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The Cellulase of *Fusarium solani*

RESOLUTION OF THE ENZYME COMPLEX

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1. Culture filtrates from *Fusarium solani* were fractionated by ion-exchange chromatography on DEAE-Sephadex, followed by gel chromatography on Sephadex G-100, into a C_1 component, a C_x component (CM-cellulase) and a β-glucosidase (cellobiase) component. 2. The individual components showed little capacity for the solubilization of cotton fibre (cellulase activity), but when recombined in their original proportions 81% of the original cellulase activity was recovered. 3. The C_1 components of *F. solani* and *Trichoderma koningii* were similar in their pH optima, heat stabilities over the pH range 5–8 and elution volumes on Sephadex G-100. 4. The C_1 component of *F. solani* synergized with the C_x component of *T. koningii* and conversely. 5. The C_1 and the β-glucosidase components of *F. solani* were devoid of the swelling-factor (S-factor) activity associated with the C_x component.

Cell-free culture filtrates of cellulolytic micro-organisms are in general incapable of extensive attack on highly ordered forms of cellulose, the archetype of which is the cotton fibre. The most notable exceptions in this regard are the culture filtrates of *Trichoderma viride* (Mandels & Reese, 1964) and *Trichoderma koningii* (Halliwell, 1965), although more recently culture filtrates of *Fusarium solani* have been shown to be equally efficient in this respect (Wood & Phillips, 1969). Culture filtrates of these fungi are remarkable in that they contain another enzyme (C_1 enzyme; Reese, Siu & Levinson, 1950) in addition to the C_x enzymes that are found in culture filtrates of all cellulolytic and pseudo-cellulolytic micro-organisms. The C_x enzymes, in isolation, are only capable of attacking soluble derivatives of cellulose such as CM-cellulose or chemically modified cellulose such as alkali- or acid-swollen cellulose. The C_1 component must also be present if refractory forms of cellulose are to be attacked. It is thought (Reese *et al.* 1950) that the C_1 enzyme attacks the cotton fibre to produce a reactive cellulose that is subsequently hydrolysed by the C_x enzyme to give soluble low-molecular-weight oligosaccharides. There is, as yet, no evidence to support this particular explanation.

A C_1 type of enzyme has now been separated from culture filtrates of *T. viride* (Mandels & Reese, 1964; Flora, 1965; Selby & Maitland, 1967; Ogawa & Toyama, 1968) as well as a culture filtrate of *T. koningii* (Wood, 1968), and has been shown to act in synergism with the C_x (CM-cellulase) and β-glucosidase or cellobiase components in effecting the solubilization of the cotton fibre.

In the present paper the separation of *F. solani* cellulase into a C_1, a C_x and a β-glucosidase (or cellobiase) component is described, and the properties of the C_1 component in relation to those of this component in *T. koningii*, are discussed.

MATERIALS

The culture of *F. solani* was kindly given by Dr J. H. Walsh of the Department of Biochemistry, University of Manchester Institute of Science and Technology. Sodium CM-cellulose (Cellofas B) was given by Imperial Chemical Industries Ltd., Nobel Division, Stevenson, Ayrshire, and the Texas cotton was obtained from the Shirley Institute, Didsbury, Manchester. Sephadex G-25, G-100, DEAE-Sephadex (A-50) and quaternary aminoethyl-Sephadex (QAE-Sephadex) (A-50) were purchased from Pharmacia (G.B.) Ltd., London W.13. *o*-Nitrophenyl β-D-glucoside was prepared by the method of Seidman & Link (1950); when recrystallized from ethanol it had m.p. 166° and $[\alpha]_D^{16}$ −98° (*c* 1·0 in water). Cellobiose was supplied by BDH (Chemicals) Ltd., Poole, Dorset. The glucose oxidase (type II) was supplied by the Sigma (London) Chemical Co., London S.W.6, and the peroxidase was purchased from the Boehringer Corp. (London) Ltd., London W.5.

METHODS

Preparation of the F. solani *cellulase.* Culture filtrates of *F. solani* were prepared by the method described by Wood (1968) for the preparation of *T. koningii* cellulase. Incubation was carried out for 25 days at 27°.

A 100-fold concentrate of the culture filtrate was prepared by precipitation with $(NH_4)_2SO_4$ between the limits of 20 and 80% saturation, followed by dissolution of the precipitate in 0·1 M-acetate buffer (acetic acid–NaOH), pH 5·0. The solution was made 5 mM with respect to NaN_3 and

stored at 1°. Enzyme assays indicated that all of the cellulase, CM-cellulase and β-glucosidase activity of the original culture filtrate was recovered.

β-Glucosidase and CM-cellulase activities. These were determined by the method described by Wood (1968).

Cellobiase activity. The incubation mixture contained 1·0ml. of 0·1M-acetate buffer (acetic acid–NaOH), pH5·0, 1·0ml. of 0·4% cellobiose soln. and enzyme and water to give a total volume of 3·0ml. After 2hr. at 37°, 3·0ml. of a glucose oxidase–peroxidase reagent [prepared by adding: (*a*) 12mg. of glucose oxidase, (*b*) 0·5mg. of peroxidase and (*c*) 0·5ml. of a 1% (w/v) solution of *o*-dianisidine in 95% ethanol to M-tris buffer, pH7·0, to give a final volume of 100ml.] was added and the solution incubated for 1hr. at 37°. The enzyme reaction was terminated by the addition of 4·0ml. of 25% (w/v) H_2SO_4 and the solution was read against a water blank on the Spekker absorptiometer with Ilford no. 604 green filters (peak transmission 515nm.).

Cellulase (solubilization) activity. The cotton fibre used in the assays was dewaxed as described by Halliwell (1957).

The incubation mixture contained 2mg. of dewaxed cotton fibre, 2·5ml. of 0·2M-acetate buffer (acetic acid–NaOH), pH5·4 (see Fig. 2 for pH optimum), 0·1ml. of 0·05M-NaN_3, water and enzyme solution to give a final volume of 5ml. Incubation was carried out for 7 days at 37° in a tube graduated at 1·8ml. The residual cellulose was sedimented by centrifugation and the supernatant was removed until a total volume of 1·8ml. was left. Approx. 10ml. of water was added and the tubes were centrifuged again. This procedure was repeated four times to remove all soluble sugars. Then 4ml. of 0·5% dichromate–H_2SO_4 reagent (Halliwell, 1958) was added and the solution heated on a boiling-water bath for 30min. After dilution with 25ml. of water, the solution was read against a water blank on the Spekker absorptiometer with Ilford no. 607 orange filters (peak transmission 600nm.). Controls were included in each experiment.

The graph of enzyme concentration plotted against percentage hydrolysis is shown in Fig. 1. The unit of cellulase (solubilization) activity is defined as the amount of enzyme required to produce 63% solubilization of dewaxed cotton fibre in the standard assay: this was produced by 2ml. of concentrated 20–80%-saturated-$(NH_4)_2SO_4$ fraction diluted 100-fold. A 2ml. portion of this diluted enzyme contained 288units of CM-cellulase activity and 6units of β-glucosidase activity.

Swelling factor (S-factor) activity (see the Results section for description). The method described by Wood (1968) was used except that the period of exposure of the cotton to the enzyme and also to the alkali was shortened to 1hr. and 30min. respectively. The pH of the buffer (0·1M-citric acid–0·2M-Na_2HPO_4) was made 3·6 to coincide with the optimum (Fig. 2). The unit of activity is defined as the amount of enzyme required to bring about an increase in swollen weight of 15mg. beyond the control value (197mg.).

Determination of protein. Protein was determined by the method of Lowry, Rosebrough, Farr & Randall (1951) as described by Wood (1968).

Preparation of Sephadex columns. (*a*) Sephadex G-100. Dry Sephadex G-100 (40–120μm.) was sieved and the material retained on an Endecotts 200-mesh sieve (particle size therefore 75–120μm.) was swollen in 0·01M-acetate buffer (acetic acid–ammonium acetate), pH5·0, and packed into a glass tube as described by Wood (1968). The final dimensions of the gel in the tube were 91cm. × 1·5cm. The void volume of the column was 48ml. when Blue Dextran was used as test substance.

(*b*) Sephadex G-25. Sephadex G-25 (20–80μm.) was used as supplied and packed into a glass column after swelling in 0·01M-acetate buffer (acetic acid–ammonium acetate), pH5·0. The final dimensions of the gel were 3·0cm. × 44cm.

Preparation of the C_1 and C_x components of T. koningii. A cell-free culture filtrate of *T. koningii* cellulase was prepared, precipitated with $(NH_4)_2SO_4$ (20–80% saturation) and concentrated (80-fold) as before.

The C_1 and C_x components were separated by chromatography (without removal of the low-molecular-weight component; Wood, 1968) on DEAE-Sephadex. The C_1 and C_x components were precipitated separately with $(NH_4)_2SO_4$ (80% saturation), desalted on a column of Sephadex G-25, freeze-dried and dissolved in 0·1M-acetate buffer (acetic acid–NaOH), pH5·0.

The individual components were further purified by rechromatography on another column of DEAE-Sephadex and precipitated, desalted and freeze-dried as before.

Portions of the C_1 and C_x components were diluted so that the volumes were equivalent in terms of the unfractionated 20–80%-saturated-$(NH_4)_2SO_4$ material, diluted 80-fold. Under these conditions 1·0ml. of the C_x component and 1·0ml. of the C_1 component solubilized cotton fibre to the extent of 7 and 1% respectively in the standard assay (see above), whereas an assay containing 1·0ml. of both produced 69% solubilization. No activity was lost during the fractionation procedures: 1·0ml. of the 20–80%-saturated-$(NH_4)_2SO_4$ material, diluted 80-fold, again produced 69% solubilization in the standard assay for cellulase activity.

The C_1 component contained only a trace amount of C_x activity. As before, all of the β-glucosidase activity of the unfractionated enzyme was found in the fraction showing C_x activity.

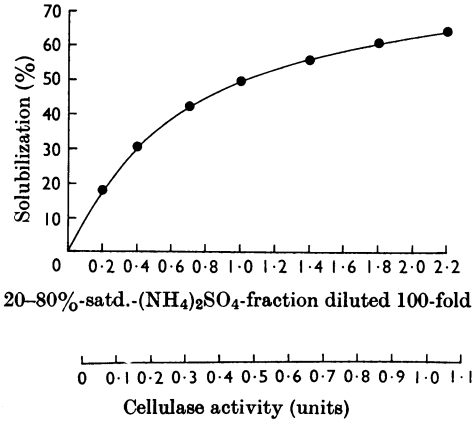

Fig. 1. Effect of enzyme concentration of *F. solani* cellulase on the solubilization of dewaxed cotton fibres. The degree of solubilization was determined as stated in the text. The enzyme used was that precipitated with 20–80%-saturated-$(NH_4)_2SO_4$ and then diluted 100-fold.

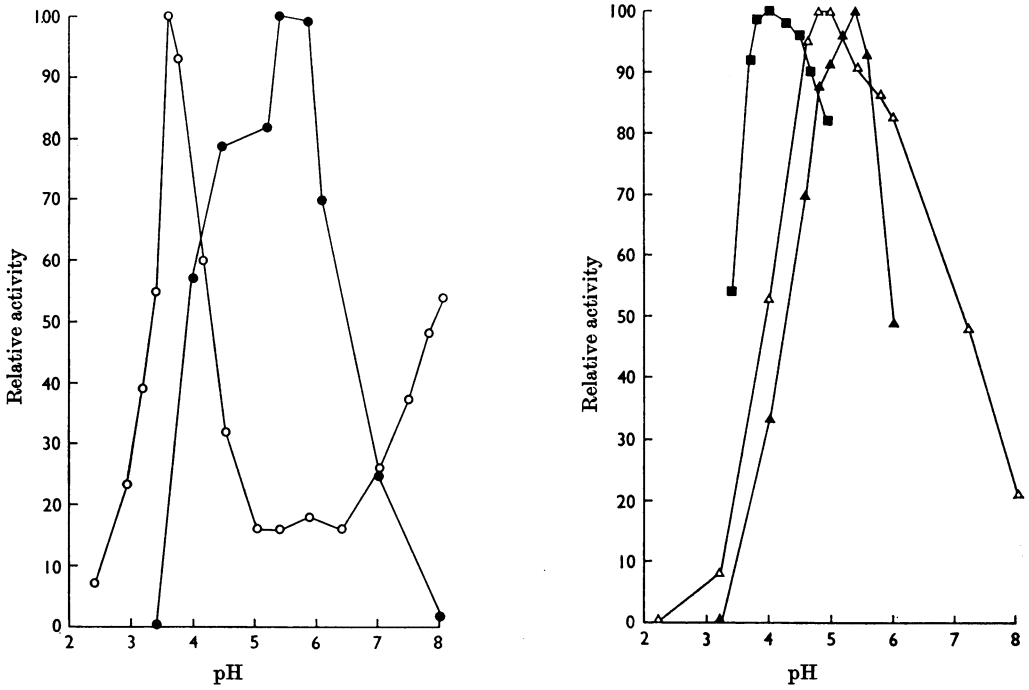

Fig. 2. Effect of pH on the activity of *F. solani* cellulase towards cotton, CM-cellulose and *o*-nitrophenyl β-D-glucoside. See the text for details of assay methods. Assays of (*a*) degree of solubilization (●), (*b*) swelling-factor (○),(*c*) CM-cellulase (▲) and (*d*) β-glucosidase (△) were carried out in phosphate–citrate buffer (0·1M-citric acid–0·2M-Na_2HPO_4). β-Glucosidase was the only enzymic activity showing an altered pH optimum in acetate buffer (acetic acid–NaOH) (■).

A 1ml. portion of the 20–80%-saturated-$(NH_4)_2SO_4$ fraction diluted 80-fold contained 1500units of C_x (CM-cellulase) activity and 150units of β-glucosidase activity.

RESULTS

Enzyme fractionation of F. solani *cellulase on ion-exchange Sephadex.* The separation of the β-glucosidase activity (portion Ba, Fig. 3) from the bulk of the CM-cellulase activity (portion Aa, Fig. 3) was brought about in a sodium chloride gradient on a column of DEAE-Sephadex, the preparation having been previously desalted on Sephadex G-25. The β-glucosidase component was associated with the major protein peak. Cellobiase activity (not shown in Fig. 3) was found only in those fractions also showing β-glucosidase activity and both peaks of activity coincided in fraction 46.

Recovery of CM-cellulase, β-glucosidase and cellobiase was 90, 110 and 100% respectively. The protein peaks coincided approximately with the enzyme peaks in portions Aa and Ba and contained 18 and 79% of the original load.

Pooled fractions Aa (4–12) and Ba (43–51) were compared with the starting material (20–80%-saturated-ammonium sulphate fraction) after dilution for assay to the same final volume in all three cases. Portions Aa and Ba were initially diluted to only half volume in each case so as to permit the addition of only 1·0ml. to the assay, as opposed to 2·0ml. of starting material. Cellulase assays carried out on the diluted fractions indicated that portion Aa produced 12% solubilization and portion Ba 23% solubilization. Portions Aa and Ba therefore contained 6 and 14% respectively of the cellulase activity (from Fig. 1) of the 20–80%-saturated-ammonium sulphate fraction. When the portions were recombined in their original proportions, 91% of the original cellulase activity was recovered (i.e. 61% solubilization as compared with 63%).

The fractionation was repeated on a larger sample of desalted concentrated culture filtrate (15ml.) with a column (15cm. × 2·8cm.) of DEAE-Sephadex. The fractions corresponding to portions Aa and Ba in Fig. 3 were combined (and designated portions Ab and Bb) and precipitated separately by the addition of ammonium sulphate until the solution was 80% saturated. Portions Ab and Bb were

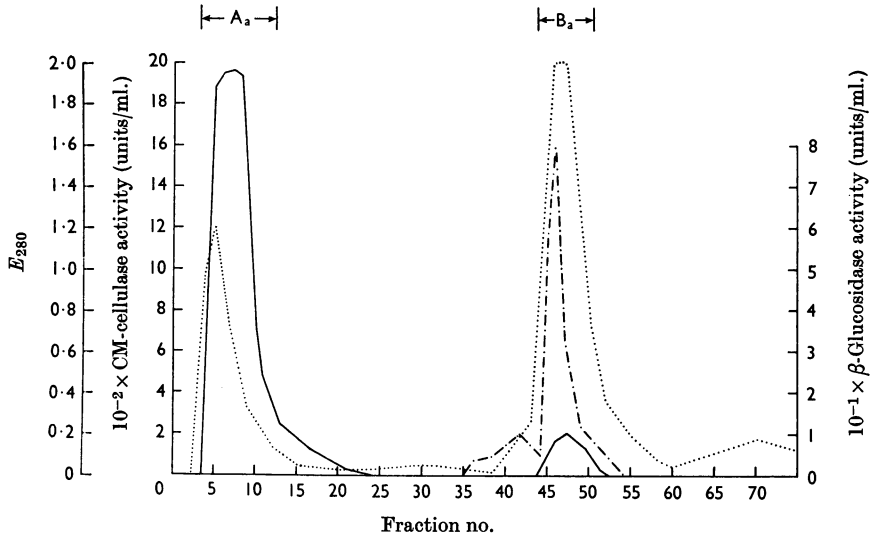

Fig. 3. Fractionation of enzymic activities of *F. solani* cellulase on DEAE-Sephadex A-50 (acetate form). The column (12·0cm. × 1·6cm.) of DEAE-Sephadex was equilibrated with 0·1M-acetate buffer (acetic acid–NaOH), pH5·0. The sample (4ml.) of concentrated (100-fold) culture filtrate [20–80%-saturated-$(NH_4)_2SO_4$ fraction, desalted on Sephadex G-25, freeze-dried and dissolved in 0·1M-acetate buffer, pH5·0] was applied to the column and eluted first with equilibrating buffer. After 20 fractions (4·4ml./fraction) had been collected, a linear gradient of NaCl (0–0·5M in 0·1M-acetate buffer, pH5·0, applied over a volume of 200ml.) was introduced. The column was finally washed with equilibrating buffer, 0·5M with respect to NaCl. The fractions were examined for E_{280} (·····), CM-cellulase (——), β-glucosidase (–·–·–·) and cellobiase (not shown) activities by the standard assays described in the text. Fractions 4–12 (portion Aa) and 43–51 (portion Ba) were pooled and assays for recovery of the various enzymic activities were carried out on these portions.

desalted on the column of Sephadex G-25, freeze-dried, dissolved in 0·1M-acetate buffer (acetic acid–sodium hydroxide), pH5·0, and rechromatographed on columns (20cm. × 2·8cm.) of DEAE-Sephadex. The enzymes were recovered from the columns as before, precipitated with ammonium sulphate (80% saturation) and dissolved in 10ml. of 0·01M-acetate buffer (acetic acid–ammonium acetate), pH5·0. The recovery of CM-cellulase and β-glucosidase activity over the fractionation procedure was 92 and 82% respectively, and 91% of the protein was recovered. It was assumed that the C_1 component must be in portion Bb.

Attempts to obtain the protein peak Bb free from CM-cellulase and β-glucosidase activities by chromatography on DEAE-Sephadex by using a pH gradient were unsuccessful. Fractionation of portion Bb or the original material on QAE-Sephadex (A-50) (acetate or phosphate form) by using a pH gradient (pH5·0–3·5), or alternatively by using a salt gradient in buffers of pH values 4·0–5·0, provided a main protein peak (C_1) with only traces of CM-cellulase and β-glucosidase remaining. In each case, however, recovery of β-glucosidase was only about 30% and the remainder of the activity could not be recovered from the column. Recovery of protein from the QAE-Sephadex columns was also low. Separation of the components of portion Bb was more efficient by using Sephadex G-100 after fractionation (as in Fig. 3) on DEAE-Sephadex.

Fractionation on Sephadex G-100. Fig. 4 shows that the β-glucosidase (portion C) and the major protein component (portion D) in portion Bb were completely separated on a column of Sephadex G-100; a small amount of CM-cellulase activity was found to be associated with both components. Cellobiase was found only in portion C. The recovery from the column of protein, CM-cellulase, β-glucosidase and cellobiase was 98, 100, 105 and 101% respectively.

Portion D was examined for C_1 activity. Cellulase assays were carried out on portion D after adjusting to the same final volume as the 20–80%-saturated-ammonium sulphate fraction diluted 100-fold. At this dilution the assay for cellulase activity contained only 15units of CM-cellulase activity, compared with 288units in the assay for cellulase activity of the 20–80%-saturated-ammonium sulphate fraction. Table 1 shows that under these

conditions of assay the component contained only 3% of the cellulase activity of the starting material (63%).

In the assay for cellulase activity of the β-glucosidase component (portion C), the β-glucosidase concentration was adjusted so that it was equivalent to that in the assay for cellulase activity of the 20–80%-saturated-ammonium sulphate fraction (6 units): the β-glucosidase component had little effect on the degree of solubilization of cotton (Table 1).

Effect of heat and pH *on the activities of the C_1 components of* F. solani *and* T. koningii. The C_1 components of *T. koningii* and *F. solani* showed heat-stabilities that were remarkably similar when heated at 80° for 10 min. (Fig. 5), *F. solani* C_1 component differing only in that the region of maximum stability was at a slightly lower pH. At pH 6·0, the activity of both C_1 components was unaffected. *T. koningii* C_1 component was completely inactivated at pH 5·0.

Of the small amount of C_x activity that remained associated with the C_1 component of *F. solani*, 70% was destroyed at pH 6·0, but the activity of the C_1 component was unchanged (see Fig. 5). In unfractionated preparations, however, C_1 component was unstable to this treatment: C_1 component in crude *T. koningii* cellulase was similarly unstable.

The residual C_x activity associated with the C_1 component of *T. koningii* showed the same heat-stability over the pH range 5–8 as the C_1 component itself: at pH 6·0 neither the C_1 nor the C_x component showed any loss in activity.

Synergistic effects among separated components of F. solani *towards cellulase and S-factor activities.* Although the C_1, C_x and β-glucosidase components of *F. solani* produced little solubilization of cotton on their own, combinations of the enzymes in their

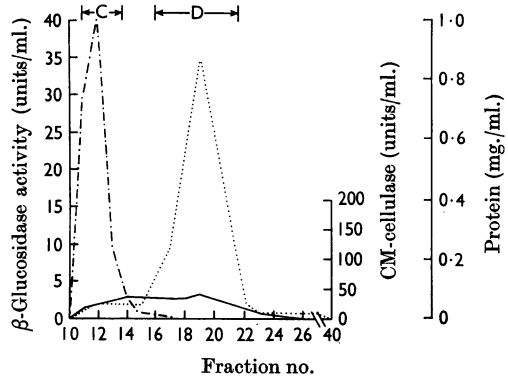

Fig. 4. Separation of the C_1 and β-glucosidase components of *F. solani* cellulase on a column (91 cm. × 1·5 cm.) of Sephadex G-100. The sample (1·0 ml.) of portion Bb [see the text for details of fractionation of 15 ml. of 20–80%-saturated-$(NH_4)_2SO_4$ fraction on DEAE-Sephadex] was eluted from the column with 0·01 M-acetate buffer (acetic acid–ammonium acetate), pH 5·0. Fractions of volume 5·2 ml. were collected. Assays were carried out as stated in the text and the results are shown as; CM-cellulase (——), β-glucosidase (–·–·–) and protein (·····) (Folin–Lowry). The void volume of the column was 48 ml. Portion C is fractions 11–14 and portion D is fractions 16–22.

Table 1. *Relative cellulase activities of the C_1, C_x and β-glucosidase components of* F. solani *cellulase, alone and in combination*

The volume of portion D (C_1 component) was adjusted so that 0·5 ml. of the solution was equivalent in terms of enzyme concentration to 2·0 ml. of the 20–80%-saturated-$(NH_4)_2SO_4$ fraction diluted 100-fold. After heat treatment to decrease the small amount of residual C_x activity (see the text), 1·0 ml. of the C_1 component (0·5 ml. of portion D + 0·5 ml. of buffer, pH 6·0) was used in the cellulase assay. Heat-treated portion D is referred to as portion D_{HT} in the table. Cellulase assays on the other portions contained the same proportion of CM-cellulase and/or β-glucosidase units that were present in 2·0 ml. of the 20–80%-saturated-$(NH_4)_2SO_4$ fraction diluted 100-fold. All portions were derived from the fractionation of 15 ml. of the 20–80%-saturated-$(NH_4)_2SO_4$ fraction (see the text).

Enzyme	Portion	Solubilization (%)	Cellulase activity (unit)	Recovery of cellulase activity (%)
C_1	D	7	0·03	3
C_1	D_{HT}	2	0·01	1
C_x	Ab	12	0·06	6
β-Glucosidase	C	3	0·01	1
C_1+C_x	D+Ab	50	0·05	50
C_1+β-glucosidase	D+C	22	0·13	13
C_x+β-glucosidase	Ab + C	24	0·14	14
C_1+C_x+β-glucosidase	D+Ab+C	59	0·81	81
20–80%-satd.-$(NH_4)_2SO_4$ fraction	—	63	1·0	100

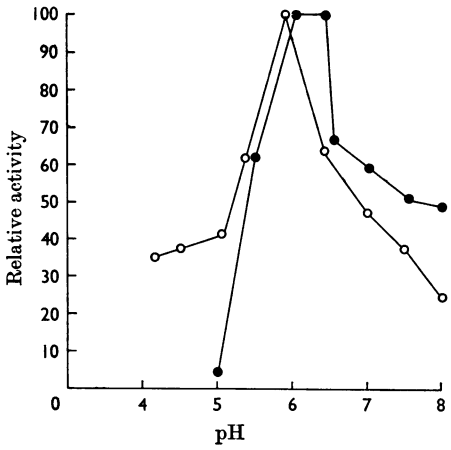

Fig. 5. Effect of heat on the activity of the C_1 components of *F. solani* and *T. koningii*. *F. solani* C_1 component was portion D from the Sephadex G-100 column. *T. koningii* C_1 component was obtained as stated in the Methods section. The volumes of the respective C_1 components were adjusted so that 0·5ml. of the solution contained the same concentration of C_1 enzyme as 2·0ml. of 20–80%-saturated-$(NH_4)_2SO_4$ fraction diluted 100-fold for *F. solani* and 1·0ml. of 20–80%-saturated-$(NH_4)_2SO_4$ fraction diluted 80-fold for *T. koningii*. In the heating experiment, 0·5ml. of the C_1 component was heated with 0·5ml. of 0·075M-phosphate buffer (Na_2HPO_4–KH_2PO_4) for 10min., cooled and 2·0ml. of 0·2M-acetate buffer (acetic acid–NaOH), pH4·8 (with *T. koningii* C_1 component) and pH5·4 (with *F. solani* C_1 component), added. Cellulase assays (see the text) were carried out after the addition of the same number of units of CM-cellulase and β-glucosidase activities that were present in the assays for cellulase activity of the respective 20–80%-saturated-$(NH_4)_2SO_4$ fractions. An activity of 100 is the degree of solubilization produced by 2·0ml. of 20–80%-saturated-$(NH_4)_2SO_4$ fraction of *F. solani* previously diluted 100-fold (63%) and 1·0ml. of the 20–80%-saturated-$(NH_4)_2SO_4$ fraction of *T. koningii* previously diluted 80-fold (69%). Heating was at 80° for 10min. ●, *T. koningii* C_1 component; ○, *F. solani* C_1 component.

original proportions always increased the degree of solubilization of the cotton (Table 1). Cellulase activity was higher with the C_1 and C_x components in admixture than it was with a combination of the C_1 and β-glucosidase components. Little synergism existed between the C_x and the β-glucosidase components. Of the cellulase activity shown by the original 20–80%-saturated-ammonium sulphate fraction 81% was reconstituted when all three components were recombined in their correct proportions. Exactly the same synergistic effects were obtained between the C_x, the β-glucosidase and the C_1 components (portion D) that had been heat-treated (80° for 10min. at pH6·0) to decrease the small amount of residual C_x activity.

Synergistic effects similar to those observed in the solubilization of cotton were apparent with S-factor activity (Table 2). C_x was the only component in isolation showing S-factor activity. Practically all (99%) of the original S-factor activity was recovered with the C_1, C_x and β-glucosidase components in combination (Table 2).

Synergism among the separated components of F. solani *and* T. koningii. The C_1 component of *F. solani*, in addition to synergizing with a mixture of the C_x and β-glucosidase components that were separated from the same culture filtrate, also synergized very effectively with a mixture of the C_x and β-glucosidase components of *T. koningii* (Table 3). *T. koningii* C_1 component behaved similarly, producing 54% solubilization with its own C_x fraction (containing both C_x and β-glucosidase activities; see the Methods section), but 79% solubilization with a mixture of the C_x and β-glucosidase components from *F. solani*.

DISCUSSION

Since the commencement of this work, Wood & Phillips (1969) have shown that cell-free culture filtrates from other strains of *F. solani* have an even higher capacity for the solubilization of cotton fibres than the culture filtrate obtained from the particular strain of *F. solani* that is dealt with here: culture filtrates from *F. solani* C.M.I. 86169 and 95994 were the most active.

The C_1, C_x and β-glucosidase components of *F. solani* resembled the C_1, C_x and cellobiase components from *T. viride* (Selby & Maitland, 1967) and the C_1 and C_x components from *T. koningii* (Wood, 1968; and also the Methods section) in that they displayed little individual capacity for the solubilization of cotton. However, when the separated components from *F. solani* were recombined in their original proportions, 81% of the cellulase activity of the unfractionated enzyme complex was recovered. Synergistic effects similar to these have been shown to exist among the C_1 component, the C_x component, and the fraction possessing both C_x and β-glucosidase activities, that were separated from *T. koningii* cellulase (Wood, 1968). In this case (Wood, 1968) 77% of the activity of the original unfractionated enzyme complex was recovered with the C_1 component in admixture with the other two fractions. Evidence that the cellulase of *T. viride* owed its activity to the combined effect of the C_1, C_x and cellobiase–β-glucosidase components, has been provided by Mandels & Reese (1964), Flora (1965), Selby & Maitland (1967) and Ogawa & Toyama (1968):

Table 2. *Swelling-factor activities of the C_1, C_x and β-glucosidase components of* F. solani *cellulase, alone and in combination*

The C_1 component was portion D (Fig. 4). The enzyme preparations used were the same as those detailed in the legend to Table 1.

Component	Increase in swollen wt. (mg.)	Activity (units)	Recovery of S-factor activity (%)
C_1	Nil	Nil	Nil
C_x	22	1·5	45
β-Glucosidase	Nil	Nil	Nil
C_1+C_x	44	3·0	90
C_1+β-glucosidase	4	0·25	8
C_1+C_x+β-glucosidase	48	3·27	99
20–80%-satd.-$(NH_4)_2SO_4$ fraction	49	3·3	100

Table 3. *Synergistic effects on cellulase activity obtained by combinations of the C_1 component of* T. koningii *with the C_x component of* F. solani *and conversely*

F. solani C_1 component was portion D (Fig. 3) with the volume adjusted as detailed in the legend to Table 1; 0·5ml. was used in the cellulase assays. *T. koningii* C_1 component (see the Methods section) was adjusted so that 1·0ml. of the solution was equivalent to 1·0ml. of starting material [20–80%-saturated-$(NH_4)_2SO_4$ fraction diluted 100-fold]. All assays contained 288units of CM-cellulase activity and 32units of β-glucosidase activity. Assays were carried out as detailed in the text. The pH optima for cellulase activity of *T. koningii* and *F. solani* were 4·8 (Wood, 1968) and 5·4 (from Fig. 2) respectively.

Source of C_1 component	Source of C_x component	Source of β-glucosidase component	Solubilization (%)	pH of cellulase assay
F. solani	*F. solani*	*F. solani*	59	5·4
F. solani	*T. koningii*	*T. koningii*	49	4·8
			51	5·4
T. koningii	*T. koningii*	*T. koningii*	54	4·8
T. koningii	*F. solani*	*F. solani*	79	4·8
			75	5·4

Selby & Maitland (1967) obtained a quantitative recovery of the cellulase activity of the unfractionated cell-free culture filtrate when these components were recombined in their original proportions.

The C_1 component separated from *T. viride* cellulase by Selby & Maitland (1967) was free from C_x activity, as were small portions of a C_1 type of component isolated by Mandels & Reese (1964) and Ogawa & Toyama (1968) from culture filtrates of the same fungus. The C_1 component of *F. solani* differed from *T. viride* C_1 component (Selby & Maitland, 1967), but resembled *T. koningii* C_1 component (Wood, 1968) in containing small amounts of C_x activity. However, the properties of these small amounts of C_x activity differed: whereas that associated with *F. solani* C_1 component could be decreased still further by heat treatment, that associated with *T. koningii* C_1 component could not. In the last case the heat-stability over the pH range 5–8 was exactly the same as that shown by the C_1 component itself, and may therefore indicate that the C_x activity is an integral part of the C_1 enzyme.

The C_1 components of *F. solani* and *T. koningii* had the same elution volume on the Sephadex G-100 column (Fig. 4) and were therefore probably of the same size and/or shape. Further, the C_1 components of both fungi were similar both in the effect of pH on the activities and also in their stabilities under the same conditions of pH and temperature. It was remarkable that the heat stabilities shown by both C_1 components were greater after purification. Additional support to the fact that the enzymes of both fungi were very similar in nature was provided by the observation that the C_1 component of either culture filtrate could synergize very effectively with the C_x component of the other. [Since this paper was first submitted the author's attention has been drawn to a preliminary report in which Selby (1968) describes similar synergistic effects between components from *T. viride* and *Penicillium funiculosum*.]

When cotton fibre that has been exposed to cell-free filtrates of many micro-organisms is subsequently immersed in 18% (w/v) sodium hydroxide, its capacity for the adsorption of the aqueous alkali increases (Marsh, Merola & Simpson, 1953). The enzyme responsible has been called 'swelling factor' or 'S-factor' (Reese & Gilligan, 1954). It has been shown that the bulk of the S-factor activity in a cellulase preparation of *T. koningii* is the result of the C_1, C_x and β-glucosidase components acting in synergism (Wood, 1968). Synergistic effects similar to those observed in *T. koningii* cellulase were apparent among the separated components of *F. solani* cellulase, 99% of the original activity of the unfractionated enzyme complex being reconstituted when all three components were mixed in their original proportions. The C_1 component of *T. koningii* was devoid of S-factor activity (Wood, 1968) as were the β-glucosidase and C_1 components of *F. solani*.

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