

26259

### Zone Electrophoresis of "Lichenase"

The hydrolysis of  $\beta$ -glucans of mixed linkage can be accomplished by either of the glucanases specific for one of the linkages. Barley glucan and lichenin, both glucose polymers of mixed  $\beta$ -(1  $\rightarrow$  3) and  $\beta$ -(1  $\rightarrow$  4) linkages, are hydrolyzed by the  $\beta$ -(1  $\rightarrow$  3) glucanase of *Rhizopus arrhizus* QM 1032 or by the  $\beta$ -(1  $\rightarrow$  4) glucanase of *Streptomyces* sp. QM B814 (1, 2). Zone electrophoresis as developed by Miller (3, 4) gives good separation of carbohydrases (5). Its application to the study of "lichenase" is being reported here.

Many fungi grow on lichenin and secrete enzymes capable of hydrolyzing it.<sup>1</sup> Fifteen culture filtrates, after dialysis against water and concentration *in vacuo*, were examined by zone electrophoresis. Most contain both  $\beta$ -(1  $\rightarrow$  3)-, and  $\beta$ -(1  $\rightarrow$  4) glucanases. However, *Rhizopus arrhizus* filtrate gives a single  $\beta$ -(1  $\rightarrow$  3) glucanase peak, coincident with the "lichenase" peak, and no  $\beta$ -(1  $\rightarrow$  4) glucanase; and a *Trichoderma viride* filtrate gives a single  $\beta$ -(1  $\rightarrow$  4) glucanase peak, coincident with the "lichenase" peak, and no  $\beta$ -(1  $\rightarrow$  3) glucanase. Where both  $\beta$ -(1  $\rightarrow$  3) and  $\beta$ -(1  $\rightarrow$  4) glucanases are present (Fig. 1), nearly all of the "lichenase" can be attributed to their combined

<sup>1</sup> Fungi grown on substrates other than lichenin also produce "lichenase."

effects. There are occasional minor "lichenase" peaks that may not be attributed to the two  $\beta$ -glucanases.

While each major  $\beta$ -(1  $\rightarrow$  4) glucanase peak has a corresponding "lichenase" peak, many of the  $\beta$ -(1  $\rightarrow$  3) glucanase peaks have not (Fig. 1). Analysis of these, by chromatography of their hydrolysis products, shows that only the endo- $\beta$ -(1  $\rightarrow$  3) glucanases hydrolyze lichenin. The exo- $\beta$ -(1  $\rightarrow$  3) glucanases are inactive. Apparently, all  $\beta$ -(1  $\rightarrow$  4) glucanases are active because all are random splitting enzymes.

The endo- $\beta$ -(1  $\rightarrow$  3) glucanases act on lichenin to produce the  $\beta$ -(1  $\rightarrow$  3) dimer, laminaribiose, and none of the  $\beta$ -(1  $\rightarrow$  4) dimer, cellobiose. Similarly, the endo- $\beta$ -(1  $\rightarrow$  4) glucanases produce cellobiose and not laminaribiose. This type of action, explained elsewhere (6), and confirmed by Cunningham *et al.* (7), simplifies the analysis of the "lichenase" peaks.

Murti and Stone (8) have noted that the "lichenase" of *Aspergillus niger* corresponds to  $\beta$ -(1  $\rightarrow$  4) glucanase in its behavior on ion-exchange columns. Our results indicate that this is likely, and that the presence of an endo- $\beta$ -(1  $\rightarrow$  3) glucanase might well account for any deviations.

While the fungal "lichenases" tested thus owe most of their activities to endo- $\beta$ -(1  $\rightarrow$  3)-glucanase and/or  $\beta$ -(1  $\rightarrow$  4) glucanase, a commercial

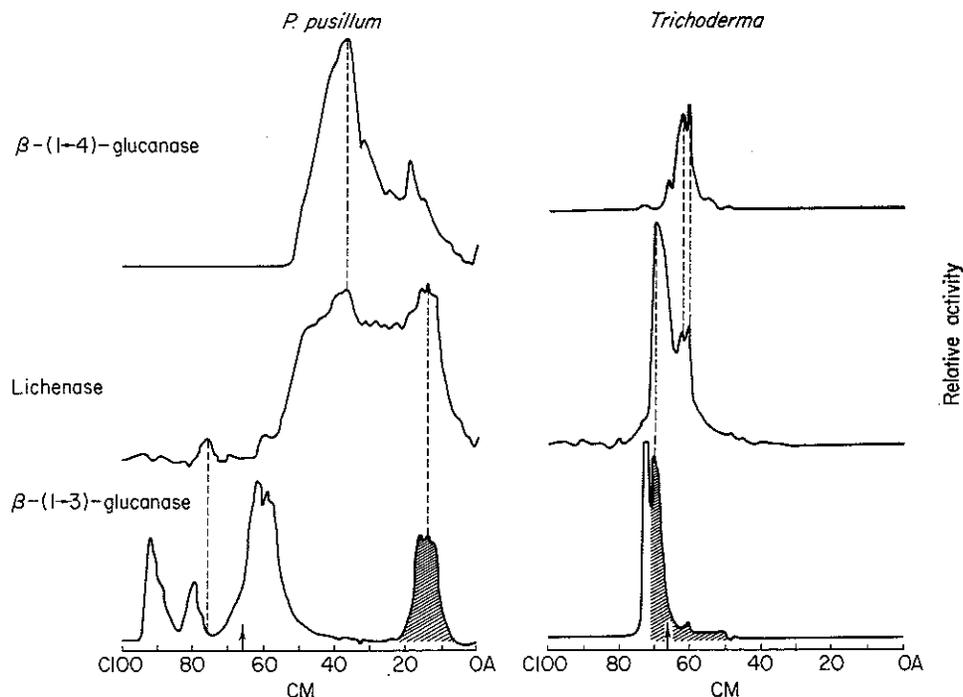


FIG. 1. Electrophoretic patterns of "lichenase,"  $\beta$ -(1  $\rightarrow$  4) glucanase, and  $\beta$ -(1  $\rightarrow$  3) glucanase. *P. pusillum* = *Penicillium pusillum*; *Trichoderma* = *Trichoderma viride*. (This is the same species referred to earlier, but the preparation is of different enzyme makeup because of differences in the substrates on which it was grown.) Dotted lines show correspondence of peaks.  $\uparrow$  shows final position of dextran marker. Abscissa shows position of component on starch block after electrophoretic run. A = anode; C = cathode. Ordinate = relative enzyme activity. Cross hatching indicates the components of  $\beta$ -(1  $\rightarrow$  3) glucanase which act in a random fashion.

diastase (Röhm and Haas Co. No. 57) of unspecified origin does contain a "lichenase" component in which the activity is not attributable to these enzymes. Under the electrophoretic conditions used (5), this "lichenase" moves strongly toward the cathode, rather than towards the anode.

#### REFERENCES

1. PARRISH, F. W., PERLIN, A. S., AND REESE, E. T., *Can. J. Chem.* **38**, 2094 (1960).
2. PERLIN, A. S., AND SUZUKI, S., *Abstr. 44th Can. Chem. Conf. Montreal, Chem. in Can.* **13**, 41 (1961).
3. MILLER, G. L., AND BLUM, R., *J. Biol. Chem.* **218**, 131 (1956).
4. MILLER, G. L., BLUM, R., AND HAMILTON, N. F., *J. Chromatog.* **3**, 576 (1960).
5. MANDELS, M., MILLER, G. L., AND SLATER, R. W., *Arch. Biochem. Biophys.* **93**, 115 (1961).
6. PARRISH, F. W., AND PERLIN, A. S., *Nature* **187**, 1110 (1960).
7. CUNNINGHAM, W. L., AND MANNERS, D. J., *Biochem. J.* **80**, 42-43 (1961).
8. MURTI, C. R. K., AND STONE, B. A., *Biochem. J.* **78**, 715 (1961).

ELWYN T. REESE  
RAY ANDREOTTI  
FREDERICK W. PARRISH

Pioneering Research Division  
Quartermaster Research and Engineering Center  
Natick, Massachusetts

Received November 1, 1961