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Application of Circular Paper Chromatography to the Study of the Mechanism of Amylolysis
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Application of circular paper chromatography to the study of the mechanism of amylolysis.

In a recent communication[1]), a simple method for the separation of sugars by circular paper chromatography was described. This note deals with the application of this technique to the study of the products formed during the enzymic hydrolysis of amylose and starch by β-amylase [prepared from sweet potatoes according to the procedure described by GIRI[2])], salivary α-amylase prepared according to HANES[3]) and TAKA diastase (Parke & Davis & Co.).

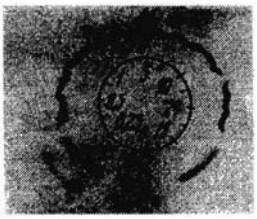

Fig. 1.

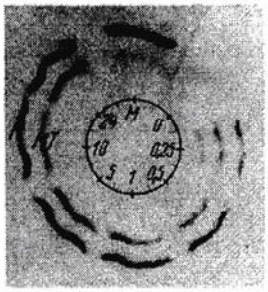

Fig. 2.

Fig. 1. Chromatogram showing the products formed by the action of sweet potato beta amylase on amylose.

Fig. 2. Chromatogram showing the products formed by the action of salivary alpha amylase on amylose.

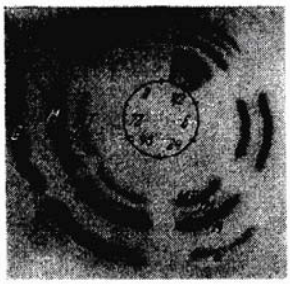

Fig. 3.

Fig. 4.

Fig. 3. Chromatogram showing the products formed by the action of TAKA diastase on amylose.

Fig. 4. Chromatogram showing the products formed by the action of salivary alpha amylase on starch.

Fig. 5. Chromatogram showing the products formed by the action of TAKA diastase on starch.

The procedure adopted was essentially the same as desribed by GIRI and RAO[4]) and the development of the chromatogram was exactly similar to that described by GIRI and NIGAM[1]). The nature of the products formed during the enzymic hydrolysis was followed by spotting about 30 µl of the reaction mixture on the paper at known intervals of time. The reaction mixture contained 1 c.c. of 2% amylose and 1 c.c. 0·1% β-amylase, salivary α-amylase or TAKA-diastase, for amylose hydrolysis. For starch hydrolysis the reaction mixture contained 1 c.c. 5% soluble starch (ZULKOWSKY) and 1 c.c. of the amylase solution of the same concentration. Buffers were not added, the p_H being adjusted to 6·8 in all cases.

Figs. 1 to 5 are the typical chromatograms showing the degradation products formed during the hydrolysis of starch and amylose by the amylases. The saccharides appear as bluish green or grey coloured bands on the paper depending on the type of sugar, against the white background. The chromatographic patterns of the hydrolysates of starch and amylose by the action of β-amylase, salivary α-amylase and TAKA-diastase show differences characteristic of the type of amylase. The chromatogram of β-amylolysis (Fig. 1) shows only one prominent maltose band, while those of salivary α-amylolysis (Figs. 2 and 4) show in addition to maltose other bands of higher saccharides.

The chromatogram of the hydrolysis of starch by TAKA-diastase (Fig. 5) shows some interesting features. At the initial stages of the hydrolysis the short dextrines are formed which are then hydrolysed yielding glucose, maltose, iso-maltose, malto-triose. At later stages of hydrolysis some of the bands relating to the dextrines (having lower R_f value than maltose) slowly disappear and again bands relating to higher saccharides appear on further hydrolysis. These changes in the oligosaccharide bands during the course of hydrolysis indicate synthetic action of the enzymes present in TAKA-diastase preparation. This observation is in conformity with the view expressed by STARK[5]). In this connection the recent findings of PAN et al[6]) on the transglycosidase activity of amylase preparations derived from mold cultures are of special significance. The TAKA-diastase preparation was found to contain isomaltose in traces. The band relating to isomaltose was identified by running chromatograms with pure isomaltose prepared from isomaltose-octaacetate, which was kindly supplied by Dr. A. THOMPSON of the Ohio State University. The isomaltose can be easily identified on the chromatogram by the yellowish brown colour of the bands similar to glucose but quite distinct from the blue colour given by maltose and other higher saccharides.

The chromatograms (Figs. 2 and 4) relating to the action of salivary amylase on amylose and starch show that the end products of the hydrolysis are maltose and malto-triose, although at the initial stages one prominent band of a higher saccharide is formed. These observations are in agreement with those of WHELAN and ROBERTS[7]), whose paper appeared after this work was completed.

The above technique can be easily adopted to various problems in carbohydrate metabolism. The sugars separated on the chromatogram can be estimated quantitatively by spraying the paper with triphenyl tetrazolium chloride reagent[8]),[9]) and estimating the colour intensity after extraction of the colour bands with 75% alcohol[10]). Attention is also directed to the possibility of its use in conjunction with the charcoal column technique[11]) for the preparative isolation of sugars from mixtures. The study of the mechanism of amylolysis by examination and characterisation of the degradation products is rendered simpler by this technique.

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