BBA Report

BBA 61257

Isoenzymes of $NADP^+$- and NAD^+-glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase in rat adipose tissue

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(Received June 22nd, 1972)

SUMMARY

Glucose-6-phosphate dehydrogenase (D-glucose-6-phosphate:$NADP^+$ oxidoreductase, EC 1.1.1.49) and 6-phosphogluconate dehydrogenase (6-phospho-D-gluconate:$NADP^+$ oxidoreductase (decarboxylating), EC 1.1.1.44) of rat adipose tissue, were shown to react not only with $NADP^+$ but also with NAD^+. Different isoenzymes were involved in the NAD^+-linked and $NADP^+$-linked reactions. The intracellular distribution of the NAD^+-linked isoenzymes differed from that of $NADP^+$-linked isoenzymes.

Glucose-6-phosphate dehydrogenase (D-glucose-6-phosphate:$NADP^+$ oxidoreductase, EC 1.1.1.49) from brewer's yeast[1], *Candida utilis*[2] and *Escherichia coli*[3] was shown to react exclusively with $NADP^+$. Levy[4], however, has reported that glucose-6-phosphate dehydrogenase from rat mammary gland, adrenal, and infant liver and brain, reacts with $NADP^+$ but also displays weak activity with NAD^+. Yoshida[5] has found that glucose-6-phosphate dehydrogenase from erythrocytes will also react with NAD^+. Investigations of the rat mammary enzyme have shown that the activities with NAD^+ and $NADP^+$ respond differently to various reagents and conditions[6–8].

In our earlier studies[9,10] we have demonstrated the presence of four isoenzymes of glucose-6-phosphate dehydrogenase and two isoenzymes of 6-phosphogluconate dehydrogenase (6-phospho-D-gluconate:$NADP^+$ oxidoreductase (decarboxylating), EC 1.1.1.44) in rat adipose tissue. On the basis of the above-mentioned findings we considered an investigation of the coenzyme specificity of these isoenzymes to be important.

Preparation of epididymal fat pads extract, and cell fractionation were carried out as described previously[10]. The electrophoretic fractionation was performed on cellogel strips using the Beckman microzone electrophoresis cell (Model R-101).

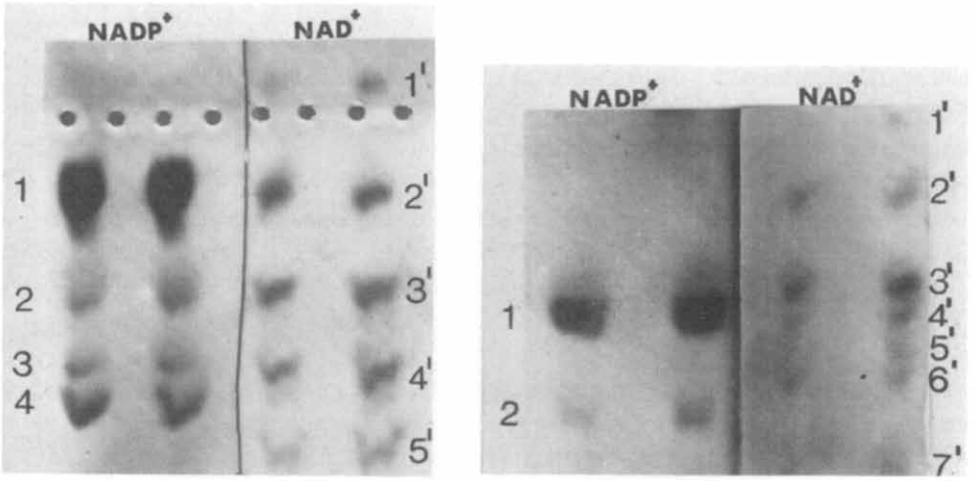


Fig. 1. Isoenzymes of $NADP^+$-glucose-6-phosphate dehydrogenase and NAD^+-glucose-6-phosphate dehydrogenase from rat adipose tissue resolved by cellogel electrophoresis. An extract from epididymal fat pads was subjected to electrophoresis. Electrophoresis was conducted on cellogel strips at 190 V for 90 min in 0.075 M Tris, 0.075 M citric acid and 0.005 M EDTA (pH 7.5). After electrophoresis the cellogels were cut in half. One half (left) was 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), 1.5 mM glucose-6-phosphate, 7 mM $MgCl_2$, 0.1 mM phenazine methosulfate, 0.4 mM nitro-blue tetrazolium, and $NADP^+$ at 0.4 mM. The other half (right) was developed in an identical medium except that it contained 0.4 mM NAD^+ instead of $NADP^+$. Both experiments were performed in duplicate. Ten experiments were carried out with identical results.

Fig. 2. Isoenzymes of $NADP^+$-6-phosphogluconate dehydrogenase and NAD^+-6-phosphogluconate dehydrogenase from rat adipose tissue resolved by cellogel electrophoresis. Electrophoresis and color development were carried out as for Fig. 1, except for the addition of 6-phosphogluconate at 1.5 mM, instead of glucose-6-phosphate. Left, color development in the presence of 0.4 mM $NADP^+$. Right, color development in the presence of 0.8 mM NAD^+ instead of $NADP^+$. Both experiments were performed in duplicate. Ten experiments were carried out with identical results.

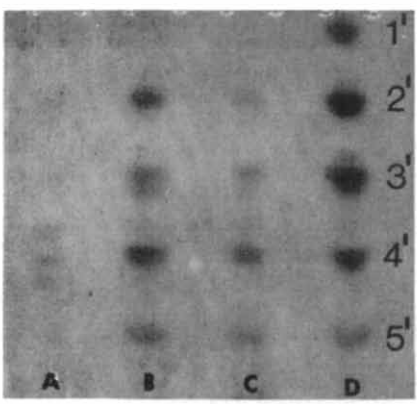
Fig. 1 reveals that rat adipose tissue glucose-6-phosphate dehydrogenase reacts not only with $NADP^+$ but also with NAD^+. Five isozymic forms were detected after cellogel electrophoresis when NAD^+, instead of $NADP^+$, was added at an equal concentration to the staining mixture. They were designated as 1′–5′ (1′, being the fastest migrating anodal form). Isoenzymes 2′, 3′ and 4′ had an electrophoretic mobility identical to that of isoenzymes 1, 2 and 3, respectively, of the $NADP^+$-glucose-6-phosphate dehydrogenase. Surprisingly, two different isoenzymes of the NAD^+-glucose-6-phosphate dehydrogenase were detected: isoenzymes 1′, which was the fastest migrating anodal form, and isoenzyme 5′, which was the slowest migrating anodal form. No change in the number of isoenzymes was observed with increase in the concentration of NAD^+. No staining was observed in control experiments in which both $NADP^+$ and NAD^+ were absent from the staining mixture.

Fig. 2 reveals that rat adipose tissue 6-phosphogluconate dehydrogenase also reacts with both $NADP^+$ and NAD^+. Different isoenzymes were involved in the $NADP^+$-

linked and the NAD^+-linked reactions. Two isozymic forms of the $NADP^+$-6-phosphogluconate dehydrogenase (designated as 1 and 2) were detected, whereas seven isozymic forms of NAD^+-6-phosphogluconate dehydrogenase (designated as 1′–7′) were found. No staining was observed in control experiments in which both $NADP^+$ and NAD^+ were absent from the staining mixture. The seven isozymic forms of NAD^+-6-phosphogluconate dehydrogenase were detected only in the presence of a doubled concentration of NAD^+ (0.8 mM). In the presence of 0.4 mM NAD^+ only isoenzymes 2′, 3′, 4′ and 6′ could be detected, indicating that the other three isoenzymes have a smaller affinity to react with NAD^+. A further increase in the concentration of NAD^+, above 0.8 mM, did not change the number of the isoenzymes.

We are unaware of any report indicating that mammalian 6-phosphogluconate dehydrogenase can react with NAD^+. We realize again the great similarity in the behavior of this enzyme and glucose-6-phosphate dehydrogenase, as already pointed out earlier[10].

An earlier study done in this laboratory[10] indicated that the different isozymic forms of the $NADP^+$-linked glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase differed in their subcellular locations. We have now extended this kind of investigation to the NAD^+-linked isoenzymes. Figs 3 and 4 show the intracellular distribution of the NAD^+-linked isoenzymes. The supernatant contained all the five isoenzymes of the NAD^+-glucose-6-phosphate dehydrogenase (Fig. 3). These isoenzymes were also detected in the mitochondrial and microsomal fractions, except for isoenzyme 1′ which



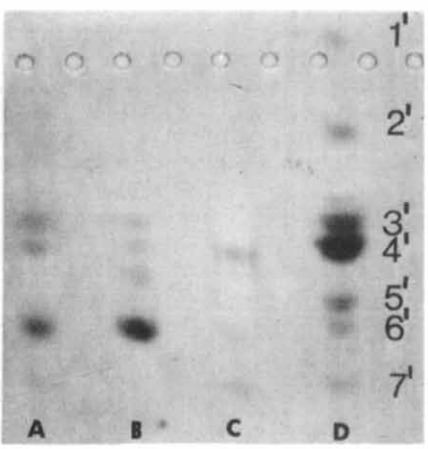


Fig. 3. Intracellular distribution of isoenzymes of NAD^+-glucose-6-phosphate dehydrogenase from rat adipose tissue resolved by cellogel electrophoresis. A, heavy mitochondrial fraction; B, light mitochondrial fraction; C, microsomal fraction; D, supernatant. Cell fractionation was carried out as described earlier[10]. The different fractions were subjected to electrophoresis which was conducted as for Fig. 1. Color development was carried out as for Fig. 1, in the presence of 0.4 mM NAD^+. Ten experiments were carried out with identical results.

Fig. 4. Intracellular distribution of isoenzymes of NAD^+-6-phosphogluconate dehydrogenase from rat adipose tissue resolved by cellogel electrophoresis. A, heavy mitochondrial fraction; B, light mitochondrial fraction; C, microsomal fraction; D, supernatant. Conditions as for Fig. 3. The cellogels were developed for 6-phosphogluconate dehydrogenase activity in the presence of 0.8 mM NAD^+. Ten experiments were carried out with identical results.

was found exclusively in the supernatant. All the seven isozymic forms of the NAD^+-6-phosphogluconate dehydrogenase were also found in the supernatant (Fig. 4). In the mitochondrial fractions, isoenzymes 3′, 4′, 5′ and 6′ were detected, whereas the microsomal fraction contained isoenzymes 4′, 6′ and 7′. Isoenzymes 1′ and 2′ were present exclusively in the supernatant.

The intracellular distribution of the NAD^+-linked isoenzymes of both glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase differed from that of the $NADP^+$-linked isoenzymes. As we have previously reported[10] only one isoenzyme of the $NADP^+$-linked glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase was found in the supernatant. In addition, specific mitochondrial and microsomal isoenzymes of the $NADP^+$-linked activities, absent from other cell fractions, were detected.

We have shown previously[10] that the particle-associated forms of the $NADP^+$-dependent enzymes, were activated by Mg^{2+}, whereas the supernatant isoenzymes were not affected by addition of Mg^{2+}. Similar experiments with the NAD^+-dependent enzymes revealed that none of the isoenzymes was affected by Mg^{2+}.

The behavior of glucose-6-phosphate and 6-phosphogluconate dehydrogenases resembles that of isocitrate and malate dehydrogenases[11]. All these four enzymes have been found to exist in separate isozymic forms in supernatant and the particulate fractions of tissue homogenates, and to react with both $NADP^+$ and NAD^+. Based on an earlier finding in which we noted intracellular compartmentation of glycolytic phosphate esters[12,13], we believe that certain glycolytic enzymes may also exist in separate isozymic forms in supernatant and the particulate fractions.

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

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