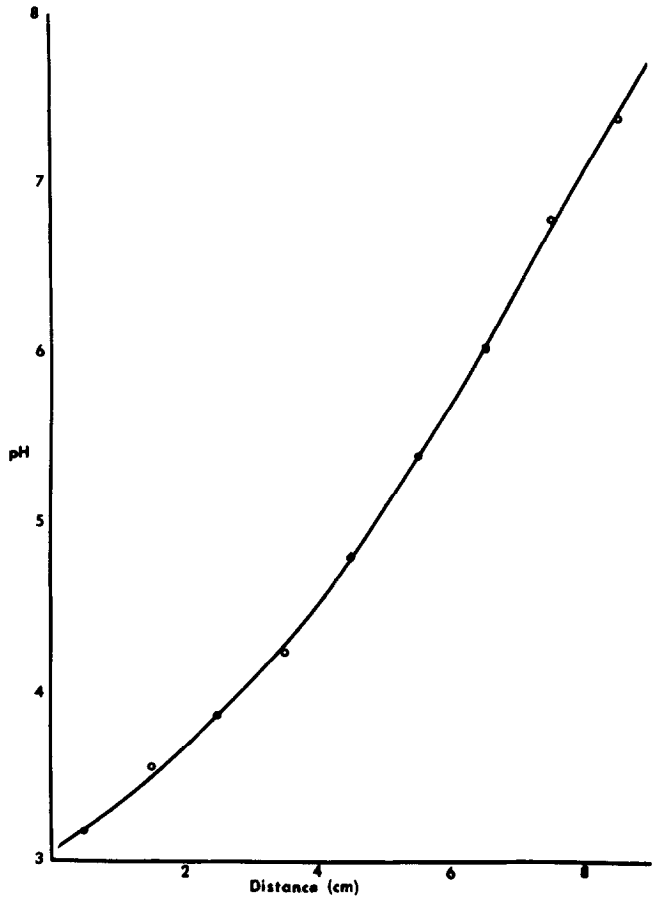

Fig. 3. pH gradient produced during isoelectric focusing.

is cytoplasmic malic dehydrogenase which catalyzes one of the reactions of the citric acid cycle but functions in the mitochondrial oxidation of cytoplasmic NADH (8).

Acknowledgement

The author gratefully acknowledges the excellent assistance of Charlotte Jensen.

References

1. Harrison, D.C. (1931). Biochem. J. 25, 1016-1027.
2. Beutler, E. and Morrison, M. (1967). J. Biol. Chem. 242, 5289-5293.

3. Bublitz, C. (1981). Biochem. Biophys. Res. Commun. 98, 455-462.
4. Yamada, K. and Shimazono, H. (1961). Biochim. Biophys. Acta 54, 205-206.
5. deDuve, C., Pressman, B.C., Gianetto, R., Wattaux, R., and Appleman, F. (1955). Biochem. J. 60, 604-617.
6. Righetti, P.G. and Drysdale, J.W. (1973). Ann. N.Y. Acad. Sci. 209, 163-186.
7. Anderson, H. (1965). Acta Histochem. 21, 120-134.
8. La Noue, K.F. and Williamson, J.R. (1971). Metabolism 20, 119-140.