A method of estimation of 2-deoxyribose

It has been proposed that malonaldehyde (propanedial) is formed during the oxidation of 2-deoxysugars by periodate[1-4]. Recently, 2-thiobarbituric acid (2-thio-4,6-dioxypyrimidine) has been employed as a reagent for identification of malonaldehyde[5,6].

In this laboratory, highly sensitive analytical procedures for 2-deoxysugars have been developed[7] which are based upon periodate oxidation and subsequent estimation of the resulting malonaldehyde with 2-thiobarbituric acid. This report describes the estimation of 2-deoxyribose.

To 3.5 ml of an aqueous solution containing 0.2–12.5 μg of 2-deoxy-D-ribose*, in a test tube, add 0.5 ml of a solution of 0.025 M periodic acid in 0.125 N sulfuric acid and mix well. After 20 min at room temperature, the mixture is shaken momentarily with 1.0 ml of a solution of 1.0 g of sodium arsenite dissolved in 50 ml 0.5 N HCl. After 2 min, an aliquot of 1.0 ml, or less, of the resulting solution is transferred to a 15 × 125 mm test tube containing 2.0 ml of aqueous 0.6% 2-thiobarbituric acid** (adjusted to pH 2.0 using 1.0 N NaOH and 1.0 N HCl). The volume is brought to 3.0 ml and the contents of the tube are mixed. A blank is prepared by mixing 2.0 ml of the thiobarbituric acid reagent with 1.0 ml distilled water. The tubes are fitted with tear-drop condensers and immersed in a boiling water bath for 20 min. After cooling in tap water at room temperature for 2 min, the intensity of the pink solution is measured at 532 mμ with a Beckman DU Spectrophotometer.

The color is stable for at least one hour. The absorption spectrum (Fig. 1), obtained with a Warren recording spectrophotometer, is in agreement with spectra reported by other workers for the condensation product of malonaldehyde and 2-thiobarbituric acid[5,6]. The optical density was proportional to concentration up to at least 2.5 μg 2-deoxy-D-ribose/ml.

Malonaldehyde bis-bisulfite, sodium salt[8] was utilized in studies demonstrating the complete conversion of 2-deoxy-D-ribose to malonaldehyde; the latter was found to be stable in the excess of periodate in the above procedure[7].

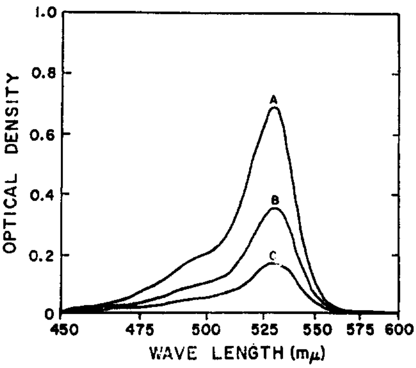

Fig. 1. Visual spectra of products obtained by heating periodate reaction mixtures with 2-thiobarbituric acid. A = 2.0 μg 2-deoxyribose; B = 2.0 μg deoxyadenosine; C = 40 μg DNA.

The characteristic spectrum (Fig. 1) has also been derived from aliquots of 5% trichloracetic acid (TCA) hydrolysates of commercial preparations of deoxyadenosine*** and highly polymerized salmon deoxyribonucleic acid (DNA)* by heating at 90° C for 15 min prior to analysis[9]. The absorbency at 532 mμ for the deoxyadenosine sample accounts for a 96% recovery of deoxyribose. The small absorbency for the DNA sample is a reflection of incomplete hydrolysis by TCA.

Further studies on the application of this procedure for the estimation of 2-deoxysugars in various biological materials are in progress.

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[1] C. F. HUEBNER, S. R. AMES AND E. C. BUBL, *J. Am. Chem. Soc.*, 68 (1946) 1621.
[2] L. A. MANSON AND J. O. LAMPEN, *J. Biol. Chem.*, 191 (1951) 95.
[3] J. T. EDWARD AND D. M. WALDRON, *J. Chem. Soc.*, (1952) 3631.
[4] F. B. CRAMER AND G. A. NEVILLE, *J. Franklin Inst.*, 256 (1953) 379.
[5] S. PATTON AND G. W. KURTZ, *J. Dairy Sci.*, 34 (1951) 669.
[6] W. G. JENNINGS, W. L. DUNKLEY AND H. G. REIBER, *Food Research*, 20 (1955) 13.
[7] V. S. WARAVDEKAR AND L. D. SASLAW (in preparation).
[8] L. D. SASLAW AND V. S. WARAVDEKAR (submitted for publication).
[9] W. C. SCHNEIDER, *J. Biol. Chem.*, 161 (1945) 293.

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** A 3% solution of 2-thiobarbituric acid (Eastman Kodak Co., Rochester, N.Y.) in hot water, is percolated through a column of Woelm acid aluminum oxide (activity grade 1) and allowed to crystallize at room temperature. The white rectangular crystals ($C_4H_4N_2SO_2 \cdot 1\frac{1}{2}H_2O$) are washed with cold water and air-dried.

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