

Oxygen Uptake and Spectrophotometric Measurement of Superoxide, Hydrogen Peroxide and Hydroxyl Radicals

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Introduction

The uptake or evolution of oxygen during a reaction can be monitored by use of a Clark-type electrode. The method is particularly useful when turbidity or interfering chromogens preclude the use of spectrophotometry [1].

Protocol

Electrode calibration

1. Add deionized water (2 mL) to the temperature-controller reaction chamber with magnetic stirring, and apply 0.7 V across the electrodes.
2. An output of 0.1 to 10 mV is used with a chart recorder set to a value of 100%.
3. Leave electrode to settle for 5 min.
4. Add a few crystals of sodium dithionate to remove O₂.
5. This value represents 0% O₂. The electrode is now calibrated from 0 to 100% oxygen.
6. Apply test samples to chamber after carefully washing each time.

Calculation

The concentration of oxygen can be calculated by use of the information that air-saturated water at 30°C contains 0.460 μmol O₂.

Spectrophotometric Measurement of Superoxide Anion Radicals

First applied to neutrophil studies in 1968, the nitroblue tetrazolium (NBT) test provides a simple assay for O₂⁻ production. NBT is reduced by O₂⁻ to a blue formazan product which is measured at 620 nm. Spectrophotometric assay techniques are covered in full [2,3]. Pulse radiolysis techniques are also described [4].

Spectrophotometric Measurement of Hydrogen Peroxide

Any system producing O₂⁻ will also produce H₂O₂ by the dismutation reaction. H₂O₂ is an uncharged molecule that can readily enter cells as a water look-alike. Most assays utilize a peroxidase with coupled oxidation of a detector molecule.

H₂O₂ can also be determined by measuring the loss of scopoletin fluorescence (460 nm), an assay covered in detail [5] or on the basis of its reaction with cytochrome-c peroxidase to form a stable complex with absorbance at 419 nm (A₄₁₉) [1]

Herein we describe a method using phenol red to measure hydrogen peroxide in breath condensates [6].

Protocol

1. Dilute stock hydrogen peroxide (30% v/v) 1:1000 with Chelex resin treated distilled water.
2. Calculate the concentration of H₂O₂ by measuring absorbance at 240 nm using a molar absorption coefficient of 43.6 M/cm.
3. Prepare buffered phenol red-peroxidase reagent containing NaCl (40 mM), potassium phosphate (pH 7.0, 10 mM), phenol red (0.1 g/L), and horseradish peroxidase (HRPO; 8.5 unit/mL), adding phenol red and HRPO to buffer just before use.
4. To 1.0 mL phenol red reagent add 0.5 mL breath condensate. Hydrogen peroxide standards are similarly treated.
5. Mix and stand for 5 min at 25°C.
6. Add NaOH (1 M, 10 μL) to give a pH of 12.5 and a stable purple color.
7. Measure absorbance at 610 nm.

Calculation

Include a blank containing H₂O₂-free water and calculate values from an H₂O₂ standard curve.

Spectrophotometric Measurement of Hydroxy Radical Damage

The hydroxyl radical is much too reactive to be measured directly by simple spectrophotometric techniques. Evidence characteristic of its damage is, therefore, usually sought by use of a detection molecular for such damage. Here we describe the use of the sugar 2-deoxy-D-ribose [7-9].

Protocol

1. Into clean glass tubes place 5-deoxy-D-ribose (5 mM, 0.2 mL) and sodium phosphate buffer (0.024 M containing 0.15 M NaCl, pH 7.4, 0.2 mL).
 2. Add OH⁻ generating system, i.e.;
- [A] 0.1 mL of ferrous salt (freshly prepared at pH <6.0) or

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[B] ferric-EDTA + ascorbate or

[C] hypoxanthine + xanthine oxidase + ferric salt.

3. Incubate reactants for 30 min at 37°C.
4. Add thiobarbituric acid (1% w/v, 0.5 mL) and trichloroacetic acid (2.8% W/V, 0.5 mL).
5. Heat tube contents at 100°C for 10 min.
6. Cool and read absorbance at 532 nm or fluorescence at 553 nm after excitation at 532 nm.

Calculation

The assessment of OH \cdot damage can be used to test the effectiveness of scavengers and inhibitors, and to determine second-order rate constants for reaction of OH \cdot with various scavenger.

Comments

OH \cdot generating systems [A] and [B] are not inhibited by SCD, whereas [C] is. All three -OH-generating systems should be inhibited by catalase or other H $_2$ O $_2$ removing enzymes. If solutions are turbid extract chromogen into butan-1-ol.

References

1. Trudgill PW (1985) Oxygen Consumption. In Cell CRC Handbook of Methods of Methods for Oxygen Radical Research, CRC Press, Boca Raton, USA 329-42.
2. Rice Evans CA, Diplock AT, Symons MCR. (1991) Techniques in Free Radical Research, Elsevier Amsterdam 85-91.
3. Greenwald RA (1995) CRC Handbook of Methods for Oxygen Radical Research. CRC Press, Boca Raton, USA 85-91.
4. PUNCHARD NA, Kelly FJ (1996) Free Radicals: A Practical Approach, IRL-Press, Oxford 11-24.
5. Rice-Evans CA, Diplock AT, Symons MCR (1991) Techniques in Free Radical Research. Elsevier, Amsterdam 91-96.
6. Pick E, Keisari Y (1980) A simple colorimetric method for the measurement of hydrogen peroxide produced by cells in culture. J Immunol Methods 38: 161-170.
7. Gutteridge JM (1981) Thiobarbituric acid-reactivity following iron-dependent free-radical damage to amino acids and carbohydrates. FEBS Lett 128: 343-346.
8. Halliwell B, Gutteridge JM (1981) Formation of thiobarbituric-acid-reactive substance from deoxyribose in the presence of iron salts: the role of superoxide and hydroxyl radicals. FEBS Lett 128: 347-352.
9. Gutteridge JM (1987) Ferrous-salt-promoted damage to deoxyribose and benzoate. The increased effectiveness of hydroxyl-radical scavengers in the presence of EDTA. Biochem J 243: 709-714.

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