Deoxyribose Assay

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Introduction

The deoxyribose assay is used to determine the reactivity of tannins toward hydroxyl radicals. The method sounds simple and straightforward, but it is technically difficult to get good results, and interpretation of the data is ambiguous especially for phenolics (which can participate not only as hydroxyl radical scavengers, but also as "pro-oxidants" and as metal ion chelators). I do not recommend this method for general use. Instead, the metmyoglobin (Randox) method is simple and yields better results.

Results we have obtained for tannins are described in (1). Original descriptions can be found in (2, 3).

Reagents

- Deaerated water. Heat an appropriate amount of distilled water at a hard boil for 5 to 10 minutes and allow to come to room temperature. Alternatively, bubble nitrogen through the water for approximately 10 minutes.
- 33.6 mM Deoxyribose. (450.7 mg deoxyribose in 100 mL of distilled water). Allow the deoxyribose solid to come to room temperature before weighing. Refrigerate the solution. The 2-Deoxy-D-Ribose is purchased from Sigma (D-2751). (Final concentration in the assay is 2.8 mM).
  - M FeCl3/0.1 M HCl (16.221 g of solid ferric chloride in 50 mL of 2N HCl and bring final volume to 1000 mL with deaerated water). Store indefinitely.
- 300 µM FeCl3 (150 µL of 0.1 M solution to a final volume of 50 mL of deaerated water). Prepare immediately before use. (Final concentration in the assay is 25 µM).
- 1.2 mM EDTA (35.1 mg disodium salt in 100 mL water). Store indefinitely. (Final concentration in the assay is 100 µM).
- 120 mM Phosphate Buffer (1.6330 g potassium phosphate monobasic in 100 mL water). Adjust pH to 7.4 with 25% KOH. Store indefinitely. (Final concentration in the assay is 10 mM).
- 1.2 mM Ascorbate (21.1 mg L-ascorbic acid in 100 mL deaerated water). Prepare immediately before use. (Final concentration in the assay is 100 µM).
• 50 mM sodium hydroxide (200 mg sodium hydroxide dissolved in 100 mL distilled water). Stable for one week.
• 33.6 mM Hydrogen Peroxide (335 μL stock 30 % w/v solution to 100 mL distilled water). Prepare immediately before use. (Final concentration in the assay is 2.8 mM).
• % w/v TBA (0.25 g thiobarbituric acid in 25 mL of 50 mM sodium hydroxide). Stir for approximately 1 hour and prepare daily.
• 2.8 % w/v TCA (1.4 mL 100% w/v trichloroacetic acid solution diluted with 50 mL water). Prepare daily.
• Tannin (approximately 10 mg tannin in 1 mL deaerated water). Make sure to record the actual concentration of tannin used in a laboratory book. If not all the tannin dissolves, vortex the solution and sonicate for about 1 minute. Remove the tube from the sonicator, vortex, and centrifuge. Transfer the solution to a new tube. Repeat the centrifugation as needed.

A 37 C water bath and a boiling water bath will be needed in this experiment.

Method

1. Start by preparing a spreadsheet of all the reagent and sample amounts. Include blanks for each set of samples and the various reagent conditions.
2. Obtain and label an appropriate number of 15 mL polypropylene screw-top clinical centrifuge tubes for each set of samples to be tested in triplicate.
3. Add 35 μL each of the buffer and the deoxyribose solutionm to the 15 mL tubes. Add the appropriate amount of water listed on the spreadsheet. Vortex the tubes.
4. Add 35 μL of H2O2 and vortex again.
5. Add 35 μL each of the EDTA and FeCl3 solutions. Vortex the tubes.
6. Finally, add the tannin and 35 μL of the ascorbate. Vortex and cap the tubes.
7. The final volume of the mixture in each tube should be 420 μL. Centrifuge @ 3200 rpm (~ 1900 g-force) for 1 minute to ensure that the entire sample is at the bottom of the tube. Vortex each tube lightly.
8. Place the tubes in the 37 C water bath for 1 hour.
9. Allow the tubes to cool for 5 minutes. Vortex and shake the tubes. Centrifuge @ 3200 rpm for 1 minute.
10. Add 350 μL each of the TBA and the TCA solutions, in that order. The volume in each tube is 1120 μL.
11. Loosely cap and vortex the tubes. Place them in the boiling water bath for exactly 20 minutes.
12. Carefully remove the tubes with a pair of tongs to avoid splashing the boiling water and cool for 20 minutes. The pink color will form.
13. Vortex and shake the tubes. Centrifuge @ 3200 rpm for 1 minute.
14. Add 1120 μL 1-butanol and shake gently to mix the two layers. Centrifuge @ 3200 rpm for 6 minutes to separate the layers.
15. Using a solvent-resistant (glass or quartz) microcuvette, blank the spectrophotometer at 532 nm with 1-butanol. Take at least 900 μL of the upper layer of each sample and record the absorbance.
16. Subtract the blank absorbance from each appropriate sample set reading.

The typical absorbance for the control (no tannin) is 1.02 (although this seems to vary substantially).
**Modifications**

Several modifications were made to the Aruoma method in (2).

- The volume of the overall reaction was reduced by 35%. This allows less tannin to be used in each sample.
- The temperature of the first water bath needs to be exactly 37°C. The second water bath was originally described as an 80°C bath, but a boiling water bath (~100°C) gives better reproducibility.
- When the ascorbate and/or the EDTA are omitted, water is used to replace the missing volume. The volume before the TBA and the TCA are added needs to be 420 μL.
- The order of addition of the reagents given in the Aruoma method is changed for our experiments. In the Aruoma method, the order of addition is FeCl₃, EDTA, buffer, water, H₂O₂, tannin, dOR, and Asc.

**References**