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IMMUNOELECTROPHORETIC CHARACTERIZATION OF CELLULOLYTIC ENZYMES OF FUNGAL ORIGIN

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SUMMARY

A crude cellulase preparation from *Gliocladium* sp. was immunochemically characterized and the components of cellulolytic activity were identified. Nineteen different antigenic components could be detected by crossed-isoelectric focusing immunoelectrophoresis. The 28 components separated by preparative isoelectric focusing from the above preparation were characterized by fused-rocket immunoelectrophoresis using polyspecific antisera. The main protein components in the enzyme complex were found to be endoglucanases with p*I* values between 3.2 and 5.3. Most of the antigenically active components proved to be glycoproteins.

INTRODUCTION

The immunoelectrophoretic methods introduced by Grabar and Williams[1] and further developed by Laurell[2] have recently been applied to the identification and characterization of minute quantities of proteins, *e.g.* enzymes, in clinical and biological laboratories.

The commercial cellulase preparation Celluclast from *Trichoderma reesei* (Novo, Copenhagen, Denmark) has been characterized by crossed-immunoelectrophoresis[3,4].

The objective of the present work is to investigate whether the immunoelectrophoretic methods are applicable to the characterization of the Gliocladium cellulolytic enzyme system obtained in our laboratory. Five of the most commonly used techniques, immunoelectrophoresis (IE)[1,5] rocket immunoelectrophoresis (RIE)[2], fused-rocket immunoelectrophoresis (FRIE)[2], crossed-immunoelectrophoresis (CIE)[4] and crossed-immunoelectrofocusing (CIEF)[6] were applied in our experiments.

MATERIALS AND METHODS

The crude cellulolytic enzyme preparation of *Gliocladium* sp. was used as antigen[7]. The isoelectric focusing separation of the Gliocladium enzyme components was detailed in a preceding paper[8]. The different cellulolytic activities, the molecular weights and the amino acid contents of the components differing in isoelectric points were also given. Nineteen of the twenty-eight components were found to be glycoproteins.

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The antisera were raised against the cellulase complex of *Gliocladium* sp. as antigen in rabbits, inoculated every two weeks during a 2-month period[9]. The immunoglobulin fraction was purified according to Steinbach and Audran[10]. The molecular weight of the purified antiserum was determined by sodium dodecyl sulphate-polyacryl amide gel electrophoresis (SDS-PAGE) using the Weber and Osborn[11,12] SDS–phosphate continuous buffer system, as described earlier[8].

The culture filtrates of *Aspergillus terreus* OKI 16/5, *Penicillium Verruculosum* WA 30, *Trichoderma viride* OKI B-1 and *Trichoderma reesei* QM 9414 were obtained by courtesy of the Technical University of Budapest, Hungary. The commercial enzyme preparations Cellulase SP 122 and Celluclast 250 S (Novo) were also used.

Agarose gels (1% in barbital buffer, pH 8.6) were cast on glass plates according to the Pharmacia manual on immunoelectrophoresis[13]. The gels, containing 100 μl of antiserum and 10 μl (0.1 mg) of protein, were run at different V/cm values and times according to the various techniques described in the manual[13].

After formation of the antigen–antibody precipitate, the gels were stained with Servablau G (Serva, Heidelberg, F.R.G.).

In CIEF, the proteins (antigens) were first separated by analytical IF according to the Pharmacia manual on IF[14], using Ampholine (LKB, Bromma, Sweden) in the pH range 3–10. The focusing was followed by a second electrophoretic run, perpendicular to the first dimension, into the antibody-containing agarose gel.

RESULTS AND DISCUSSION

Polyspecific antibodies with subunit molecular weights (67 000–10 000 Dalton) were produced against the complex enzyme system of *Gliocladium* sp. Of the 28 enzyme components with different activities and isoelectric points, separated according to a preceding paper[8], several were found to act as antigens. Therefore, the evaluation of RIE is limited, although the individual precipitation zones are proportional to the concentrations of the corresponding antigens (Fig. 1). To identify the immunologically active enzyme components (*e.g.* antigens), samples from the 28 Gliocladium fractions, obtained during preparative IF, were run in FRIE (Fig. 2).

The fractions with p*I* values in the range 3.2–5.3 were found to be the strongest antigens. These fractions showed the highest cellulolytic activities and proved to be glycoproteins as well[8], which may be related to their positive antigenic behaviour[15].

Gliocladia are related to the Trichodermae[16], not only morphologically and biochemically but immunologically as well, as shown in FRIE. The Trichoderma

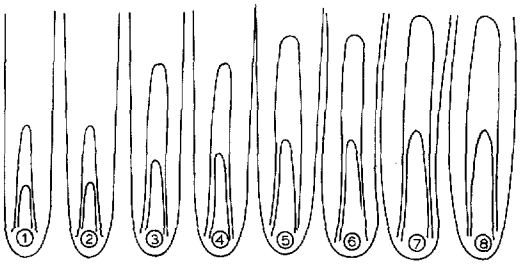

Fig. 1. Rocket immunoelectrophoresis. Quantitation of Gliocladium cellulases. Wells 1–8: 0.2; 0.5; 1.0; 1.5 μl of Gliocladium culture filtrate in two parallels. Pattern developed for 24 h at 50 V and 5°C.

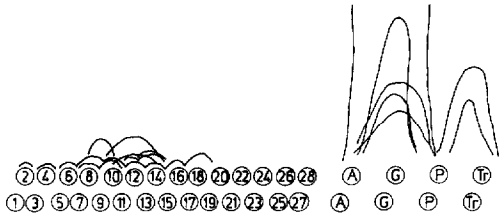

Fig. 2. Fused-rocket immunoelectrophoresis. Wells 1–28: 5 μl of the Gliocladium fractions separated by preparative IF; A, G, P, Tr: 5 μl of the Aspergillus, Gliocladium, Penicillium and *Trichoderma reesei* culture filtrates, respectively. Samples were run for 20 h at 50 V and 5°C in an agarose gel containing 100 μl of antiserum.

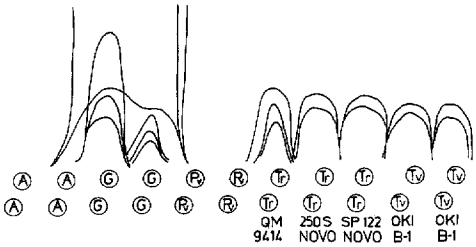

Fig. 3. Fused-rocket immunoelectrophoresis of cellulases. Wells A; G; Pv; Tr QM 9414; Tr 250 S NOVO; Tr SP 122 NOVO; Tv OKI B-1: 5 μl of culture filtrates *Aspergillus terreus* OKI 16/5; *Gliocladium* sp.; *Penicillium verruculosum* WA 30; *Trichoderma reesei* QM 9414; *Trichoderma viride* OKI B-1, respectively, in four parallels; 5 μl of commercial enzyme preparations *Trichoderma reesei* 250 S (Celluclast, Novo); *Trichoderma reesei* SP 122 (Cellulase, Novo) respectively, in two parallels. Samples were run in an agarose gel containing 100 μl of antiserum against *Gliocladium* sp.

cellulases formed precipitates with the antisera produced against the Gliocladium cellulase (Fig. 3).

No relation was found between the Gliocladium and Aspergillus or Penicillium cellulases, respectively. At least two antigenically active components were found in the CIE characterization of *Trichoderma reesei* QM 9414 (Fig. 4).

Trichoderma cellulases also contain carbohydrates[16]. A more accurate iden-

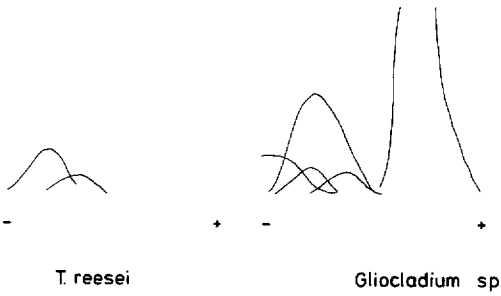

Fig. 4. Crossed-immunoelectrophoresis of *Gliocladium* sp. and *Trichoderma reesei* QM 9414 culture filtrates (5 μl each, first dimension 1.5 h, 200 V, 5°C; antiserum against *Gliocladium* sp., second dimension 20 h, 50 V, 5°C).

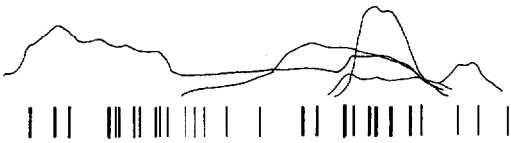

Fig. 5. Crossed-isoelectric focusing immunoelectrophoresis. First dimension: 10 μl of *Gliocladium* sp. culture filtrate, separated by IF for 3.5 h at 1000 V, 15°C; second dimension: CIEF against 100 μl of antiserum for 20 h at 50 V, 5°C.

tification of the Gliocladium components of antigenic activity was obtained by CIEF (Fig. 5).

Ten glycoprotein components with p*I* values in the range 3.2–5.3, and nine components of p*I* 5.8, 6.6, 7.3, 7.5, 7.6, 7.7, 8.0, 8.4 and 9.1, gave the strongest precipitation zones. However, after preparative IF, the main part of the cellulolytic activities and the protein content were found in the fractions of p*I* values as indicated above[8].

In order to investigate whether the immunoactivity can be explained by high enzymic activity, the enzyme complex of *Gliocladium* sp. was treated with 10 *M* sodium hydroxide for 60 min. The denaturated enzyme components formed almost symmetrical precipitation lines, even though the components boiled with strong alkali for 10 min gave distorted zones (Fig. 6).

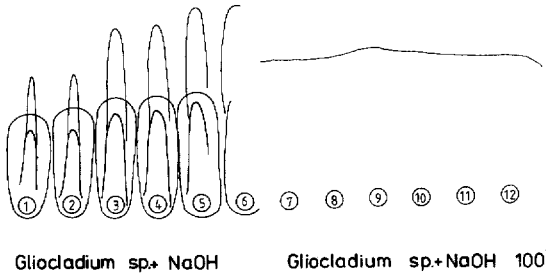

Fig. 6. Rocket electrophoresis of denaturated antigens. Wells 1–6: 5 μl of *Gliocladium* sp. culture filtrate, denaturated by sodium hydroxide; wells 7–12: 5 μl of *Gliocladium* sp. culture filtrate, boiled with sodium hydroxide.

These results are in agreement with the theory that immunoactivity and protein structure are closely related. Indications can be found in the literature[15], such that the immunologically active part of glycoproteins is the carbohydrate moiety. This might explain why the samples boiled with 10 *M* alkali still show some reaction with the antigen. However, this point will be further investigated in forthcoming work.

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