BBA Report

BBA 61250

Intracellular distribution of isoenzymes of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase in rat adipose tissue

RIVKA BEITNER and ZWI NAOR

Department of Life-Sciences, Bar-Ilan University, Ramat-Gan (Israel)

(Received May 3rd, 1972)

SUMMARY

Four isoenzymes of glucose-6-phosphate dehydrogenase (D-glucose-6-phosphate: $NADP^+$ oxidoreductase, EC 1.1.1.49) and two isoenzymes of 6-phosphogluconate dehydrogenase (6-phospho-D-gluconate:$NADP^+$ oxidoreductase (decarboxylating), EC 1.1.1.44) were found in rat adipose tissue. The different isozymic forms of both activities differed in their subcellular locations. Different isoenzymes were detected in the mitochondrial, microsomal and supernatant fractions. The particle-associated forms of both enzymes were activated by Mg^{2+}, whereas the supernatant isoenzymes were not affected by addition of Mg^{2+}.

An earlier study done in this laboratory[1] indicated that in order to demonstrate all the isoenzymes of glucose-6-phosphate dehydrogenase (D-glucose-6-phosphate:$NADP^+$ oxidoreductase, EC 1.1.1.49) from rat tissues, specific methods of extraction are required, namely, addition of Triton X-100 to the homogenization medium, and freezing and thawing of the tissues[2]. This led us to the conclusion that certain isoenzymes of glucose-6-phosphate dehydrogenase may be associated with particulate components, being fully released only by the above mentioned methods. A similar observation was obtained for the isoenzymes of 6-phosphogluconate dehydrogenase (6-phospho-D-gluconate:$NADP^+$ oxidoreductase (decarboxylating), EC 1.1.1.44). The present investigation was intended to define the intracellular distribution of the different forms of the two enzymes in rat adipose tissue. A specific association of a given molecular form of the enzymes with particular subcellular organelles may help to clarify the physiological role of the isoenzymes.

Cell fractionation was carried out by means of a slightly modified procedure of Hogeboom[3]. Epididymal fat pads from 120–150 g male albino rats were homogenized in

9 parts of cold 0.25 M sucrose, containing 1 mM EDTA. Cell debris, unbroken cells, and nuclei were removed by centrifuging twice at 700 × *g* for 10 min. The supernatant was then subjected to successive centrifugation at 5000 × *g* for 10 min, at 15 000 × *g* for 20 min and at 105 000 × *g* for 60 min. The sediments were washed twice with sucrose and designated as heavy mitochondrial, light mitochondrial and microsomal fractions, respectively. To release the enzymes, these particulate fractions were then frozen and thawed and suspended in 3 parts of cold distilled water containing 1 mM EDTA, 5 mM $MgCl_2$ and 0.5% Triton X-100. These suspensions, as well as the supernatant from the microsomal fraction, were subjected to electrophoresis.

For preparation of whole tissue extract, whole epididymal fat pads were immediately frozen and thawed and homogenized in cold distilled water containing 1 mM EDTA, 5 mM $MgCl_2$ and 0.5% Triton X-100. This extract was centrifuged for 30 min at 100 000 × *g* and the supernatant was subjected to electrophoresis. For experiments in which the effect of Mg^{2+} was studied, the $MgCl_2$ was omitted from the extraction medium. The electrophoretic fractionation was performed on cellogel strips using the Beckman microzone electrophoresis cell (Model R-101).

The intracellular distribution of the different forms of glucose-6-phosphate dehydrogenase of the rat adipose tissue is shown in Fig. 1. In the whole tissue extract four isozymic forms were detected. They were designated as A–D (A, being the fastest migrating anodal band). The four isoenzymes differed in subcellular location. Isoenzyme D was found primarily in the mitochondrial fractions. Isoenzyme C was detected exclusively in the microsomal fraction. Isoenzyme B was detected in both mitochondrial

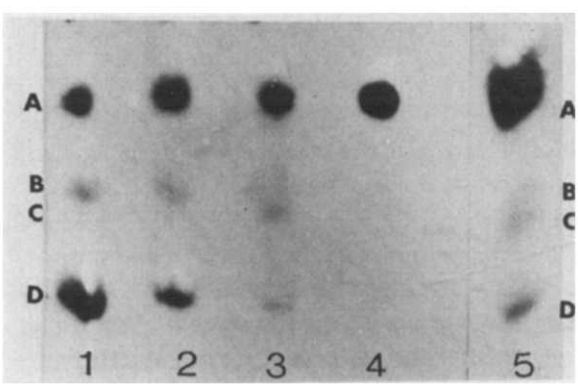


Fig. 1. Electrophoretic separation of molecular forms of glucose-6-phosphate dehydrogenase in rat adipose tissue. 1, heavy mitochondrial fraction; 2, light mitochondrial fraction; 3, microsomal fraction; 4, supernatant; 5, whole tissue extract. Electrophoresis on cellogel strips for 90 min, in 0.075 M Tris, 0.075 M citric acid and 0.005 M EDTA, pH 7.5. The cellogels were developed for glucose-6-phosphate dehydrogenase activity at 37°C for 30 min in an incubation medium consisting of 0.3 M Tris–HCl buffer, pH 7.6, 7 mM $MgCl_2$, 0.4 mM $NADP^+$, 0.1 mM phenazine methosulfate, 0.4 mM nitro-blue tetrazolium and glucose-6-phosphate at 1.5 mM. 15 experiments were carried out with identical results.

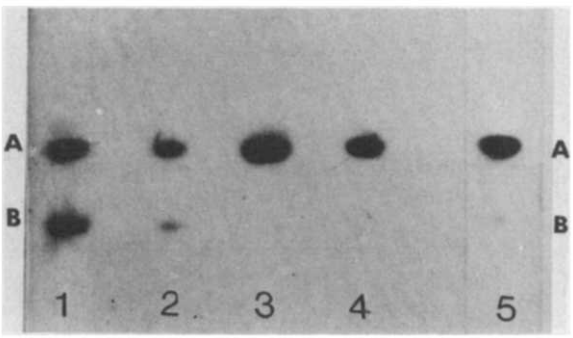


Fig. 2. Electrophoretic separation of molecular forms of 6-phosphogluconate dehydrogenase in rat adipose tissue. 1, heavy mitochondrial fraction; 2, light mitochondrial fraction; 3, microsomal fraction; 4, supernatant; 5, whole tissue extract. Electrophoresis and color development were conducted as for Fig. 1, except for addition of 6-phosphogluconate at 1.5 mM, instead of glucose-6-phosphate. 15 experiments were carried out with identical results.

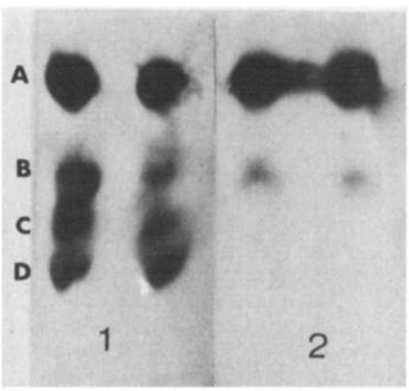
and microsomal fractions whereas isoenzyme A was found in the supernatant as well as in other cell fractions.

The intracellular distribution of the different forms of 6-phosphogluconate dehydrogenase of the rat adipose tissue is shown in Fig. 2. In the whole tissue extract two isozymic forms were detected, designated as A and B. Isoenzyme B was detected exclusively in the mitochondrial fractions, whereas isoenzyme A was found in the supernatant as well as in other fractions.

The question now arises whether these different isozymic forms of the two enzymes, differing in their intracellular location, also respond differently to various regulatory conditions. As $MgCl_2$ is routinely employed in assaying these enzymes, we found it of interest to study its effect on the different isozymic forms of the two enzymes. As shown in Fig. 3, omission of Mg^{2+} from the incubation medium, caused a complete disappearance of bands D and C and reduction in band B of glucose-6-phosphate dehydrogenase activity, whereas band A was unaffected.

We also found that the isoenzymes of 6-phosphogluconate dehydrogenase respond differently to Mg^{2+} (Fig. 4). Omission of Mg^{2+} from the incubation medium caused a complete disappearance of isoenzyme B, whereas isoenzyme A was unaffected.

There is a striking similarity between the different isozymic forms of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase with regard both to their intracellular locations and their responses to Mg^{2+}. Specific mitochondrial isoenzymes were detected in both instances. The supernatant, in both cases, contained only one isoenzyme. This isoenzyme was also present in other cell fractions. The specific particle-associated isoenzymes of both glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase were activated by Mg^{2+}, whereas the supernatant isoenzymes did not require presence of Mg^{2+}.



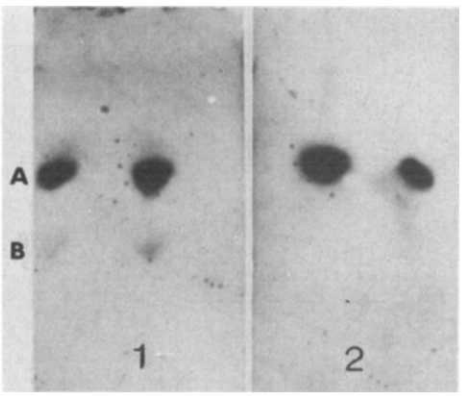


Fig. 3. The effect of Mg^{2+} on the different forms of glucose-6-phosphate dehydrogenase of the rat adipose tissue. 1, with Mg^{2+}; 2, without Mg^{2+}. Whole tissue extract was subjected to electrophoresis. Electrophoresis was conducted as for Fig. 1. After electrophoresis the cellogels were cut to two halves. One half was developed for glucose-6-phosphate dehydrogenase activity in an incubation medium containing 7 mM $MgCl_2$, corresponding to Fig. 1, while the other half was developed in an identical medium, except for absence of $MgCl_2$. Both experiments were performed in duplicates. 10 experiments were carried out with identical results.

Fig. 4. The effect of Mg^{2+} on the different forms of 6-phosphogluconate dehydrogenase of the rat adipose tissue. 1, with Mg^{2+}; 2, without Mg^{2+}. Conditions as for Fig. 3. After electrophoresis the cellogels were cut to two halves. One half was developed for 6-phosphogluconate dehydrogenase activity in the presence of 7 mM $MgCl_2$, while the other half, in the absence of $MgCl_2$. Both experiments were performed in duplicates. 10 experiments were carried out with identical results.

Several other enzymes have been found to exist in separate isozymic forms in supernatant and the particulate fractions of tissue homogenates[4-6]. We are trying to extend this kind of investigation to other glycolytic enzymes as well, in the hope that these studies may throw some light on an earlier finding in which we noted intracellular compartmentation of glycolytic phosphate esters[7,8].

This research was supported in part by the Research Committee, Bar-Ilan University.

REFERENCES

1 Lahat, N., Beitner, R. and Pinsky, A., *Nature*, in the press
2 Morton, R.K. (1955) in *Methods in Enzymology* (Colowick, S.P. and Kaplan, N.O., eds), Vol. 1, pp. 25–51, Academic Press, New York
3 Hogeboom, G.H. (1955) in *Methods in Enzymology* (Colowick, S.P. and Kaplan, N.O., eds), Vol. 1, pp. 16–19, Academic Press, New York
4 Henderson, N.S. (1968) *Ann. N.Y. Acad. Sci.* 151, 429–440
5 Domenech, E.M., Domenech, C.E. and Blanco, A. (1970) *Arch. Biochem. Biophys.* 141, 147–154

6 Agostoni, A., Vergani, C. and Villa, L. (1966) *Nature* 209, 1024–1025
7 Kalant, N. and Beitner, R. (1971) *J. Biol. Chem.* 246, 504–507
8 Beitner, R. and Kalant, N. (1971) *J. Biol. Chem.* 246, 500–503

The Author and Subject index of this volume can be found on p. 741 and p. 746, respectively.

ANNOUNCEMENT

REFERENCES

As from the present date Elsevier Publishing Company, publisher of Biochimica et Biophysica Acta, intends to implement the recommendations of the Commission of Editors of Biochemical Journals of the International Union of Biochemists concerning references.

The recommended style is as follows:

Pispa, J.P. and Buchanan, F.M. (1971) *Biochim. Biophys. Acta* 247, 181–184

It should be noted that first and last pages are cited; this will be optional for papers published during 1972, but will become mandatory in 1973.

In the reference list, titles of journals should be abbreviated in accordance with the Chemical Abstracts Service (American Chemical Society) system.

Examples of the style to be used in the reference list for references to books are:

Dixon, M. and Webb, E.C. (1964) *Enzymes*, 2nd edn, p. 565, Longmans Green and Co., London

Grant, J.K. (1969) in *Essays in Biochemistry* (Campbell, P.N. and Greville, G.D., eds), Vol. 5, pp. 1–58, Academic Press, London and New York

Contrary to paragraph 5, p. 6, of "Information to Contributors" of Biochimica et Biophysica Acta, unpublished work of one or more of the authors should not be listed under References, but should be mentioned in the text as follows:

(Spector, R. and Glimcher, J., unpublished)