# Glycolaldehyde

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During studies of the decarboxylation of hydroxypyruvate by yeast enzymes it was observed that the glycolaldehyde formed was reduced by reduced diphosphopyridine nucleotide (DPNH). This reduction is catalysed by yeast alcohol dehydrogenase[1]. The reaction can be used to determine glycolaldehyde by a spectrophotometric method (see *O. Warburg* for a summary[2]).

## Principle

Crystalline alcohol dehydrogenase (ADH) from baker's yeast catalyses the reaction:

(1) Glycolaldehyde + DPNH + $H^+$ $\rightleftharpoons$ ethyleneglycol + $DPN^+$

The equilibrium of the reaction lies to the right, but under suitable conditions it is possible to demonstrate the oxidation of ethyleneglycol[1]. Since the affinity of glycolaldehyde for ADH is low (for characterization of the affinity ratios, see[1]), it is necessary to work with high ADH concentrations so as to guarantee a quantitative reduction. In addition to glycolaldehyde, a large number of other aldehydes are reduced by DPNH in the presence of ADH (see "Specificity").

## Reagents

1. Citric acid, A. R.
2. Sodium hydroxide, A. R., 20% in water
3. Reduced diphosphopyridine nucleotide, DPNH
   disodium salt, DPNH-$Na_2$; commercial preparation, see p. 1011.
4. Yeast alcohol dehydrogenase, ADH
   crystalline suspension in 2.4 M ammonium sulphate solution; commercial preparation, see p. 969.

   **Purity of the enzyme preparation**

   Commercially available crystalline ADH preparations satisfy the requirements of the method.

## Preparation of Solutions

I. Citrate buffer (0.33 M; pH 6.0):
Dissolve 17.5 g. citric acid · $1 H_2O$, A. R., in about 100 ml. doubly distilled water, adjust pH to 6.0 (glass electrode) with *ca.* 45 ml. 20% NaOH and dilute with doubly distilled water to 250 ml.

II. Reduced diphosphopyridine nucleotide (*ca.* $2 \times 10^{-2}$ M β-DPNH):
Dissolve 10 mg. DPNH-$Na_2$ in 1.0 ml. doubly distilled water.

III. Yeast alcohol dehydrogenase, ADH (30 mg. protein/ml.):
Dilute suspensions of higher concentration with 2.4 M $(NH_4)_2SO_4$ solution.

## Procedure

For preparation and extraction of experimental material (blood, tissue, *etc.*) see determination of pyruvate (p. 254).

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[1] *H. Holzer, H. W. Goedde* and *S. Schneider*, Biochem. Z. *327*, 245 [1955].
[2] *O. Warburg:* Wasserstoffübertragende Fermente. Dr. W. Saenger GmbH, Berlin 1948.

**Spectrophotometric measurements**

Wavelength: 366 mμ (glass cuvettes) or 340 mμ (silica cuvettes) light path: 1 cm.; final volume: 3.0 ml. Light path and final volume can be varied so that, if necessary, the test can be made more sensitive.

Read experimental and control cuvettes against a water blank.

Bring the buffer and sample to room temperature. Pipette successively into the cuvettes:

| *Experimental cuvette* | *Control cuvette* |
|---|---|
| 1.00 ml. buffer (solution I) | 1.00 ml. buffer (solution I) |
| 0.03 ml. DPNH solution (II) | 0.03 ml. DPNH solution (II) |
| sample + water to 2.94 ml. | water to 2.94 ml. |

Mix, read optical densities of both cuvettes. If the change in optical density in both cuvettes is not greater than 0.001 to 0.002 per 30 seconds, mix into both cuvettes

0.06 ml. ADH suspension (III) (*ca.* 2 mg. protein).

The reaction is considered to have stopped (usually after 15—20 minutes) when the same very small decrease in optical density occurs in both cuvettes. A control containing all components of the assay system except the enzyme, usually gives no significant change in optical density with time. The difference in optical density between sample and control at the end of the reaction minus the difference in optical density between sample and control before addition of ADH gives the ΔE required for the calculations.

The change in optical density caused by the absorption of the enzyme and by dilution of the assay mixture on addition of the enzyme solution is obtained by addition of the enzyme to the control cuvette, or addition of the enzyme to the experimental cuvette after completion of the reaction. This change in optical density can be either positive or negative according to the magnitude of the initial optical density and the absorption of the enzyme solution; usually it can be ignored.

**Calculations**

$$\frac{\Delta E \times V}{\varepsilon \times d} = \mu\text{moles glycolaldehyde/cuvette}$$

ΔE is the decrease in optical density occurring on addition of ADH, corrected as described above. The extinction coefficient ε (cm.²/μmole) of DPNH is 3.3 at 366 mμ, 6.2 at 340 mμ and 5.9 at 334 mμ. d is the light path of the cuvette in cm., V is the final volume of the assay mixture in ml.

## Other Determinations

If the test is carried out at pH 7.4 instead of pH 6.0, pyruvate and hydroxypyruvate (with crystalline lactic dehydrogenase from skeletal muscle) (see p. 253) and L-erythrulose (with polyol dehydrogenase from sheep liver) (see p. 208) may be determined in the same cuvette used to estimate the glycolaldehyde. This three-fold combined test is described under[3]. To carry out the test at pH 7.4, 1.5 ml. 0.2 M triethanolamine buffer (pH 7.4) is used instead of 1.0 ml. 0.33 M citrate buffer (pH 6.0), otherwise the execution of the test is exactly as at pH 6.0.

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[3] *H. Holzer* and *H. W. Goedde*, Biochim. biophysica Acta *40*, 297 [1960].

Since under slightly acidic conditions[1] low concentrations of acetaldehyde are reduced faster than glycolaldehyde it should be possible, by addition of ADH in low concentration to a mixture of acetaldehyde and glycolaldehyde, to selectively reduce the former and then later to reduce the glycolaldehyde by addition of a larger amount of ADH.

## Specificity

With the high ADH concentration required in the test as described above the following aldehydes, other than acetaldehyde and glycolaldehyde, are reduced by DPNH: formaldehyde, propionaldehyde, butyraldehyde, valeraldehyde, isobutyraldehyde and glyceraldehyde.