DEOXYRIBONUCLEASE-I ASSAY METHODS

The original Kunitz[1] procedure is well suited for the assay of DNase-I when applied to the assay of purified preparations of DNase-I. This method is not suitable when applied to crude preparations such as tissue homogenates because of the presence of other ultraviolet-absorbing constitutents[2]. Kurnick[3,4] devised an assay procedure which measures changes in the absorbance (A) in the visible spectrum (640nm) employing DNA-methyl green as substrate. Highly polymerized DNA has a strong affinity for methyl green and forms a complex with this dye. The action of DNase on this complex results in the loss of this affinity for methyl green. Furthermore, the visible color of the complex is stable at pH 7.5 whereas the color of free methyl green fades to colorless in about 12 hours. These characteristics form the basis for the colorimetric assay of DNase by measuring the rate of disappearance of the color of the DNA-methyl green complex.

Since the activity observed with a given amount of DNase varies with each lot of DNA-Methyl Green Substrate, DNase-I Vial, (D 4263) is used as a control.

Kurnick[3] recommends this procedure for the assay of DNase in crude extracts and even heterogeneous systems. He also suggests that it is well suited for the study of inhibitors of DNase.

Assay of DNase-I Using DNA-Methyl Green

The principles of the procedure have been discussed above. An additional comment is in order to explain the use of sodium citrate. The citrate is used to stop enzyme activity, thus permitting time necessary for the color of free methyl green (which is liberated by the enzyme) to fade.

REAGENTS

A. Substrate Solution

1. Suspend 20mg of DNA-Methyl Green (Prod. No. D-2376) in 100ml of 0.05M Tris buffer, pH 7.5 at 37°C, containing 7.5mM $MgSO_4$.
2. To solubilize, stir overnite with magnetic stirrer, and then allow it to stand at 37°C for several hours before use.

B. Inhibiting Solution

0.083M Sodium Citrate in 0.04M Tris buffer, pH 7.5 at 25°C

C. Known Enzyme Solution

1. Reconstitute DNase-I Vial, (D 4263) with 1.0ml cold water (store frozen when not in use).
2. Just before use, dilute 0.1ml to 5ml with cold water (40 Kunitz units/ml).

   Use this solution in Step 2 of procedure below. Discard excess.

D. Unknown Enzyme Solution

1. Prepare dilution which will contain 20 to 100 Kunitz units per ml.
2. Use this solution in Step 2 of procedure below. Record dilution factor used in preparing the solution.

(over)

PROCEDURE

Step 1. Pipet 15ml of substrate solution (Reagent A) into test tube. Place tube in water bath at 37°C for a few minutes to warm up.

Step 2. At zero time, add 1.0ml of enzyme solution (Reagent C or D). Mix quickly by inversion several times and return to water bath.

Step 3. At two minute intervals, pipet 2.0ml aliquot of reaction mixture into 3.0ml of inhibiting solution (Reagent B). After a total of five aliquots have been taken and well mixed, stopper them and allow them to stand at room temperature in the dark for a minimum of 12 hours.

Step 4. Transfer solutions to cuvets of known light path; measure and record the absorbance (A) of each at 640nm, using water as reference.

Calculate the ΔA per minute for the Known and the Unknown from the initial linear rate.

Kunitz Units per ml of Enzyme Solution used in Step 2

$$\frac{\Delta A \text{ per min. of Unk.}}{\Delta A \text{ per min. of Known.}} \times 40$$

REFERENCES

1. Kunitz, M., J. Gen. Physiol, **33**, 349 & 363, (1950).
2. Kovacs, E., J. Pediat., **46**, 691, (1955).
3. Kurnick, N.B., in "Methods of Biochemical Analysis", **9**, 1, Edited by D. Glick, Interscience Publishers, N.Y., (1962).
4. Kurnick, N.B., Arch. Biochem., **29**, 41, (1950).