THE CELLULOLYTIC COMPLEX OF *TRICHODERMA REESEI* QM 9414

An immunochemical approach

Lars G. FÄGERSTAM and L. Göran PETTERSSON
Institute of Biochemistry, University of Uppsala, Biomedical Center, Box 576, S-751 23 Uppsala, Sweden

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1. Introduction

In many laboratories cellulolytic enzyme components from culture filtrates of different species of the fungus *Trichoderma* have been isolated and characterized [1–10]. These components have shown great diversity as regards both enzymic and physicochemical properties. More than a dozen 'different' enzymes have been isolated. The great diversity among these enzymes is somewhat puzzling in view of the fact that they must perform their functions outside the organism in a rather hostile environment that would not be expected to favour a complex multienzyme system sensitive to minor changes in the conditions.

The aim of the present work is therefore to investigate the relationships among components present in culture filtrates of the enhanced mutant strain QM 9414 of *Trichoderma reesei* using a new approach based on immunochemical techniques.

2. Methods

2.1. *Ion-exchange chromatography*

To a column (2.6 × 30 cm) packed with DEAE–Sepharose CL 6B (Pharmacia Fine Chemicals, Uppsala) 'equilibrated' in water, 1 g freeze-dried culture filtrate dissolved in 100 ml water, was applied. After washing the column with 100 ml water, elution was done with a linear ionic strength gradient from 0–0.5 M ammonium acetate buffer (pH 5.0) with total vol. 1100 ml. Fractions (6 ml) were collected at a 180 ml/h flow rate.

2.2. *Isoelectric focusing*

Isoelectric focusing was done in a polyacrylamide gel slab with the composition $T = 5\%$, $C = 3\%$ [11] containing 2.4% Ampholine (LKB Produkter AB, Bromma) in the pH-range 3.5–5.0. After electrofocusing, strips were cut from the gel for further analysis. Two 2 mm wide strips were used for crossed immunoelectrophoresis experiments (see below). A 5 mm wide strip was sectioned into 2 mm wide fractions which were transferred to test tubes containing 200 μl 0.1 M ammonium acetate buffer (pH 5.0). After standing overnight the supernatant in each tube was assayed for endoglucanase activity. The remaining gel slab was fixed and stained with Coomassie brilliant blue.

2.3. *Enzyme assays*

The enzymic activities against Avicel (cellobiohydrolase activity, avicelase) and carboxymethyl cellulose (endoglucanase activity, CM-cellulase) were assayed as in [8].

2.4. *Immunoelectrophoresis*

All procedures and reagents were according to [12]. The antisera used in these experiments were produced against homogeneous components isolated as in [6,7]. The method of antiserum production was as in [12].

2.4.1. Fused rocket immunoelectrophoresis

Aliquots (5 μl) from every second fraction of the DEAE–Sepharose chromatography were analysed by fused rocket immunoelectrophoresis using antisera against the two types of enzymes. The components

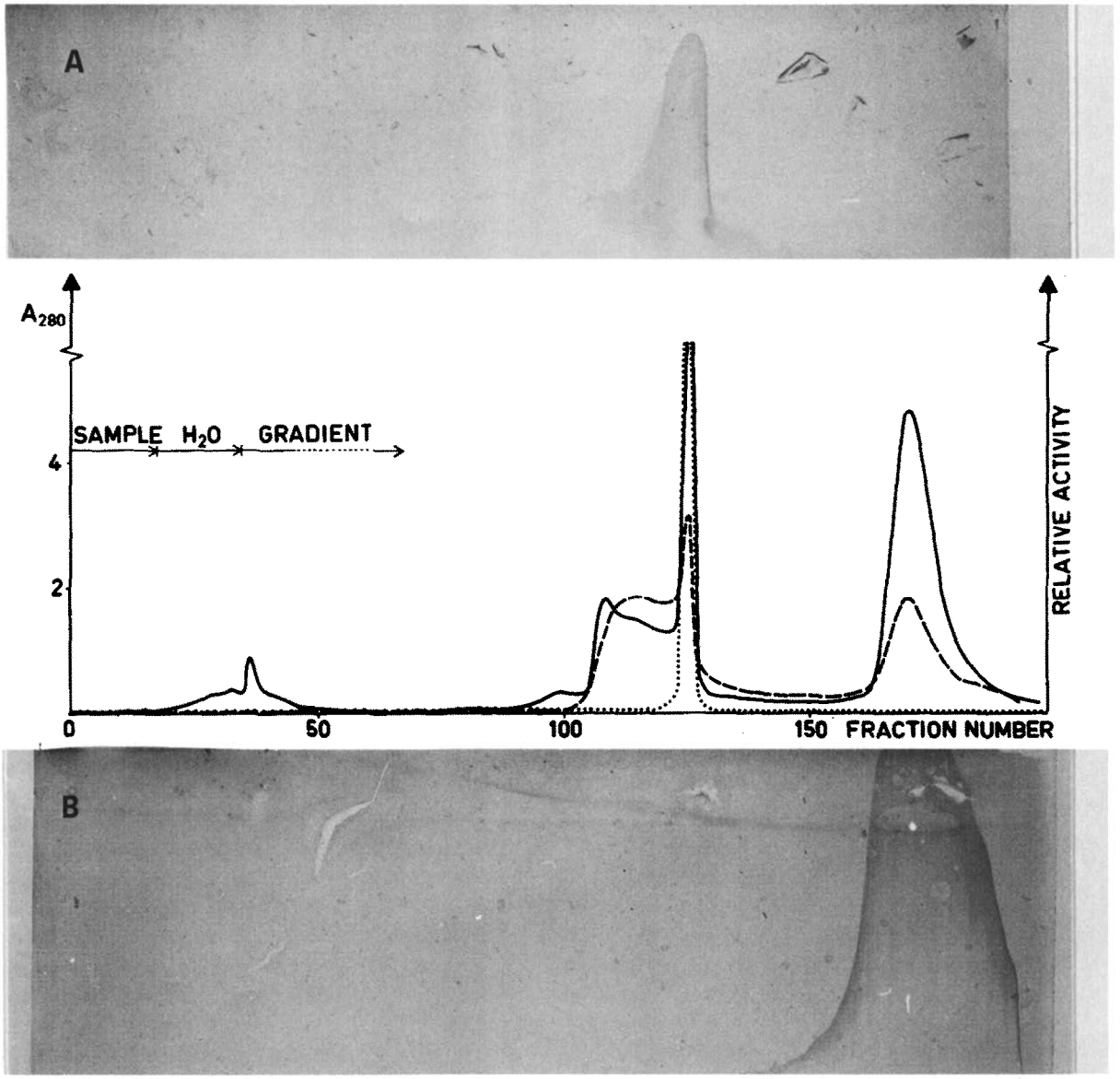

Fig.1. Ion-exchange chromatography of crude filtrate from *T. reesei* on DEAE–Sepharose CL 6B. Freeze-dried filtrate (1 g) dissolved in 100 ml water was applied to the column (2.6 × 30 cm) equilibrated in water. Elution was done with a linear gradient from 0–0.5 M ammonium acetate buffer (pH 5.0). A_{280} (——); CM-cellulase activity (· · ·); Avicelase activity (- - -). Fused rocket immunoelectrophoresis was done using in (A) anti-CM-cellulase antiserum and in (B) anti-Avicelase antiserum.

originally used for production of the antisera are indicated by arrows in fig.2.

2.4.2. Crossed immunoelectrofocusing

In the first dimension the components in the original filtrate were resolved by isoelectric focusing in a polyacrylamide gel slab (0.2 cm thick, 10 cm long) in the pH-range 3.5–5.0. After completion of the electrofocusing a 2 mm wide strip was cut from the gel and transferred to a glass plate (10.5 X 12.5 cm) on which a bisectioned 1%-agarose gel had been cast (total vol. 20 ml). The polyacrylamide gel strip was placed on the lower antibody-free part of the gel and left there for the first 30 min of immunoelectrophoresis (see fig.2). The other part of the gel contained 1000 μl anticellobiohydrolase antiserum or 500 μl antiendoglucanase antiserum. After electrophoresis for 16 h at 2 V/cm the gel plates were stained as in [12].

3. Results and discussion

Isoelectric focusing of whole filtrate from *Trichoderma reesei* QM 9414 reveals a rather complex composition (fig.4a). About 30 components are readily detectable. Most of these focus in the pH-range 3.5–6.

Ion exchange chromatography on DEAE–Sepharose CL 6B affords a rapid and simple group separation of the two main types of activities present in the crude filtrate. By dissolving the freeze-dried filtrate in deionized water and applying it to the ion exchanger equilibrated in water the ionic strength can be held low enough to allow adsorption of the glucanohydrolase, which otherwise has proven to be difficult to adsorb to any ion exchanger [6]. When the column is eluted with a linear ionic strength gradient the glucanohydrolase activity is recovered in one extremely sharp peak (fig.1). The material in this activity peak can be resolved into several subcomponents by electrofocusing. Nevertheless, the peak is very narrow in the chromatographic experiment, which indicates that all of the subcomponents are very similar as regards the distribution of the exposed charges on the surface of the molecules, which are available for interaction with the ion exchanger.

The Avicelase activity, however, is subdivided into two fractions, one eluting at the beginning of the gradient and the other at the very end. Fused rocket immunoelectrophoresis using antiserum prepared against one of the Avicelase components in the second Avicelase peak further indicates that the Avicelase activity in the first peak is due to an enzyme component that is structurally unrelated to the material in the second peak.

By isoelectric focusing both the CM-cellulase activity and the Avicelase activity can be subfractioned into several components, indicating differences in ionizable amino acid sidechain content (fig.2). That this multiple nature of the enzymes is not an artefact produced during the electrofocusing but is due to true heterogeneity can be seen from the fact that, e.g., the Avicelase-components in the last peak in the chromatogram (fig.2) elute as expected, i.e., the lower the pI of the component – the higher

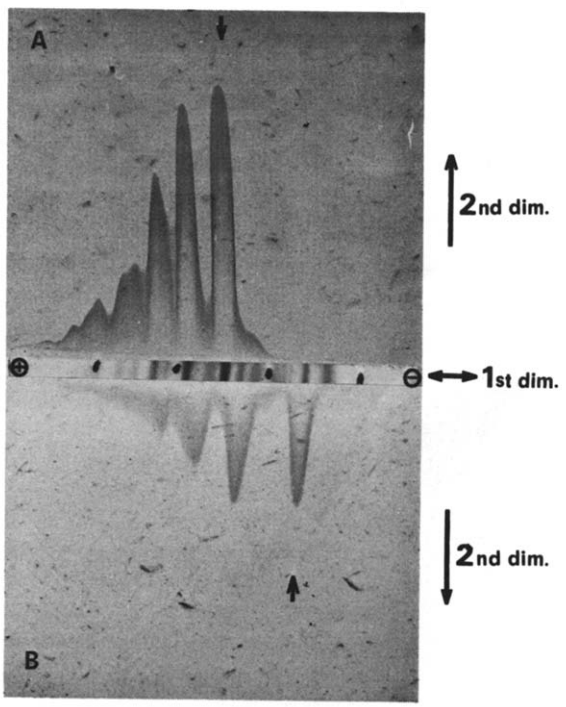

Fig.2. Crossed immunoelectrofocusing of whole filtrate. 1st dimension: Isoelectric focusing in the pH-range 3.5–5.0. 2nd dimension: (A) against anti-Avicelase antiserum. (B) against anti-CM-cellulase antiserum. The components originally used for preparation of antisera are indicated by arrows.

the ionic strength needed for elution from the ion exchanger. This is illustrated in fig.4b–e. When these components are tested for antigenicity by crossed immunoelectrofocusing towards antibodies raised against one originally isolated component (fig.2), they all show crossreactivity. Since the antibodies, like the ion exchanger, only interact with the sterically-available portions of the surfaces of the molecules, the surface topography of the components must be very similar. The appearance in the chromatogram of a component of the Avicelase type that does not crossreact with antiserum against the Avicelase in the second peak indicates that there are major differences between these two enzymes. As can be seen in fig.1 the specific activity of this component is strikingly higher (3–4 times) than that of the component in the second Avicelase peak. The nature of this component will be studied further, as it makes a substantial contribution to the total Avicelase activity.

The relationship between the CM-cellulase activity and the ability of the CM-cellulase subcomponents to form immunoprecipitates is demonstrated in fig.3. There is a very good coincidence between activity peaks and immunoprecipitates. Due to the low sensitivity of the Avicelase assay this was only demonstrated for the CM-cellulase.

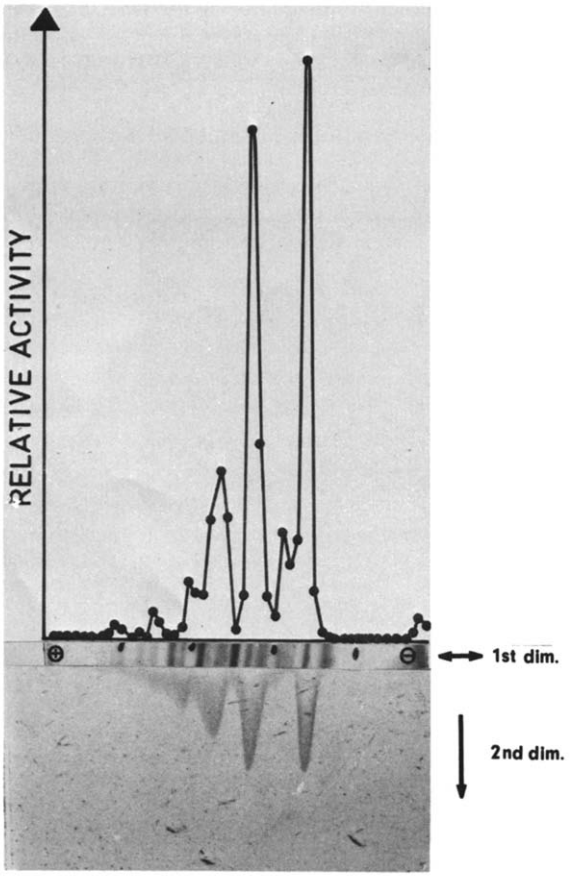

Fig.3. The correspondence between CM-cellulase activity and the ability of the subcomponents to form immunoprecipitates. After focusing a strip of the gel was sectioned and by elution of the sections with buffer the relative activity in each section could be determined. 1st dimension: Isoelectric focusing in the pH-range 3.5–5.0. 2nd dimension: Anti-CM-cellulase antiserum.

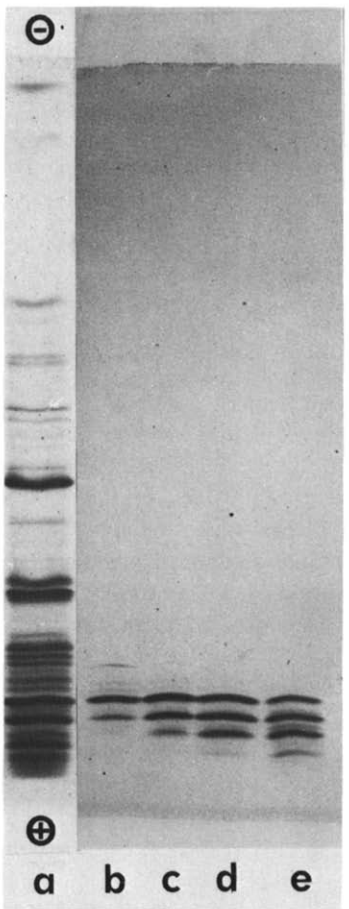

Fig.4. Isoelectric focusing in the pH-range 3.5–9.5. (a) Crude filtrate. (b–e) Fractions 165, 168, 175 and 180 from the DEAE–Sepharose chromatography.

From the results presented here it is evident that the immunochemical technique is a powerful method for studying the cellulase complex and that the immunological identity found both among the endoglucanase and the cellobiohydrolase components probably also reflects a functional identity.

One should bear in mind that the amount and distribution of cellulolytic enzyme components probably depends on the conditions of growth, so that the picture given here is valid only for the specific growth conditions used in this case. The low molecular weight endoglucanase component described [8] is for instance not present here.

Acknowledgements

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References

[1] Wood, T. M. (1968) Biochem. J. 109, 217–227.
[2] Okada, G. and Nisizawa, K. (1968) J. Biochem. (Tokyo) 63, 591–607.
[3] Halliwell, G. and Riaz, M. (1970) Biochem. J. 116, 35–42.
[4] Berghem, L. E. R., Pettersson, L. G. and Axiö-Fredricksson, U.-B. (1976) Eur. J. Biochem. 61, 621–630.
[5] Gum, E. K. and Brown, R. D. (1976) Biochim. Biophys. Acta 446, 371–386.
[6] Fägerstam, L. G., Håkansson, U., Pettersson, L. G. and Andersson, L. (1977) Proc. Bioconversion Symp. 165–178.
[7] Håkansson, U., Fägerstam, L. G., Pettersson, L. G. and Andersson, L. (1979) Biochem. J. in press.
[8] Håkansson, U., Fägerstam, L. G., Pettersson, L. G. and Andersson, L. (1978) Biochim. Biophys. Acta 524, 385–392.
[9] Wood, T. M. and McCrae, S. I. (1978) Biochem. J. 171, 61–72.
[10] Shoemaker, S. P. and Brown, R. D. (1978) Biochim. Biophys. Acta 523, 147–161.
[11] Hjertén, S., Jerstedt, S. and Tiselius, A. (1965) Anal. Biochem. 11, 219–223.
[12] Manual of Quantitative Immunoelectrophoresis (1973) Scand. J. Immunol. 2, suppl. 1.