Glucose-6-phosphate and gluconate-6-phosphate dehydrogenase in worms

From recent investigations it becomes clear that the hexose-monophosphate oxidative route is an important and wide-spread pathway of carbohydrate metabolism. This pathway has been detected in several tissues: mammalian organs[1], angiosperms[2], yeast[3,4] and some bacteria[5,6]. Its presence in other phyla of the animal and plant kingdom has not yet been studied. This would be of interest for comparative biochemistry.

We studied the presence of glucose-6-phosphate (G6P)- and gluconate-6-phosphate (GA6P)-dehydrogenase in several worms, most of them parasitic ones. The latter were isolated from freshly killed, infected animals. All the worms were washed in physiological saline or distilled water, suspended in twice their weight 0.05 *M* Tris buffer pH 7.46 and disrupted in an all-glass homogenizer in the cold room for a few minutes until a uniform paste was obtained. Some species of worms were too hard to be completely crushed by this procedure and were previously treated in a micro-blendor or crushed by grinding with sand. Extraction proceeded always with Tris buffer at 0° for 30 minutes. The turbid mass was centrifuged at 0° at 5000 *g* for 1–2 hours and the clear or opalescent supernatant was used at once for the enzyme determinations.

The dehydrogenase activity determinations were carried out at room temperature (22° C) in the Beckman spectrophotometer model DU at 340 mμ. The enzyme mixture contained 0.04 *M* Tris buffer pH 7.46, 0.02 *M* Mg^{++} 0.30 μmole TPN^+, 2 μmole sodium gluconate-6-phosphate or 4.5 μmole sodium glucose-6-phosphate and 0.1 ml supernatant; final volume 3.1 ml. The results are collected in Table I.

TABLE I

The activity of soluble glucose-6-phosphate- and gluconate-6-phosphate-dehydrogenases in several worms, expressed as enzyme units per gram living material (a unit is the amount of enzyme able to reduce 1 μmole TPN^+ per minute in the conditions of our experiments) and corrected for the blank value.

Organisms	*TPN*		*DPN*	
	G6P	*GA6P*	*G6P*	*GA6P*
I. Platyhelminthes				
Free-living: planarians				
Polycelis nigra	0.185	0.050	0	0
Euplanaria torva	0.050	0.025	0	0
Parasitic: trematodes				
Fasciola hepatica (cattle)	0.070–0.170	0–0.035	0	0
Dicrocoelium dendriticum (sheep)	0.100	0.065	0	0
Parasitic: cestodes				
Anoplocephala perfoliata (horse)	0.300–1.530	0.120–0.240	0	0
Moniezia benedeni (sheep)	0.080–0.105	0–0.020	0	0
*Taenia saginata** (man)	0.040	0.025		
Taenia pisiformis (dog)	0.200	0.075	0	0
Echinococcus granulosus (hydatid cysts from horse-liver)	0	0		
Dipylidium caninum (dog)	0.200	0.100	0	0
II. Parasitic nematodes				
Ascaris lumbricoides (pig)				
(dermo-muscular layer)	0.200–0.270	0.090–0.100	0.015	0
(female reproductive system)	0.500–1.600	0.120–0.230	0	0
Parascaris equorum (horse)				
(dermo-muscular layer)	0.690	0.240	0.110	0
(female reproductive system)	0.830	0.230	0.015	0
Toxacara canis (dog)	0.710	0.430	0	0
Ascaridia galli (fowl)	0.195	0.130	0	0
Ascaridia columbae (pigeon)	0.300	0.125	0.020**	0
Heterakis gallinae (fowl)	0.106	0.034	0	0
Strongylus edentatus (horse)	0.065–0.100	0.030–0.045	0	0
III. Annelida				
Tubifex tubifex	0.260	0.055**	0	0
Lumbricus terrestris	0.580	0.190	0	0

* obtained from an individual, by anthelminthical treatment with ricinus oil and pumpkin seed.
** the reaction stopped before complete reduction was obtained.

It appears that a G6P-dehydrogenase is present in all species, except in the larval stage of *Echinococcus*. This enzyme is TPN-linked. In the few cases where a weak reaction with DPN^+ was obtained, this must probably be attributed to the fact that G6P is also decomposed by way of the Embden-Meyerhof glycolytic pathway. GA6P-dehydrogenase is also present in all species, except in *Echinococcus*-larvae. It is specifically TPN-linked and always less active than G6P-dehydrogenase. The amount of both enzymes is relatively low in planarians and trematodes and very variable among cestodes. *Annelida* and especially parasitic nematodes have a higher content of both dehydrogenases. The enzyme content in the latter group is comparable to that of the bacterium *Aerobacter cloacae*, which is known to have an active system of the hexose-monophosphate oxidative route[6]: these bacteria contain about 1.2 units soluble G6P-dehydrogenase and about 0.3 units of soluble GA6P-dehydrogenase per gram living material[7].

In a system containing 0.05 *M* Tris buffer pH 7.46, 35 μmole sodium gluconate-6-phosphate, 0.8 μmole TPN^+, 44 μmole pyruvate, 40 units rabbit-muscle lactic dehydrogenase and 0.2 units GA6P-dehydrogenase from the female genital tract of *Ascaris lumbricoides*, sedoheptulose was formed, and detected by paper chromatography.

These results show that a number of worms, free living and parasitic, contain the enzyme system for the hexose-monophosphate oxidative route, or at least a closely related one.

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Enzymic synthesis of guanosine and cytidine triphosphates: A note on the nucleotide specificity of the pyruvate phosphokinase reaction

The recent isolation of the phosphorylated derivatives of uridine, cytidine and guanosine raises the question of the exact nucleotide specificity of a number of enzymes which have been studied employing usually adenosine, and sometimes inosine, nucleotides. Pyruvate phosphokinase, the enzyme catalyzing the transfer of phosphate from phosphopyruvate to a nucleoside diphosphate, has already been shown to be active with ADP*, IDP and UDP[1-3]. It has now been found that phosphorylation of GDP and CDP is also catalyzed by this enzyme.

In this study pyruvate phosphokinase was prepared from rabbit muscle as described by KORNBERG AND PRICER[4]. The material obtained by this preparation approximated in purity the material reported by them. Activity was measured by coupling the reaction to lactic dehydrogenase[4]. The suitability of a nucleotide as an acceptor in this reaction was, therefore, observed as the oxidation of reduced diphosphopyridine nucleotide (DPNH) measured at 340 mμ, in the coupled reaction. Phosphopyruvic acid (barium salt) was the gift of Mr. W. E. PRICER, Jr., and DPNH was prepared chemically with sodium hydrosulfite[5].

It is believed that each of the nucleotides employed was free of other nucleotides, except where indicated. Where possible, several independent samples of each nucleotide were employed. The sources of the nucleotides were as follows:

ADP, obtained from Sigma Chemical Co., St. Louis;

IDP, (1) from Sigma Chemical Co., and (2) kindly supplied by Drs. K. KURAHASHI AND M. UTTER[6];

UDP, (1) prepared from UTP[7] (Pabst Laboratories, Milwaukee, Wisconsin) by incubating with a crude yeast hexokinase preparation and isolating the products by anion exchange chromatography**, and (2) prepared by hydrolysis of UDP N-acetylamino sugar compounds from *Staphylococcus aureus*[8];

GDP, (1) by hydrolysis of guanosine diphosphate mannose from hen's oviduct[9], and (2) from Sigma Chemical Co***;

* AMP, ADP, ATP, IMP, IDP, ITP, UMP, UDP, UTP, CMP, CDP, CTP, GMP, GDP, GTP are the 5'-mono-, di- and triphosphates of adenosine, inosine, uridine, cytidine and guanosine respectively.
** This preparation of UDP contained about 5% UMP.
*** Both preparations of GDP contained 5–10% GMP.