SIZE OF SAMPLE IN STARCH ELECTROPHORESIS OF CELLULASE

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(Received March 30th, 1961)

The present paper reports a feasibility study with regard to size of test sample in starch block zone electrophoresis. A wide range was made possible through the use of cellulase as the test material, the assay for which is very sensitive[1,2]. Evidence was obtained in the course of the work which suggested the occurrence of dissociable complexes in the enzyme preparation which was used.

MATERIALS AND METHODS

Starch block zone electrophoresis of cellulase from *Myrothecium verrucaria* QM 460[3] was carried out by procedures described elsewhere[4,5]. The buffer used was 0.1 ionic strength pH 7 sodium phosphate, the field 8 V/cm, and the duration of the runs 60 h.

TABLE I

CONDITIONS USED FOR EXTRACTION OF STARCH SECTIONS AND ASSAY OF ENZYMIC ACTIVITY

Amount of enzyme mg	*Volume of extract per section of starch* ml	*Aliquot of extract for assay* ml	*Time of incubation with CMC** min
20	8	0.125	20
10	8	0.25	20
2.5	4	1	20
0.5	2	1	50
0.1	2	1	410
0.02	2	1	2400

* CMC = carboxymethylcellulose.

At the ends of the runs the starch blocks were cut into 0.5-cm sections, the sections were extracted with appropriate volumes of extracting fluid, and the extracts were analyzed for units of enzymic activity by procedures using carboxymethylcellulose

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as the substrate[1,2]. Water or 0.01 % bovine serum albumin solution was used as extracting fluid in different tests. Details of the conditions used for extraction of the starch sections and for assay of extracts for enzymic activity are given in Table II.

RESULTS

Results obtained with 20-, 10-, 2.5-, and 0.5-mg quantities of the cellulase preparation used as the test sample are compared in Fig. 1. In the experiments carried out with these quantities of cellulase, water was used for extracting the sections of the starch blocks at the ends of the runs. The notations which are given for the different peaks

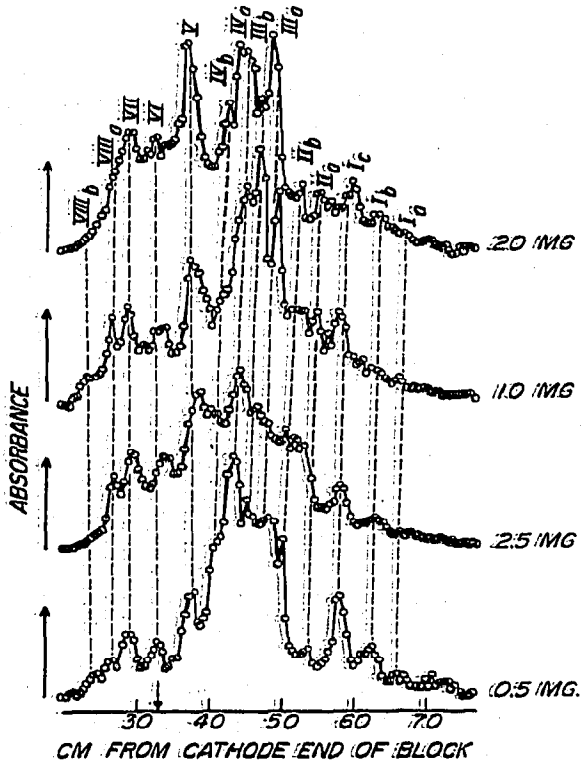

Fig. 1. Comparison of electrophoretic diagrams of cellulase using different sizes of sample. The location of the zone of dextran used as a marker is indicated by the arrow on the abscissa.

correspond to those given in a previous paper[3]. The resolution is seen to be generally less complete at the 20-mg level than at the lower levels, an observation which was confirmed repeatedly.

When the quantity of test sample was reduced to 0.1 mg, resolution of peaks became more irregular and the recovery of enzymic activity in the extracts appeared lower than expected, as shown by the upper diagram of Fig. 2. When sections of starch blocks at the end of such runs were extracted with 0.01 % bovine serum albumin instead of with water, more favorable results, shown in the lower diagram of the figure, were obtained. In a separate study[2] this effect was shown to be due to a protective action of the albumin on the enzyme during the assay for activity. Entirely

analogous results were observed in comparable runs with 0.05-mg quantities of cellulase.

Results obtained with 0.02 mg of cellulase, however, showed poor resolution of peaks, even when the sections of the starch block at the end of the run were extracted

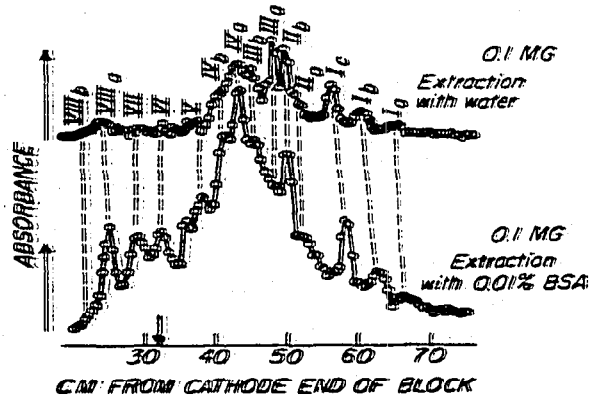

Fig. 2. Effect of extraction of starch sections with bovine serum albumin solution on electrophoretic diagrams obtained with 0.1 mg of cellulase.

with bovine serum albumin solution (upper diagram, Fig. 3). The inclusion of 0.2 mg of the albumin with the 0.02-mg quantity of cellulase in the test sample brought about considerable improvement in the data (lower diagram, Fig. 3). Here it would appear that the albumin protected the enzyme from inactivation at low concentration within the starch block during the period of electrophoresis. The use of amounts of test sample

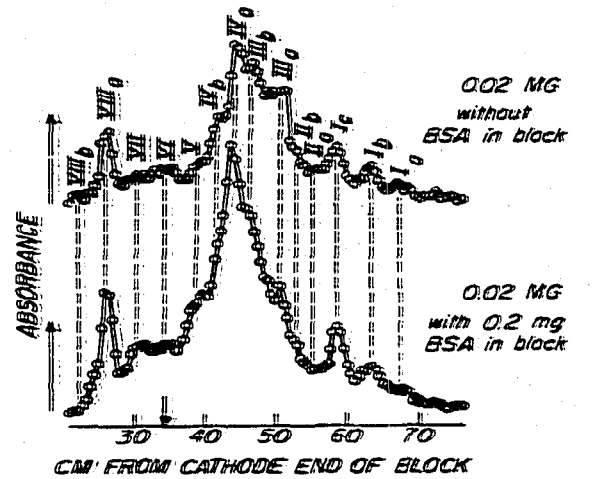

Fig. 3. Effect of presence of bovine serum albumin in test sample on electrophoretic diagrams obtained with 0.02 mg of cellulase.

lower than 0.02 mg was found to be less feasible, mainly because of the excessively long periods of incubation required for the assays of enzymic activity.

To obtain further information regarding quality of resolution when different sizes of samples are used, re-runs were made on fractions of cellulase taken from

20- and 2.5-mg runs, respectively. The fractions which were tested for the re-runs were selected at the centers of nine of the most prominent zones observed in the original runs and the sizes of the aliquots which were taken were based on equal con-

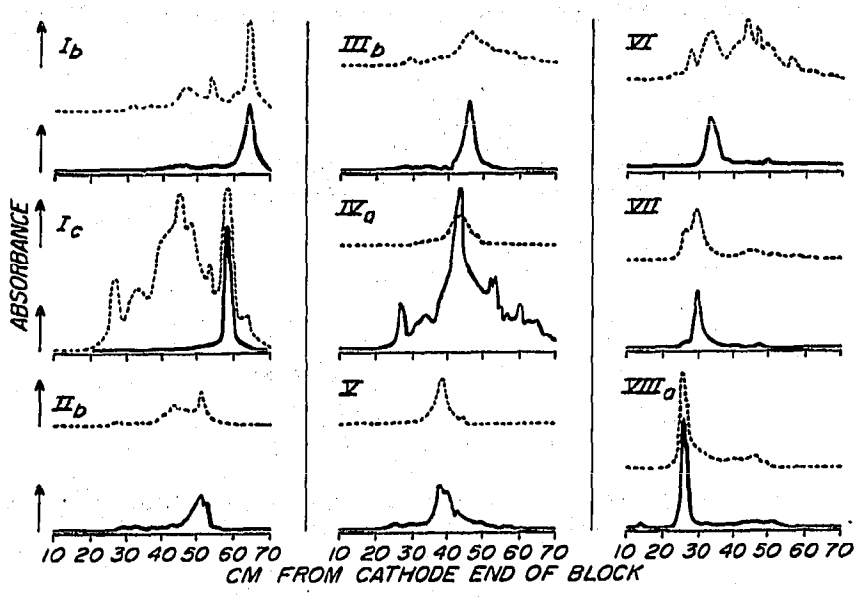

Fig. 4. Electrophoretic diagrams obtained in re-runs of electrophoretic fractions. Dotted lines represent fractions obtained from original run with 20 mg of cellulase; solid lines, fractions from run with 2.5 mg.

tents of enzymic activity (0.07 unit per aliquot). In each of the re-runs, 0.2 mg of albumin was included with the test sample, 0.01 % albumin solution was used to extract the sections of starch block, and the same period of incubation was employed in the assays for enzymic activity. The results (Fig. 4) show that the fractions taken

TABLE II

RECOVERY OF ENZYMIC ACTIVITY IN RE-RUNS OF ELECTROPHORETIC FRACTIONS

Fraction tested	*Recovery of enzymic activity*	
	20 mg run %	*2.5 mg run* %
Ib	202	137
Ic	1000	114
IIb	111	94
IIIb	164	103
IVa	93	776
V	87	139
VI	266	76
VII	132	80
VIIIa	175	119

from the 2.5-mg run were generally much more homogeneous than those taken from the 20-mg run. A minor exception obtained with Fraction V; a major, with Fraction IVa. Zones of dextran (not shown), which were used as markers in the tests, were uniformly sharp and undisturbed. Recoveries of enzymic activity (Table II) show that

in those instances where the fractions were especially contaminated with extraneous enzyme components, the recoveries were several fold higher than the theoretical. The recovery of activity in the original 20-mg run was found to be 65 %, while that in the 2.5-mg run was close to 100 %.

DISCUSSION

It is noteworthy that although the same peaks are revealed in runs carried out with different sizes of test sample, their relative quantities, as indicated by areas under the peaks (Fig. 1), appear to vary considerably. For example, the proportion of component VIIIa appears to increase as the size of sample decreases, while the proportions of components V, VI and VII decrease; other components show irregular changes. One or several of a number of factors could be responsible for this effect, such as a variation in resolution of adjacent components, differences in relationships between amounts of enzyme and amounts of reducing sugar produced by the different components[6], or the presence or absence of dissociable complexes of the type suggested below.

It was to be expected that the fractions selected for the re-runs would not be completely homogeneous, because of overlapping of zones of adjacent components. It was further to be expected that fractions taken from the 2.5-mg run might reveal greater homogeneity than those taken from the 20-mg run, since the resolution of peaks was generally sharper with the smaller-sized test samples. However, the contamination of certain of the fractions with components having widely differing mobilities, together with the unexpectedly high degree of apparent contamination and abnormally high recovery of enzymic activity in particular instances, requires special explanation. Available evidence from the present and previous work[4,5] indicates that these unusual results, wherever they did occur, did not arise from mechanical or electrical disturbances. The possible existence in the cellulase preparation of complexes which dissociate on electrophoresis in dilute solution could, however, provide a reasonable explanation. Such complexes might consist of varying combinations of enzyme components with one another, possibly through association with a polysaccharide to which they are mutually attracted. The polysaccharide might originate from the cellulase preparation itself, which is composed of carbohydrate to the extent of 50 %[3], or from the starch supporting medium. The different complexes would be required to migrate at different rates and to show low enzymic activities prior to dissociation. The observation that the recovery of enzymic activity in the original 2.5-mg run was greater than that in the corresponding 20-mg run might be taken as support for the hypothesis. It has been proposed by some investigators[7] that the multiple components in cellulase may themselves consist of complexes of enzyme with polysaccharide; possibly this is true in the sense just suggested.

ACKNOWLEDGEMENT

Technical assistance was given by RAY ANDREOTTI.

SUMMARY

Amounts of cellulase varying over a 1000-fold range (0.02 to 20 mg) gave comparable patterns when subjected to starch electrophoresis. Resolution of components appeared to be better, however, with small- than with large-sized test samples. Evidence was also obtained which suggested the occurrence of dissociable complexes in the enzyme.

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