Guanosine

Alan Coddington

The method described here was developed by *Kalckar*[1]. It has only been used for pure, aqueous solutions of guanosine.

Principle

Nucleoside phosphorylase catalyses the reaction:

(1) Guanosine + phosphate $\rightleftharpoons$ guanine + ribose-1-phosphate

In the presence of excess phosphate the reaction proceeds quantitatively from left to right. The guanine formed is oxidized further:

(2) Guanine + H_2O $\longrightarrow$ xanthine + NH_3

(3) Xanthine + H_2O + O_2 $\longrightarrow$ uric acid + H_2O_2

(4) Uric acid + O_2 + 2 H_2O $\longrightarrow$ allantoin + CO_2 + H_2O_2

The reactions (2), (3) and (4) are catalysed by guanase, xanthine oxidase and uricase, respectively. The formation of uric acid in reaction (3) results in an increase of optical density at 293 mμ. The decrease of optical density at 293 mμ on addition of uricase is measured.

Reagents

1. Potassium dihydrogen phosphate, KH_2PO_4
2. Disodium hydrogen phosphate, $Na_2HPO_4 \cdot 7\ H_2O$
3. Ethylene-diamine-tetra-acetic acid, EDTA
   disodium salt, EDTA-$Na_2H_2 \cdot 2\ H_2O$.
4. Guanosine
   commercial preparation, see p. 1020.
5. Tris-hydroxymethyl-aminomethane, tris
6. Hydrochloric acid, 0.2 N
7. Xanthine oxidase
   prepared from milk according to[2]; (see p. 499).
8. Nucleoside phosphorylase
   prepared from calf spleen according to[3]; isolation, see p. 504.
9. Guanase
   prepared from rat liver according to[4]; commercial preparation, see p. 982.
10. Uricase
    prepared from pig liver according to[5]; commercial preparation, see p. 1000.

---

[1] *H. M. Kalckar*, J. biol. Chemistry *167*, 429 [1947].
[2] *H. Klenow* and *R. Emberland*, Arch. Biochem. Biophysics *58*, 276 [1955].
[3] *V. E. Price*, *M. C. Otey* and *P. Plesner* in *S. P. Colowick* and *N. O. Kaplan:* Methods in Enzymology. Academic Press, New York 1955, Vol. II, p. 448.
[4] *L. Shuster* in *S. P. Colowick* and *N. O. Kaplan:* Methods in Enzymology. Academic Press, New York 1955, Vol. II, p. 480.
[5] *H. R. Mahler*, *G. Hübscher* and *H. Baum*, J. biol. Chemistry *216*, 625 [1955].

Purity of the enzyme preparations

The purity of the enzyme preparations obtained according to [2-5] or of the commercial preparations described on p. 499 and 982 satisfies the requirements.

Preparation of Solutions

I. Phosphate buffer (0.05 M; pH 7.9):
   a) Dissolve 27.2 g. KH_2PO_4 in doubly distilled water and make up to 1000 ml.
   b) Dissolve 53.6 g. $Na_2HPO_4 \cdot 7\ H_2O$ in doubly distilled water and make up to 1000 ml. Mix 7.0 ml. solution a) with 93.0 ml. solution b) and dilute to 400 ml. with doubly distilled water.

II. Tris buffer (0.05 M; pH 7.9):
   Dissolve 1.21 g. tris-hydroxymethyl-aminomethane in 50 ml. doubly distilled water, adjust to pH 7.9 with 29.7 ml. 0.2 N HCl and dilute to 200 ml. with doubly distilled water. Check the pH (glass electrode).

III. Ethylene-diamine-tetra-acetate, EDTA (0.01 M):
   Dissolve 0.372 g. EDTA-$Na_2H_2 \cdot 2\ H_2O$ in doubly distilled water and make up to 100 ml.

IV. Guanosine standard solution (5×10^{-3} M):
   Dissolve 28.3 mg. guanosine in 20 ml. doubly distilled water with warming.

V. Nucleoside phosphorylase (129000 units*)/ml.):
   Dilute the preparation obtained according to[3] with doubly distilled water.

VI. Xanthine oxidase (14000 units*)/ml.):
   Dilute the preparation obtained according to[2] or the commercial preparation with doubly distilled water.

VII. Guanase (14000 units**)/ml.):
   Dilute the preparation obtained according to[4] or the commercial preparation with doubly distilled water.

VIII. Uricase (42000 units†)/ml.):
   Dilute or dissolve the preparation obtained according to[5] or the commercial preparation with doubly distilled water.

Stability of the solutions

Store the enzyme solutions at $-17°$ C. They are stable for several years. Repeated freezing and thawing leads to the customary loss of activity. Store all other solutions at 0° C and prepare freshly every three months.

Procedure

Experimental material

The method described here has so far only been used for the analysis of pure, aqueous solutions of guanosine.

---

*) Definition of units, see p. 503.

**) Definition and measurements as for nucleoside phosphorylase (see p. 503), but with 1.5 μmoles guanosine as substrate and nucleoside phosphorylase in excess (unpublished).

†) A unit is the amount of enzyme which decreases the optical density at 293 mμ by 0.001 in 1 min. at 20° C, with 0.15 μmoles uric acid in 3 ml. reaction mixture (0.05 M tris buffer, pH 7.9; light path: 1 cm.) (refer to the conventional uricase units, p. 500).

Spectrophotometric measurements

Preliminary remarks: Warm all the reagents to room temperature before the determination. Thaw the enzyme solutions and allow to stand in an ice bath.

Method: Wavelength: 293 mμ; light path: 1 cm.; final volume: 3.0 ml.; measure against the control cuvette.

Pipette successively into the cuvettes:

Experimental cuvette	*Control cuvette*
2.400 ml. tris buffer (solution II)	2.400 ml. tris buffer (solution II)
0.300 ml. phosphate buffer (solution I)	0.300 ml. phosphate buffer (solution I)
0.030 ml. EDTA solution (III)	0.030 ml. EDTA solution (III)
0.020 ml. sample (containing *ca.* 0.1 μmoles guanosine) or guanosine standard solution (IV)	0.270 ml. doubly distilled water
0.165 ml. doubly distilled water	
0.035 ml. *) xanthine oxidase solution (VI)	
0.005 ml. *) nucleoside phosphorylase solution (V)	
0.035 ml. *) guanase solution (VII).	

Mix (blow air through the solutions), read the optical density at 5 min. intervals until constant: E_1. Mix into the experimental cuvette

0.010 ml. *) uricase solution (VIII),

observe the optical density until constant: E_2.

The optical density of the uricase solution (VIII) at 293 mμ must be determined separately: prepare two cuvettes similar to the control cuvette, mix 0.01 ml. uricase solution (VIII) into one cuvette and measure the increase in optical density E_U against the enzyme-free cuvette. E_U is small and need only be determined once for each uricase preparation.

Calculations

$$\frac{(E_1 - E_2 - E_U) \times 3}{12.15} = \text{μmoles guanosine/reaction mixture}$$

where

E_1 = optical density before addition of the uricase solution
E_2 = optical density after addition of the uricase solution
E_U = optical density of the uricase solution
3 = assay volume [ml.]

12.15 = extinction coefficient of uric acid at 293 mμ. [cm.²/μmole].

*) = *ca.* 500 units.