Galactose-1-phosphate Uridyl Transferase

Kurt J. Isselbacher

The enzyme galactose-1-phosphate uridyl transferase is found in many mammalian tissues, for example, erythrocytes, liver, mammary gland and brain. It is also found in bacteria, especially in *Escherichia coli*. The enzyme catalyses the reaction:

(1) Galactose-1-phosphate + UDPG $\rightleftharpoons$ UDPGal + glucose-1-phosphate

(Abbreviations: UDPG = uridine diphosphoglucose; UDPGal = uridine diphosphogalactose).
There are several methods of determining the activity of this enzyme. One method, which is frequently used in the diagnosis of galactosaemia, utilizes UDPG dehydrogenase to measure the rate of disappearance of UDPG[1]. UDPGal epimerase interferes with this method, but it is valid in erythrocytes because without added diphosphopyridine nucleotide (DPN) there is no interfering epimerase activity.

Another method measures the rate of formation of glucose-1-phosphate (G-1-P) with phosphoglucomutase (PGluM) and glucose-6-phosphate dehydrogenase (G6P-DH)[2]. UDPGal epimerase does not interfere with this method and therefore it has wider applicability. This method is described below.

Principle

The G-1-P formed in reaction (1) is converted to glucose-6-phosphate (G-6-P) with PGluM, and the G-6-P is oxidized with triphosphopyridine nucleotide (TPN) and G6P-DH to 6-phosphogluconate (6-PG):

(2) Glucose-1-phosphate $\rightleftharpoons$ glucose-6-phosphate

(3) Glucose-6-phosphate + TPN^+ $\overset{\text{G6P-DH}}{\rightleftharpoons}$ 6-phosphogluconate + TPNH + H^+

1 μmole of TPNH is formed for each μmole hexose phosphate oxidized. The rate of increase of optical density at 340 mμ due to the formation of TPNH is measure of the galactose-1-phosphate uridyl transferase activity.

Optimum Conditions for Measurements

Galactose-1-phosphate uridyl transferase from mammalian tissues and bacteria has an activity optimum[2,3] at pH 8.7. The substrates and indicator enzymes are added in excess.

Reagents*)

1. Glycine
2. Cysteine hydrochloride·H_2O
3. Magnesium chloride, $MgCl_2 \cdot 6\ H_2O$
4. Sodium hydroxide, A. R., 5 N

---

*) Complete reagent kits are available commercially, see p. 1036.

1) *E. P. Anderson, H. M. Kalckar, K. Kurahashi* and *K. J. Isselbacher*, J. Lab. clin. Med. *50*, 469 [1957].
2) *K. Kurahashi* and *E. P. Anderson*, Biochim. biophysica Acta *29*, 498 [1958].
3) *H. M. Kalckar, K. Kurahashi* and *E. Jordan*, Proc. nat. Acad. Sci. USA *45*, 1776 [1959].

5. Sodium sulphate, A. R., $Na_2SO_4 \cdot 10\ H_2O$, 10% solution
6. Galactose-1-phosphate, Gal-1-P
   barium or potassium salt; prepared according to[4] or commercial preparation (see p. 1016).
7. Uridine diphosphoglucose, UDPG
   sodium salt; commercial preparation, see p. 1031.
8. Triphosphopyridine nucleotide, TPN
   sodium salt, TPN-NaH_2; commercial preparation, see p. 1029.
9. Phosphoglucomutase, PGluM
   prepared from rabbit muscle according to[5] or commercial preparation, see p. 992.
10. Glucose-6-phosphate dehydrogenase, G6P-DH
    prepared from yeast according to[6] or commercial preparation, see p. 975.

Purity of the enzyme preparations

The indicator enzymes must not be contaminated with 6-phosphogluconic acid dehydrogenase. They are checked with known amounts of G-1-P and G-6-P. Only 1 μmole of TPNH should be formed per μmole hexose phosphate oxidized. A direct assay for 6-phosphogluconic dehydrogenase is also recommended.

A suitable G6P-DH preparation can be prepared according to[6]. The commercial G6P-DH Type III of the Sigma Chemical Co. was also found to be satisfactory.

The PGluM prepared according to[5] contains sufficient of the cofactor, glucose-1,6-diphosphate.

Preparation of Solutions

I. Glycine buffer (1 M; pH 8.7):
Dissolve 7.5 g. glycine in 75 ml. distilled water, adjust to pH 8.7 with *ca.* 1.4 ml. 5 N NaOH and dilute to 100 ml. with distilled water.

II. Cysteine (*ca.* 0.2 M):
Dissolve 350 mg. cysteine hydrochloride $\cdot H_2O$ in 9 ml. distilled water. Immediately before use adjust to pH 8.5 (indicator paper) with *ca.* 0.7 ml. 5 N NaOH.

III. Magnesium chloride (0.1 M):
Dissolve 2.03 g. $MgCl_2 \cdot 6\ H_2O$ in 100 ml. distilled water.

IV. Galactose-1-phosphate (0.01 M Gal-1-P):
Dissolve 34 mg. potassium salt in 10 ml. distilled water. Or dissolve 40 mg. barium salt in 5 ml. distilled water, add 0.4 ml. 10% Na_2SO_4 solution, centrifuge and wash the precipitate with distilled water. Combine the supernatant and washings, and dilute to 10 ml. with distilled water.

V. Uridine diphosphoglucose, UDPG (0.01 M; pH 8.7):
Dissolve 6 mg. sodium salt in 1 ml. distilled water.

VI. Triphosphopyridine nucleotide (0.025 M β-TPN):
Dissolve 20 mg. TPN-NaH_2 in 1 ml. distilled water.

---

[4] *S. P. Colowick*, J. biol. Chemistry 124, 557 [1938].
[5] *V. A. Najjar* in *S. P. Colowick* and *N. O. Kaplan:* Methods in Enzymology. Academic Press, New York 1955, Vol. I, p. 294.
[6] *A. Kornberg* and *B. L. Horecker* in *S. P. Colowick* and *N. O. Kaplan:* Methods in Enzymology. Academic Press, New York 1955, Vol. I, p. 323.

VII. Phosphoglucomutase (1 unit*)/ml.):
Dilute the product prepared from rabbit muscle according to[5] or the commercial preparation with 2.5 M ammonium sulphate solution.

VIII. Glucose-6-phosphate dehydrogenase, G6P-DH (*ca.* 1 unit*)/ml.):
Dilute the product prepared from yeast according to[6] or the commercial preparation with 3.3 M ammonium sulphate solution.

Stability of the solutions

The galactose-1-phosphate, buffer and $MgCl_2$ solution and the suspension of PGluM and G6P-DH are stable for months at 0—4° C. Prepare the UDPG, TPN and cysteine solutions freshly each week, and store cold or frozen.

Procedure

Experimental material

Erythrocyte haemolysates (preparation, see p. 747) or soluble fraction of rat liver (supernatant after centrifuging a homogenate at *ca.* 100000 g).

Spectrophotometric measurements

Wavelength: 340 mμ; silica cuvettes, light path: 1 cm.; cuvette capacity: 1 ml.; final volume: 0.62 ml.; temperature: 25° C. Measure against water.

Pipette into the experimental and control cuvettes:

0.03 ml. cysteine solution (II)
0.01 ml. $MgCl_2$ solution (III)
0.06 ml. glycine buffer (solution I)
0.01 ml. TPN solution (VI)
0.02 ml. UDPG solution (V)
0.01 ml. PGluM suspension (VII)
0.01 ml. G6P-DH suspension (VIII)
sample (containing 50 to 100 μg. transferase)
distilled water to 0.59 ml.

Mix and follow the optical density at 340 mμ until constant (usually 2 min.). Mix into the control cuvette

0.03 ml. distilled water

and into the experimental cuvette

0.03 ml. Gal-1-P solution (IV).

Read the optical density every 30 sec. for about 3 min. The initial rate of TPNH formation is a measure of the galactose-1-phosphate uridyl transferase contained in the sample.

Calculations

As the volume of the reaction mixture in the experimental cuvette is 0.62 ml., $\Delta E = 10$ corresponds to the formation of a μmole of TPNH. A unit of galactose-1-phosphate uridyl transferase is the amount of enzyme which, under the conditions described here, reduces 1 μmole TPN/min. It therefore follows that:

$$\frac{\Delta E}{10} = \text{units/assay mixture}$$

*) 1 unit is the amount of enzyme which, under the conditions described, here catalyses the formation of 1 μmole TPNH/min.

Example

The supernatant (0.1 ml.) of a rat liver homogenate (after centrifuging at 100000 g) was analysed.

	Time [min.]	*Control cuvette*	*Experimental cuvette*
Before the addition of galactose-1-phosphate	0	0.208	0.210
	0.5	0.230	0.230
	1.0	0.235	0.235
	1.5	0.240	0.240
	2.0	0.240	0.240
After addition of		H_2O	Gal-1P
	3.0	0.238	0.352
	3.5	0.240	0.412 $\Delta E = 0.118$/min.
	4.0	0.242	0.470
	4.5	0.245	0.528
	5.0	0.245	0.585 $\Delta E = 0.115$/min.
	5.5	0.245	0.640
	6.0	0.245	0.698 $\Delta E = 0.113$/min.
Mean:		$\Delta E = 0.007$/3 min.	$\Delta E = 0.346$/3 min.
		$\Delta E = 0.002$/min.	$\Delta E = 0.115$/min.
		$0.115 - 0.002 = 0.113$	

The activity of the galactose-1-phosphate uridyl transferase in the reaction mixture was therefore:

$$\frac{0.113}{10} = 0.0113 \text{ units or } 0.113 \text{ units/ml. supernatant.}$$

Stability of the Enzyme

The enzyme is relatively stable at $-10°$C. When lyophilized and stored *in vacuo* it is stable for several months without loss of activity. Glutathione increases the stability[2].