A New Type of Reagent for the Detection of Molecular Varieties of some Hydrolytic Enzymes: Detection of α -Amylase Isoenzymes

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Many enzymes are known to exist in various molecular forms and their separation and identification may be achieved by a number of techniques. The present paper describes the identification of α -amylase isoenzymes present in normal human saliva and urine. The detection compound is a water-insoluble cross-linked and coloured starch substance that is incorporated into agar medium (Ceska, Hultman & Ingelman, 1969*a*; Ceska, Birath & Brown, 1969*b*; Ceska, Brown & Birath, 1969*c*). The molecular varieties of some other hydrolytic enzymes, e.g. dextranase, lysozyme, papain etc., may be detected by this technique by using their respective insoluble and specific substrates incorporated into agar media.

The enzyme in question is first subjected to electrophoresis in agar gel. Next a strip of cellulose acetate is layered over the agar gel, and this adsorbs the separated isoenzymes. The cellulose acetate strip is then placed on a glass plate covered with a solidified layer of agar containing the enzyme-specific, water-insoluble and coloured substrate. The separated isoenzymes are desorbed from the cellulose acetate strip into the agar–substrate layer and start to hydrolyse the water-insoluble and coloured substrate, leaving clear hydrolytic zones in places where the isoenzymes were located. The water-soluble blue starch breakdown products released by the action of α -amylase are from agar plates partly adsorbed into the cellulose acetate strips in places where the separated isoenzymes were located. Therefore isoenzymes may also be detected by localizing the blue spots on the cellulose acetate strips.

The present paper shows the identification by the above method of α -amylase isoenzymes in saliva and urine from normal human beings.

The agar–substrate mixture is prepared as follows. Agar is solubilized at about 95°C in 20 mM-sodium phosphate buffer, pH 7.0, to give a 1% solution. The blue starch polymer (prepared as described by Ceska *et al.* 1969*a*) is added under strong agitation to hot agar solution to give a 0.5% suspension. This agar–substrate mixture is applied on clean microscope slides to give a thickness of about 1 mm. After being cooled the plates are stored in a wet chamber at about 4°C.

The α -amylase isoenzymes were separated by an electrophoretic technique. The separation was effected in 1% agar medium (in sodium veronal buffer, pH 8.67 and *I* 0.05). The samples were placed into wells with the help of 6 mm-diameter filter paper (Whatman no. 1). A good separation was obtained after 1 h of electrophoresis with a constant current of 20 mA/plate and under a voltage of 110–120 V.

After the electrophoresis the cellulose acetate strip was layered over the agar gel to adsorb the

Fig. 1. Hydrolysed zones due to α -amylase in human urine (right) and saliva (left). The urine sample was tenfold concentrated, whereas saliva was diluted 1:100. The amount of sample used was 1.5 μ l. The arrows indicate the place of application of the samples on the agar gel. The incubation time was 8 h at 37°C.

separated isoenzymes. The cellulose acetate strip was then layered over the agar-substrate layer and incubated in a wet chamber at 37°C or at a higher temperature. The course of the enzymic hydrolysis of the blue starch polymer by the separated isoenzymes could be followed visually. The hydrolysis was terminated by careful washings with distilled water and then by drying at about 65°C. The dried layer of agar-substrate attaches firmly to a clean glass surface and slides can then be kept as a permanent record.

In the work of Hobbs & Aw (1968), in 90% of examined human urine samples only two α -amylase isoenzymes were found. Fig. 1 shows the hydrolytic areas due to the activity of α -amylase isoenzymes from human saliva and urine by using the new technique described above: human saliva shows only one molecular variety of α -amylase, whereas two α -amylase isoenzymes were detected in the urine sample. The slower-migrating urinary

α -amylase isoenzyme coincided with the migrating speed of the salivary α -amylase. The urinary isoenzyme showing faster movement may be of pancreatic origin (Hobbs & Aw, 1968).

This technique offers advantages over that of presently being used, which is based on the iodine-starch reaction. First, other proteins do not interfere. Secondly, the colour of the blue starch polymer is very stable; photography for the purpose of keeping a record is therefore not necessary, since the dried slides keep indefinitely.

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