Zone electrophoresis of cabbage enzymes in starch gels*

A method of zone electrophoresis using a starch gel as supporting medium has recently been developed by one of us[1,2]. The present communication describes the application of this method to the separation of some enzymes from cabbage juice.

Procedure. The enzyme preparations investigated were prepared from crude cabbage juice, using calcium phosphate adsorption and elution for partial purification[3]. After dialysis against distilled water each preparation was dried from the frozen state. For the electrophoretic studies a starch gel 7.5 mm in depth, 39.5 mm in width and 300 mm in length was used. The depth is such that, at the voltage gradients employed, heating effects were negligible. The width was selected as convenient for use with a multi-blade cutter made from single-edged razor blades. The gels were made as previously described[2] using a boric acid/sodium hydroxide buffer of pH approximately 8.8 and total borate concentration 0.02 *M*. The pH of the resulting gels was 8.0 to 8.2.

Before electrophoresis a suitable amount of dried enzyme preparation (usually 5 mg) was dissolved in a minimum of the 0.02 *M* borate buffer just before use, and a piece of Whatman No. 3 filter paper (7 mm × 39 mm) was soaked in the resulting solution. The filter paper was introduced into a transverse slit in the gel. Thick filter-paper bridges were used to make electrical contact with the ends of the gel (see ref. [2]), which was then covered with liquid paraffin oil to prevent evaporation from the surface during the electrophoresis. A voltage gradient of 3 V/cm was applied for times up to 23 hours according to the type of separation required. The experiments were usually carried out in a cold room.

To isolate the different enzyme fractions after the electrophoresis the gel was sliced serially into transverse slices of convenient thickness (3.2 mm) using a multi-blade cutter. Each transverse slice was further cut into several pieces for the estimation of different enzyme activities. The small gel pieces were placed in shallow cup-like depressions arranged in rows across a plastic sheet (one row for each substrate mixture to be studied). Each cup was sealed with adhesive cellulose tape immediately the gel piece was in place. The whole sheet was then put into a freezer at —20° C which converts the gel to a sponge-like mass[2]. The substrates were later added at room temperature and were worked into the sponges with a glass rod. After incubation for the requisite time a small known amount of the contents of each cup was withdrawn for quantitative chromatography, according to the method of CONNELL, DIXON AND HANES[4], to determine the extent of the reaction and the nature of the products. Since the progress of the reactions was determined usually by a single estimation, the results must be regarded as only semi-quantitative.

Results and discussion. The results of an experiment in which four enzyme functions were investigated by this procedure are illustrated in the figure. Electrophoresis was carried out for 23 hours in this experiment. Slicing was started from the origin (towards the anode) but quantitative studies were made only from slice # 20 since it was known that the activities of most interest were beyond this position.

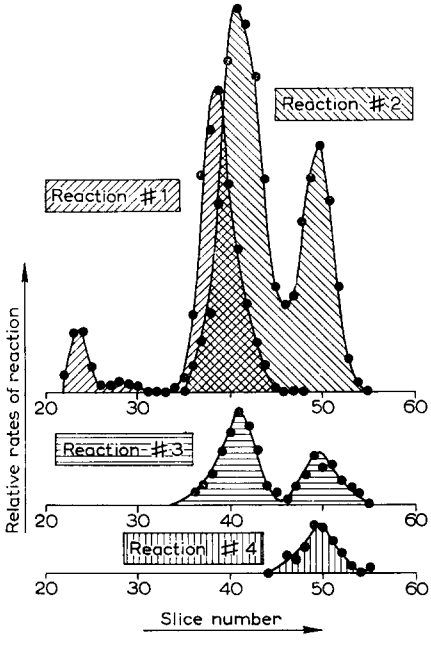

Fig. 1. Distribution of enzyme activities in the starch gel after electrophoresis. Reaction # 1: Hydrolysis of leucyl-glycine. Substrate leucyl-glycine. Reaction #2: Hydrolysis of glycyl-glycine. Substrate glycyl-glycine alone. Reaction #3: Hydrolysis of glycyl-glycine in the presence of phenylalanine. Substrate glycyl-glycine and phenylalanine. Reaction #4: Synthesis of glycyl-phenylalanine due to the transfer of a glycyl residue from glycyl-glycine to phenylalanine. Substrate glycyl-glycine and phenylalanine.

The cabbage enzyme preparations investigated in this work had initially peptidase activities towards leucyl and glycyl peptides and glycyl-transfer activity[3]. Of these activities simple glycyl-

* At the time this work was done one of us (G.H.D.) was supported by a grant from the Ontario Research Council.

glycine (GG) hydrolysis, and glycyl transfer from GG to phenylalanine (P) were of most interest. It had proved difficult by other methods to separate these hydrolytic and transferring activities, and the questions had consequently arisen whether they were two inseparable functions of a single enzyme, or whether an enzyme (or enzymes) might exist able to catalyse one of the reactions but not the other. The electrophoretic analysis illustrated in the figure provides a partial answer to both these questions. Thus the results show that one enzyme is present (with its maximum activity at slice #41) that will catalyse *only* hydrolysis of GG and is unable to catalyse the transfer reaction in the presence of P under the conditions used. A different enzyme is present (maximum at slice #50) that will catalyse *both* the hydrolysis of GG and the transfer of a glycyl residue to P, although this faster migrating enzyme still shows GG hydrolytic activity in the presence of P. Nevertheless this faster migrating enzyme shows a greater ratio of transferring to hydrolytic activity than did any preparation purified by other means, and the results suggest (although they do not prove) that it is a single enzyme able to catalyse both reactions.

The present observations do not exclude the possibility that the solely hydrolytic enzyme is derived from an enzyme with catalytic power for both types of reaction, which has lost one function of its activity during preparation (with a simultaneous change in its electrophoretic properties). However the electrophoretic analysis demonstrates that hydrolytic activity in an enzyme is not always accompanied by transferring activity, and serves to illustrate the value of such an analysis in investigations of enzyme function. The symmetry of the zones and their discreteness indicate the freedom from troublesome adsorption effects which makes starch gel such a satisfactory medium for electrophoretic analyses of protein mixtures.

We should like to point out that this type of investigation is not restricted to enzyme or protein studies but may be applied to the analysis of any property or substance in complex mixtures for which a suitable micro-method of assay is available.

We wish to thank Dr. C. S. HANES for his helpful discussion of this work.

G. H. DIXON*
O. SMITHIES

Department of Biochemistry, and Connaught Medical Research Laboratories, University of Toronto (Canada)

1 O. SMITHIES, *Nature*, 175 (1955) 307.
2 O. SMITHIES, *Biochem. J.*, 61 (1955) 629.
3 G. H. DIXON, *Ph.D. Thesis*, University of Toronto, 1956.
4 G. E. CONNELL, G. H. DIXON AND C. S. HANES, *Can. J. Biochem. and Physiol.*, 33 (1955) 416.

Received October 10th, 1956

* Present address: Department of Biochemistry, University of Washington, Seattle, Wash. (U.S.A.).

The reaggregation of the A-protein of tobacco mosaic virus

SCHRAMM and his co-workers[1,2,3] have made detailed studies of the break-down of tobacco mosaic virus (TMV) and of the reaggregation of the protein obtained in this way. They have observed a series of discrete fractions in the disaggregation and reaggregation experiments, one of which, common to both processes, consists of disc-shaped particles in which a central hole is clearly visible in the electron micrographs. The particles have a rather uniform molecular weight of about one million, and from the length of shadows on the electron micrographs, the thickness of the discs has been estimated to be about 70 A. Discs with central holes similar to these have also been observed by Berkeley workers[4,5] in reaggregation experiments.

The disc thickness therefore appears to be similar to the axial repeat period of 69 A determined by X-ray diffraction[6,7] and SCHRAMM has already drawn attention to this fact. The 69 A axial period, however, relates to the helical arrangement of protein sub-units in the TMV particle[7], and there is no obvious reason why this length of particle should form a stable fragment. 69 A is simply the shortest length of the virus rod which contains both a whole number of turns of the protein helix and a whole number (or very nearly a whole number[8]) of protein sub-units. In the present paper we put forward a hypothesis that would account for the relative stability of a disc of this length.

Reaggregation to discs. The molecular weight of the protein sub-units of TMV is approximately 17,000[9]. However, under the conditions used by both the Tübingen and Berkeley workers to disaggregate TMV, the end-product is a protein of mol.wt. about 100,000, which SCHRAMM has called A-protein. It is this protein that has been used as the starting material in the reaggregation experiments[3,5]. There thus appears to be a stable protein molecule of this size. It is relevant