NADPH-D-GLYCERALDEHYDE 3-PHOSPHATE OXIDOREDUCTASE ACTIVITY IN MUSCLE AND OTHER TISSUES OF THE RAT

Terry Wood

Department of Biochemistry, McGill University, Montreal, Quebec, Canada

Received July 9, 1973

SUMMARY: Rat muscle was found to contain a NADPH-D-glyceraldehyde 3-phosphate oxidoreductase. Of the ten tissues studied, muscle contained the highest activity at 0.90 ± 0.06 units/g. The activity was not due to L-glycerol 3-phosphate-NAD^+ oxidoreductase utilising NADPH as a hydrogen donor, nor to coupling of the latter with a NADPH-NAD^+ oxidoreductase. After partial inhibition of contaminating triose phosphate isomerase with glycidol phosphate, the oxidoreductase rate was faster with glyceraldehyde phosphate than with dihydroxyacetone phosphate as a substrate. Apparent K_m values of 14 μM for D-glyceraldehyde 3-phosphate and 0.7 μM for NADPH were determined.

In the course of experiments to investigate the operation of the Pentose Phosphate Pathway in rat tissues, muscle extract was observed to catalyse the reduction of D-glyceraldehyde 3-phosphate in the presence of NADPH to form glycerol 3-phosphate. This reaction appeared to be due to the presence of a hitherto undescribed NADPH-D-glyceraldehyde 3-phosphate oxidoreductase. The present communication describes the distribution of activity in rat tissues and some of the properties of the enzyme from muscle.

EXPERIMENTAL

Preparation of tissue supernatants. Tissues were cooled in ice and homogenised in 4 ml/g of ice-cold triethanolamine-HCl buffer containing 2 mM dithiothreitol, pH 7.4. The homogenates were centrifuged for 90 min at 4° and 58,000 g and the clear supernatants collected.

Enzyme assays. The activity was measured spectrophotometrically at 37° in 50 mM triethanolamine-HCl buffer, pH 7.4, containing 1 mM dithiothreitol and 0.1 mM NADPH. The rate of NADPH oxidation with enzyme alone was first recorded, then DL-glyceraldehyde 3-phosphate was added to 0.4 mM and the rate measured again, and the activity was

Table 1. Distribution of NADPH-D-glyceraldehyde 3-phosphate oxidoreductase activity in the soluble fraction of rat tissues

Values are means ± S.E.M. units/g. tissue for 4 animals. Assays were performed at 37° and pH 7.4 as described in the text.

	activity (units/g. tissue)		activity (units/g. tissue)
muscle	0.90 ± 0.03	lung	0.05 ± 0.01
liver	0.16 ± 0.02	spleen	0.05 ± 0.01
heart	0.17 ± 0.02	intestine	< 0.02
kidney	0.22 ± 0.03	uterus	< 0.02
brain	0.07 ± 0.01	blood	< 0.02

calculated from the difference of the two rates. L-Glycerol 3-phosphate-NAD^+ oxidoreductase (EC 1.1.1.8) activity was measured by substituting NADH for NADPH, and 0.2 mM dihydroxyacetone phosphate for the glyceraldehyde 3-phosphate. NADPH-NAD^+ oxidoreductase (EC 1.6.1.1) activity was assayed as described by Kaplan et al. (1). The rate of the NADPH-D-glyceraldehyde 3-phosphate oxidoreductase was also measured at 22° in a Turner 450 spectrofluorometer attached to a recorder. The reaction mixture contained 50 mM triethanolamine-HCl buffer, pH 7.4, 0.1 mM dithiothreitol, 100 μg/ml bovine serum albumin, the enzyme sample, and between 1.5 and 8 μM NADPH. The reaction was initiated by the addition of triose phosphate and corrected for the rate in its absence.

RESULTS

Enzyme assays. The activity of the enzyme in the supernatant fraction of various rat tissues is given in Table 1. The positions of the activity and that of L-glycerol 3-phosphate-NAD^+ oxidoreductase, following chromatography of a muscle supernatant on DEAE-Sephadex, is shown in Fig. 1. The NADPH-NAD^+ oxidoreductase in a muscle supernatant was less than 1% of its NADPH-D-glyceraldehyde 3-phosphate oxidoreductase activity.

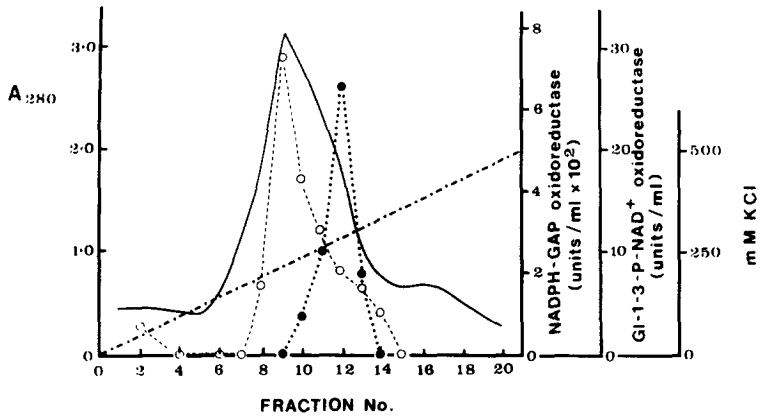

Fig. 1. Enzyme distribution after chromatography on DEAE-Sephadex A-50. A three-fold diluted muscle supernatant (45ml) was passed through a 2 x 4 cm column of absorbent packed in 20mM tris-HCl buffer, pH 9.0, containing 1mM EDTA and 1mM mercaptoethanol. The protein was washed through with 30ml of the same buffer before elution with a linear gradient of 50ml of the buffer containing 500mM KCl at pH 8.5 running into 50ml of the buffer, pH 8.5. Fractions of 5 ml were collected.
o-----o NADPH-glyceraldehyde 3-phosphate (GAP) oxidoreductase activity
●·····● L-glycerol 3-phosphate (Gl-3-P)-NAD^+ oxidoreductase activity
——— Absorbance at 280nm in a 1 cm cell.
—·—·— KCl gradient

Nature of the substrate. Experiments with a muscle supernatant indicated that the initial reaction was slightly faster with glyceraldehyde phosphate than with dihydroxyacetone phosphate and that, with the former substrate, subsequent addition of triose phosphate isomerase slowed the reaction, an effect that was not reproduced by addition of the amount of ammonium sulphate present in the isomerase solution. The product of the reaction co-chromatographed with glycerol-3-phosphate in three different solvent systems (2) and the product formed from 0.18 μmole D-glyceraldehyde 3-phosphate gave 0.21 μmole glycerol 3-phosphate when assayed with L-glycerol 3-phosphate dehydrogenase (3). Further evidence that glyceraldehyde 3-phosphate was the true substrate was obtained after partial inhibition of triose phosphate isomerase as follows:-

A fraction of muscle supernatant precipitating between 50 and 85% saturation with ammonium sulphate was incubated at 37° with 4 mM glycidol phosphate in 50 mM triethanolamine-HCl buffer, pH 7.5. At the end of 15 min, an aliquot was withdrawn

Table 2. Rates of reaction with D-glyceraldehyde 3-phosphate and with dihydroxyacetone phosphate after partial inhibition of triose phosphate isomerase in the oxidoreductase preparation.
Further details are given in the text.

	Substrate	Rate (fluorescent units/3 min)
Inhibited enzyme batch 1		
	20μM dihydroxyacetone phosphate alone	4.7
	20μM D-glyceraldehyde 3-phosphate alone	7.5
Inhibited enzyme batch 2		
(a)	20μM D-glyceraldehyde 3-phosphate	12.3
plus	20μM dihydroxyacetone phosphate	7.0
(b)	20μM dihydroxyacetone phosphate	6.9
plus	20μM D-glyceraldehyde 3-phosphate	10.7
(c)	20μM D-glyceraldehyde 3-phosphate	12.0
plus	20μM dihydroxyacetone phosphate	6.6

and the rate was measured fluorometrically at 25° and 1.6 μM NADPH. A higher rate was obtained with D-glyceraldehyde 3-phosphate as substrate than with dihydroxyacetone phosphate and addition of the latter inhibited the reaction (Table 2).

Properties. The activity of a muscle supernatant was increased by the addition of 1mM dithiothreitol and this compound was consequently included in the assay medium. There was no inhibition by 0.1 mM sodium cyanide, but with 0.1 mM p-chloromercuribenzoate complete inhibition was obtained that could be reversed by 1 mM dithiothreitol. Ammonium sulphate inhibited the enzyme by 60% at a 20 mM concentration. The enzyme could be precipitated between 50 and 85% saturation with ammonium sulphate but was easily inactivated with this reagent. Muscle supernatants and other preparations of the enzyme lost activity on storage for 3-4 days at 2° and 75% of the activity was lost when a 0-50% v/v acetone fraction was heated for 1 min at 45°. Initial reaction velocities were measured fluorometrically at various concentrations of each substrate and apparent K_m values of 14 μM for D-glyceraldehyde 3-phosphate and 0.7 μM for NADPH were calculated from Lineweaver-Burk plots (Fig. 2).

DISCUSSION

Of the tissues examined, the highest NADPH-D-glyceraldehyde 3-phosphate

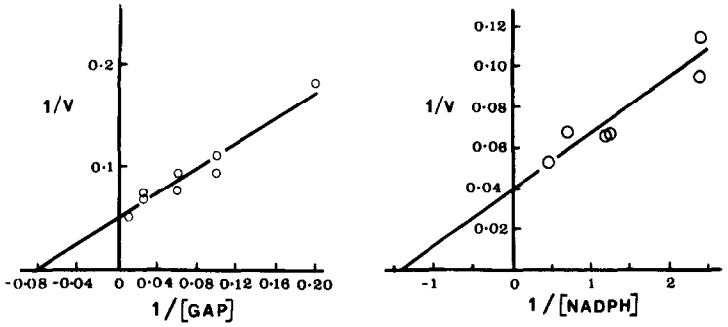

Fig. 2. Lineweaver-Burk plots of reciprocal rate against reciprocal substrate concentration at 22°. (A). At a fixed concentration of 8μM NADPH, [GAP] = micromolecular concentration of D-glyceraldehyde 3-phosphate, v = fluorescent units/6min; K_m = 12μM D-glyceraldehyde 3-phosphate. (B). At a fixed concentration of 200μM DL-glyceraldehyde 3-phosphate, [NADPH] = micromolecular concentration of NADPH, v = fluorescent units/1.5min; K_m = 0.7μM NADPH.

oxidoreductase activity was found in muscle which contained 0.90 ± 0.06 units/g, a level greater than the sum of the activities of glucose 6-phosphate and 6-phosphogluconate dehydrogenases in this tissue (4). The activity was not due to L-glycerol 3-phosphate-NAD^+ oxidoreductase utilising NADPH as a hydrogen donor as suggested by Borrebaek et al. (5), since there was no correlation between the distribution of activity and that of L-glycerol 3-phosphate-NAD^+ oxidoreductase as determined by Shonk and Boxer (6), and a distinct separation of the two enzymes was obtained by chromatography of a muscle extract on DEAE-Sephadex. Nor was the activity due to a coupling of L-glycerol 3-phosphate-NAD^+ oxidoreductase with the NADPH-NAD^+ oxidoreductase described by Colowick et al. (7) since the activity of the latter enzyme in a muscle supernatant was less than 1% of the activity under discussion.

The demonstration that D-glyceraldehyde 3-phosphate, and not dihydroxyacetone phosphate, was the substrate of the enzyme was hindered by the large amount of triose phosphate isomerase present. However, glycidol phosphate is a potent inhibitor of

triose phosphate isomerase (8) and a 97% inhibition of the rabbit muscle enzyme was obtained following incubation at 37° with a 0.4 mM concentration for 10 min. Treatment of a preparation from rat muscle with 4 mM glycidol phosphate led to a clear-cut difference in the rates of NADPH oxidation between glyceraldehyde phosphate and dihydroxyacetone phosphate and indicated that the latter compound probably acted as a competitive inhibitor. Although in the present experiments racemic DL-glyceraldehyde 3-phosphate was used, the reactivity of dihydroxyacetone phosphate in the presence of triose phosphate isomerase implied that the enzyme acted upon the D-form.

The enzyme was rather unstable and activity was easily lost by heating, treatment with ammonium sulphate, and on storage. It behaved as a typical -SH enzyme, being sensitive to the effects of both p-chloromercuribenzoate and dithiothreitol. The affinity for its substrates was high as shown by the low values of the apparent K_m's for D-glyceraldehyde 3-phosphate and NADPH. The function of the enzyme remains to be investigated but probably resides in providing muscle with a mechanism for reoxidising NADPH and maintaining a flow of material through the Pentose Phosphate Pathway even in the absence of concomitant biosynthesis.

ACKNOWLEDGMENT

I am very grateful to Dr. I.A. Rose for the generous gift of glycidol phosphate.

REFERENCES

1. Kaplan, N.O., Colowick, S.P., and Neufeld, E.F. (1952) J. Biol. Chem. 195, 107-119.
2. Wood, T. (1961) J. Chromatog. 6, 142-154.
3. Hohorst, H.-J. (1965) in Methods in Enzymatic Analysis (Bergmeyer, H.-U., ed). pp. 215-219, Academic Press, New York and London.
4. Glock, G.E., and McLean, P. (1954) Biochem. J. 56, 171-175.
5. Borrebaek, B., Abraham, S., and Chaikoff, I.L. (1965) Biochim. Biophys. Acta 96, 237-247.
6. Shonk, C.E., and Boxer, G.E. (1964) Cancer Res. 24, 709-721.
7. Colowick, S.P., Kaplan, N.O., Neufeld, E.F., and Ciotti, M.N. (1952) J. Biol. Chem. 195, 95-105.
8. Rose, I.A., and O'Connell, E.L. (1969) J. Biol. Chem. 244, 6548-6550.