

THE ZONE ELECTROPHORESIS OF *IRPEX* CELLULASE

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(manuscript received Sep. 20, 1958)

Many works have been carried out on cellulase. Few of the works, however, have been performed with unpurified enzyme preparations. At present, purified preparations are much required. Though NISHIZAWA (1), (2), FUKUMOTO (3), and others have purified *Irpex* cellulase, purified preparations have not been obtained in an amount enough to be stored and used for further study.

In the present study, it has been tried to separate *Irpex* cellulase, cellobiase, and amylase from one another by means of the zone electrophoresis. Some findings are presented.

MATERIALS and METHODS

Enzyme Preparations.....Enzyme solutions used for electrophoresis were prepared by concentrating culture fluid of *Irpex lacteus*. The culture medium was that of NISHIZAWA and others (4). After cultivating at 30° for two weeks, the medium was filtrated by suction. 10 l. of the filtrate was concentrated at 30-40° under a reduced pressure and dialyzed in a bladder membrane of bull against distilled water. After repeated concentration and dialysis, the final volume was 10 ml.

Zone Electrophoresis.....The electrophoresis was carried out with potato starches as a supporting medium. Changes of pH at the electrodes were avoided by renewing all 500 ml. of buffer in the electrode vessels every 5 hours.

The runs were made at 10°. The starting sample, which had been prepared by adding 1.5 ml. of buffer of twice the concentration used for the runs to 1.5 ml. of the concentrated enzyme solution, was introduced into a 1 cm. slit cut crosswise in a 40 × 5 × 1 cm. block of starch.

After the completion of each run, 1 cm. wide strips cut from each of the long sides of the block were discarded. The remaining strip of the block was then cut crosswise into sections 2 cm. wide. Each section was extracted three times with 20 ml. of water and the extracts were concentrated to 10 ml. These concentrated extracts (fraction number 1-20) were used for the assay of activity.

Enzyme Activity.....The assay procedure consisted of adding 1 ml. of each of the above extracts to the mixture of 1 ml. of the substrate (1% carboxymethylcellulose, 1% starch, and 0.048 M salicin) and 2 ml. of acetate buffer, 0.1 M, pH 4.0 (4.8 for starch and salicin), incubating at 30°, and measuring the amounts of reducing sugars found in 1

ml. of this reaction by the SCHAFER - SOMOGYI - HARTMAN'S method after incubation for a suitable time (20 hours for carboxymethylcellulose and 10 hours for starch and salicin.)

RESULTS and DISCUSSION

The results of the runs at M/20 acetate buffer, pH 4.8, 250 volts, for 24 hours, and at M/30 phosphate buffer, pH 7.2, 200 volts, for 16 hours are shown in Figs. 1 and 2 respectively.

In Figs. 1 and 2, the vertical axis indicates ml. of N/200 sodium thiosulfate solution consumed in the SCHAFER - SOMOGYI - HARTMAN'S method.

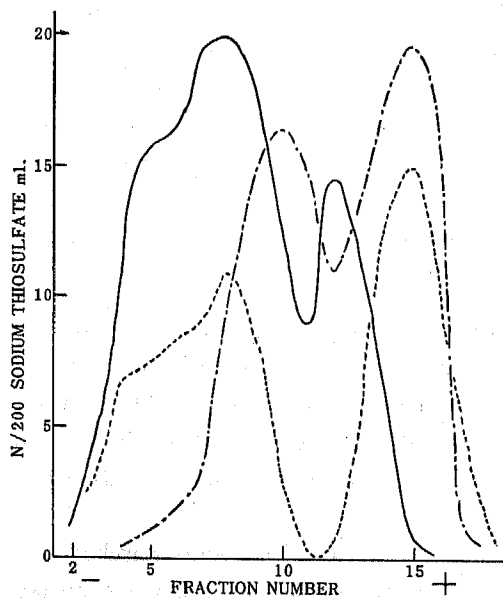


Fig. 1. Distribution of enzyme activity at pH 4.8.cellulase, ----cellobiase, —— amylase.

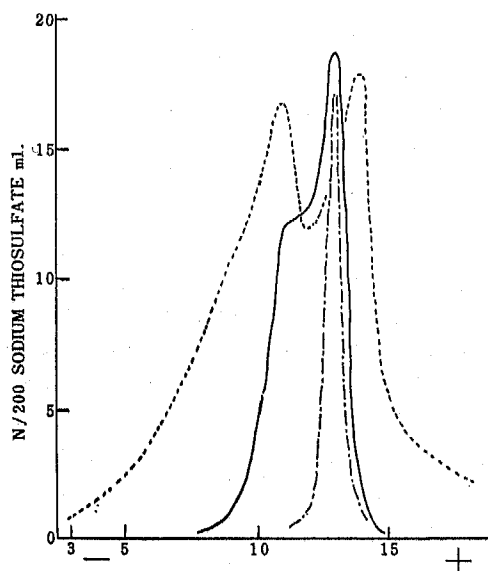


Fig. 2. Distribution of enzyme activity at pH 7.2.cellulase, ----cellobiase, —— amylase.

In Fig. 1, starting sample was introduced into the slit of fraction number 10 and in Fig. 2, fraction number 11 was so.

In Fig. 1, the pattern of the distribution of cellulase activity resembles that of amylase. However, cellulase component at the peak of fraction number 15 is separated from the amylase component, while it overlaps with the cellobiase component. In Fig. 2, the two components of cellulase are successfully separated from cellobiase, while these overlap with the amylase pattern. Therefore, a combination of electrophoresis at pH 4.8 with that at pH 7.2 makes it possible to separate at least each one of the components of cellulase and cellobiase from each other. The separated components are amylase-free.

At the other pH (between 4.8 and 8.4) these three enzymes were not separated from

one another.

Since the removal of other protein impurities from the separated cellulase and cellobiase is relatively easy, it will be of interest and importance to study the specificity, physical properties, and other properties using these preparations.

SUMMARY

The zone electrophoresis of the culture fluid of *Irpex lacteus* has been carried out. Cellulase, cellobiase, and amylase have been separated from one another.

It has been shown that cellulase and cellobiase have the respective two components in the pattern of the activity distribution, and amylase has three components.

REFERENCES

1. NISHIZAWA, K., *J. Biochem.*, **42**, 825 (1956).
2. NISHIZAWA, K., KOBAYASHI, T., & ICHIKAWA, N., *KOSOKAGAKU SHINPOJUM*, **10**, 6 (1954)
3. FUKUMOTO, J., KISHI, K., OSAKA KOSHI. HO., **26**, 295 (1952).
4. NISHIZAWA, K., TANAKA, H., & ICHIKAWA, N., *Research Reports of the Faculty of Textiles and Sericult.*, Shinshu Univ., **4**, 56 (1954).