Dr Vijay Kumar
24/7/2017

Acid hydrolysis of DNA and separation of bases by paper chromatography

PRINCIPLE:

RNA and DNA can be hydrolysed to the constituent bases by treatment with 72% perchloric acid for one hour. The method is not completely quantitative since some thymine is lost. The resulting bases are then separated by paper chromatography and detected with ultraviolet light.

Principle of paper chromatography:
Chromatography is a powerful technique to separate closely related substances on the basis of their physicochemical properties. The compounds are separated on the basis of their partition or distribution coefficients between two immiscible phases. Cellulose in the form of paper sheets is an ideal support medium where water is adsorbed between the cellulose fibres and forms the stationary or static hydrophilic phase. An appropriate solvent, which functions as a mobile phase, is then allowed to flow over the sample spot. On coming in contact with the mobile phase, the various components of the sample get partitioned between the stationary and mobile phases. Those constituents having a higher affinity for the stationary phase move less rapidly as compared to those having higher affinity for the mobile phase.l

MATERIALS

Chemicals

1. DNA (commercial sample) — 100mg
2. Perchloric acid (72%) — 1ml
3. Marker bases (adenine, guanine, cytosine and thymine) — 5mg each
4. HCl
5. Iso propanol

Glassware and equipment

- Boiling water bath
- Table centrifuge
- Fine glass capillaries
- Paper chromatography chamber with lid
- Whatman No. 1 filter paper
- Ultraviolet lamp
- Hair dryer

PREPARATION OF SOLUTIONS.

Standard Base

To prepare standard base solution, dissolve 5 mg of the base in 1ml of 0.1 N HCl.

0.1 N HCl

To prepare 0.1 N HCl, add 0.8 ml of concentrated HCl (37.5%, sp.gr. 1.19) to 99.2ml distilled water.

Chromatographic solvent (isopranol:water:conc. HCl::130:37:33)

To prepare the chromatographic solvent, mix isopropanol and water in a ratio of 130 volumes: 37 volumes. Then add 33 volumes of concentrated HCl.

METHOD

1. Mix 100mg of the nucleic acid with 1 ml of 72% perchloric acid in a long test tube and screw on the cap tightly. Heat on a boiling water bath for one hour. Do not heat to dryness.
2. Cool the tube, add 1 ml of water, and centrifuge the contents at 3000 rpm for 10 mins.
3. Use the clear supernatant for ascending paper chromatography. Using a fine glass capillary, apply 3-4 drops of the supernatant on the starting line of a Whatman No. 1 filter paper sheet cut to a suitable size (e.g. 15cms x 19cms)
4. Also spot 3-4 drops of each of the bases as the reference standards about 2.5 cms apart from each other on the starting line.
5. Allow the spots to dry completely and develop the chromatogram in the chromatographic chamber. When the solvent has reached 2-3 cms from the other end of the paper (takes approximately two hours), remove it from the chromatographic chamber, draw a line with a lead pencil to mark the solvent front and dry in a current of cold air/ leave overnight to dry completely.
6. Detect the spots by examining the dried paper in a dark room under UV light (256 nm). Draw an outline of the dark spots due to the bases with a lead pencil.
7. Identify each spot by comparing its Rf value with the standard bases.

$$Rf = \frac{\text{Distance travelled by the component from the base line}}{\text{Distance travelled by the solvent from the base line}}$$

PRECAUTIONS

1. Do not let the DNA-perchloric acid mixture heat to dryness. It is preferable to carry out the hydrolysis in a fume chamber.
2. Hold the chromatographic paper only by the corners.
3. Put the solvent in the chromatographic jar 30 minutes before use so that the chamber gets saturated.
4. UV radiation is extremely harmful for eyesight. Care should be taken so that UV light does not fall directly on the eyes or any other part of the body.

REFERENCES

1. Plummer D.T.1979. An Introduction to Practical Biochemistry. Tata McGraw-Hill Publishing Company Ltd.
2. Sadasivam S. and Manickam A. 1992. Wiley Eastern Limited.
3. Sawhney S.K. and Singh R. 2000 Eds. Introductory Practical Biochemistry. Narosa Publishing House.