PAPER CHROMATOGRAPHY OF SOME ENZYMES AND THE PLASMA PROTEINS

by

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Several recent communications deal with paper chromatography of proteins, with buffers[1,2,3,4], or ammonium sulphate solutions[5] as solvents. In the investigation presented here, water miscible organic solvents have been applied to the separation of enzymes and the plasma proteins by paper chromatography.

Partially purified yeast extracts were submitted to chromatography using mixtures of ethanol with phosphate buffers of p_H 7. The ascending technique was employed, and the runs were carried out in the cold, to avoid denaturation of the enzymes. The distribution of the enzymatic activities along the paper was ascertained, cutting it into transversal strips, which were placed in test tubes containing a suitable substrate and incubated.

When extracts from *Saccharomyces fragilis* were chromatographed, it was found that a definite separation between the enzymes phosphoglucomutase and invertase could be obtained with a buffered 20–25% ethanol solvent. Invertase moved faster than phosphoglucomutase, and the R_F of both enzymes was lower at higher alcohol concentrations.

The experiments were repeated with extracts from brewer's yeast; in this case two separate zones of invertase activity were found along the paper. The possibility that the two zones might correspond to different saccharases, was investigated analyzing the products of the reaction and also using maltose, α-methyl-glucoside and raffinose as substrates. All the experiments failed to show any difference between the two enzymatic activities.

Ethanol-water and ethanol-buffer mixtures were also employed for the chromatography of serum and plasma of different mammals, using bromophenol blue[6] as the colour reagent. With aqueous ethanol the chromatograms showed a more or less elongated streak, starting from the origin, and a spot, with R_F ranging from about 0.4 to 0.8, as the alcohol concentration decreased from 40 to 20%. The albumin and globulin fractions of serum were prepared by precipitation with ammonium sulphate and chromatographed simultaneously with the untreated serum. It was found that the streak was due to the globulins and the fast running spot to albumin.

An interaction between albumin and the globulins was indicated by the diminished R_F and the marked tailing of the albumin spot when an artificial mixture of both fractions (or the untreated serum) was run, as compared with chromatograms of the albumin alone.

The use of acetone instead of alcohol in the solvent also yielded good separations. No improvement was obtained by addition of buffers of several p_H values.

A full account of the work will be published in a later issue of this Journal*.

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