Methylglyoxal

Helmut Klotzsch and Hans-Ulrich Bergmeyer

If methylglyoxal is distilled, the methylglyoxal content of the distillate varies with the conditions of the distillation. In certain cases, for example in the determination of glutathione (see p. 363), it is important to know the content of such distillates accurately.

Principle

Methylglyoxal and glutathione (GSH) are quantitatively converted to *S*-lactyl-GSH in the reaction catalysed by glyoxalase I (Gl-I):

(1) $$GSH + CH_3-CO-CHO \longrightarrow S\text{-lactyl-GSH}$$

Lactyl-GSH is measured directly at 240 mμ. The reaction proceeds quantitatively under the conditions given below[1].

Reagents

1. Potassium dihydrogen phosphate, KH_2PO_4, A. R.
2. Dipotassium hydrogen phosphate, K_2HPO_4, A. R., anhydrous
3. Glutathione, GSH
   crystalline; commercial preparation, see p. 1018.
4. Glyoxalase I, Gl-I
   from yeast, solution in 30% glycerol; specific activity at least 300 units*)/mg.; commercial preparation, see p. 981.

   Purity of the enzyme preparation
   Glyoxalase I must be completely free from glyoxalase II.

Preparation of Solutions

I. Phosphate buffer (0.1 M; pH 6.8):
   a) Dissolve 1.36 g. KH_2PO_4 in doubly distilled water and make up to 100 ml.
   b) Dissolve 1.74 g. K_2HPO_4 in doubly distilled water and make up to 100 ml.
   Mix 50 ml. solution a) with 61 ml. solution b). Check the pH (glass electrode).

II. Glutathione (*ca.* 0.03 M GSH):
   Dissolve 10 mg. glutathione in 1 ml. doubly distilled water.

III. Glyoxalase I, Gl-I (1 mg. protein/ml.):
   Dilute the stock solution with 30% glycerol (v/v).

Stability of the solutions

Store all solutions and suspensions, stoppered, in a refrigerator at 0–4° C. They keep for several weeks in this state.

Procedure

Experimental material

So far the method has only been carried out on pure solutions. Dilute the distillate of a 30% commercial product (refer to p. 363) 1:50 with doubly distilled water.

---

*) A unit is the amount of enzyme which converts 1 μmole of substrate in 1 min. at 25° C.

[1] *E. Racker* in *S. P. Colowick* and *N. O. Kaplan:* Methods in Enzymology, Academic Press, New York 1957, Vol. III, p. 296.

Spectrophotometric measurements

Wavelength: 240 mμ; light path: 1 cm.; final volume: 2.99 ml.; room temperature. Measure against the blank.

Pipette successively into the cuvettes:

Blank: 2.90 ml. phosphate buffer (solution I)
0.05 ml. GSH solution (II)

Experimental: 2.90 ml. phosphate buffer (solution I)
0.05 ml. GSH solution (II)
0.02 ml. Gl-I solution (III)

Mix thoroughly with a glass or plastic rod flattened at one end and read the optical density E_1.

Mix into both cuvettes

0.02 ml. sample

Read the optical density E_2 after 8, 10 and 12 min. $\Delta E = E_2 - E_1$ is used for the calculations.

Calculations

According to *E. Racker*[2] the extinction coefficient of *S*-lactyl-GSH $\varepsilon_{240} = 3.37$ cm.^{2}/μmole. Therefore with a final volume in the cuvette of 2.99 ml.:

$$\frac{\Delta E \times 2.99}{3.37} = \text{μmoles methylglyoxal/assay mixture.}$$

To obtain the methylglyoxal content per ml. of sample, it is necessary to multiply by 2500, because of the dilution of the sample 1:50 and the fact that 0.02 ml. of the sample is taken for analysis. Therefore

$$\frac{\Delta E \times 2.99 \times 2500}{3.37} = \Delta E \times 2220 = \text{μmoles methylglyoxal/ml. sample.}$$

---

[2] *E. Racker*, J. biol. Chemistry *190*, 685 [1951].