The Partial Purification and Properties of Endopolygalacturonase and α -L-Arabinofuranosidase secreted by *Sclerotinia fructigena*

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SUMMARY

An endopolygalacturonase (PG) and an α -L-arabinofuranosidase (AF) in culture filtrates of the fungus *Sclerotinia fructigena* were partially purified by ion-exchange chromatography on CM-Sephadex and Ecteola-cellulose columns, and characterized. Molecular weight estimations by gel-filtration and electrophoresis on cellulose acetate strips indicated that both enzymes existed in multiple forms. The PG showed unusual stability to extreme pH values, to classical inhibitors, and to proteolytic enzymes; in the crude filtrate a bimodal temperature/stability curve was obtained.

INTRODUCTION

The role of pectolytic enzymes in the pathogenicity of fungi and bacteria towards higher plants has been reviewed by Wood (1960) and by Bateman & Millar (1966). Pectolytic enzymes are believed to be involved in the brown rots of plant tissues caused by three species of the genus *Sclerotinia*. The importance of 'cytolytic' enzymes in the pathogenicity of fungi was first put forward in the classical paper of de Bary (1886) about *S. libertiana*; Bruschi (cited by Valteau, 1915) was the first to suggest the action of pectolytic enzymes in a fruit-rotting species. Subsequent work with the brown rot fungi was reviewed by Wormald (1954). Cole (1956), who studied the secretion by *S. fructigena* of extracellular pectolytic enzymes, showed a strong parallel between the plant-macerating properties of his preparations and their ability to decrease the viscosity of pectate solutions. In further studies, however, Cole & Wood (1961) concluded that the degree of macerating activity was not always directly related to the degree of polygalacturonase activity alone or together with pectin methyl esterase. They also showed the presence of traces of polygalacturonase in infected apples: an appreciable amount was also detected by Calonge, Fielding, Byrde & Akinrefon (1969).

Much of the earlier work on the pectolytic enzymes of *Sclerotinia* species and other fungal pathogens was done with crude culture filtrates or with ethanol or acetone precipitates from them; the present work aimed at a fuller purification of the polygalacturonase of *S. fructigena*. During this purification, maceration was found to be associated with a component distinct from polygalacturonase (Byrde & Fielding, 1962); this was later identified as pectin methyl-*trans*-eliminase (Byrde & Fielding, 1968). The presence of an enzyme capable of hydrolysing phenyl- α -L-arabinofuranoside, and initially thought to be involved in maceration, was also demonstrated in these preparations (Byrde & Fielding, 1965) and in infected apple (Calonge *et al.*

1969). The secretion of arabanases by plant pathogenic fungi was reported by Japanese workers (e.g. Kaji, Tagawa & Motoyama, 1965); Fuchs, Jobson & Wonts (1965) surveyed arabanase production by a range of plant pathogens and drew attention to the relatively high secretion of these enzymes by pathogens of leguminous plants rich in arabans. There have been subsequent reports of the secretion of arabanase by *Penicillium* species (Bush & Codner, 1968) and of an α -L-arabinofuranosidase by *Phytophthora palmivora* (Akinrefon, 1968).

The present paper describes the partial purification and some properties of the polygalacturonase (poly- α -1,4-galacturonide glycanohydrolase; EC. 3.2.1.15) and of the α -L-arabinofuranosidase (α -L-arabinofuranoside arabinohydrolase) secreted by *Sclerotinia fructigena* Aderh. & Ruhl.

METHODS

Materials. Pectinol 10 M (Rohm and Haas, U.S.A.) was used as a reference source of polygalacturonase. Sodium polypectate (Exchange Lemon Products Co., California), 'Brown Ribbon' pectin (12.5% methoxyl) (Union Crystalex Gelatine Ltd., London) and *p*-nitrophenyl- α -L-arabinofuranoside (Fielding & Hough, 1965) were used as substrates. For analytical studies, the pectic materials were purified by washing with acidified 80% (v/v) ethanol in water, followed by ethanol to remove the acid, and dried with ethanol+ether (1:1, v/v). For chromatographic studies, Araban (Koch-Light, Colnbrook, Bucks) and apple fibre (Cole & Wood, 1961) were also used as substrates. Sephadex cross-linked dextrans for gel-filtration and ion-exchange chromatography were obtained from Pharmacia AB, Uppsala. Whatman Ecteola-cellulose powder ET 30 and other modified celluloses were also used. The enzyme inhibitors tested were from commercial sources.

Culture of the fungus. The methods used were as described by Byrde & Fielding (1968) except that, in some of the earlier work, sporulating cultures were grown on potato plugs soaked for 72 hr in aqueous malic acid solution (7.5%, w/v) before autoclaving (Wiltshire, 1920).

Concentration of the enzyme. When desirable, the enzymes in the crude culture filtrate were concentrated by partial 'freezing-out' (Dixon & Webb, 1958) or by tannic acid precipitation followed by washing with cold acetone (Shibata & Nisizawa, 1965). The latter method gave a pale buff-coloured powder which retained high enzyme activities for many months when stored at 4°.

Estimation of enzyme activities. *Polygalacturonase* (poly- α -1,4-galacturonide glycanohydrolase, PG) (EC. 3.2.1.15), was estimated by one of three methods: (a) reducing group assay, based on the estimation of residual iodine with sodium thiosulphate (Jansen & MacDonnell, 1945). Activity was expressed as micro-equivalents of reducing groups liberated per min. at 25°; (b) viscosity assay, based on the loss of viscosity of aqueous solutions of pectin or sodium polypectate following decrease in chain length (Wood, 1955). The test solution comprised aqueous pectin or sodium polypectate solution (1% w/v, 5 ml.), appropriate buffer (2 ml.), water (2 ml.) and enzyme preparation (1 ml.). Activity was expressed as $1/t$ where t was the time (min.) for 50% decrease in viscosity; (c) an agar plate assay, as described by Dingle, Reid & Solomons (1953) was extensively used for semi-quantitative estimations during purification. The external diameter of the white ring obtained on the pectate agar plate was proportional to the

logarithm of the enzyme concentration over a range of dilutions. Activities were expressed relative to an aqueous solution (1 mg./ml.) of Pectinol 10 M, defined arbitrarily as having 100 units (u.) of activity/ml. Results obtained by this method were shown to be well correlated with those obtained by the viscosity method (see Results).

α -L-Arabinofuranosidase (α -L-arabinofuranoside arabinohydrolase) (AF), *pectin methyl-trans-eliminase* (PTE) and *macerating activity* were estimated as described by Byrde & Fielding (1968), who detected no polygalacturonate *trans*-eliminase in the culture filtrate.

Gel filtration. For molecular weight determinations 0.1 M-KCl in 0.05 M-tris buffer (pH 7.4) was used for elution of a Sephadex G 75 column (bead form) following the method of Andrews (1964) and basing the standard curve on nine proteins; 3 ml. fractions were collected. In other experiments, water was used for elution and 10 ml. fractions were collected.

Ion-exchange chromatography. This was done with CM-Sephadex gel (C.50) (Byrde & Fielding, 1968) or Ecteola-cellulose. Before use, Ecteola-cellulose was washed successively with N-NaOH, M-KH₂PO₄ and 0.01 M-phosphate buffer (pH 7.0) to remove impurities showing high absorption at 280 m μ (Bradley & Rich, 1956). Elution was with water and NaCl solutions.

Paper chromatography. Breakdown products were examined on Whatman no. 54 paper with solvents ethyl acetate + acetic acid + water (2 + 1 + 2, by vol.; Jermyn & Isherwood, 1949) or 80% (w/v) phenol in water. Spots were developed by spraying the papers with *p*-anisidine hydrochloride (3%, w/v, in butan-1-ol; Hough, Jones & Wadman, 1950) or with silver nitrate (Trevelyan, Procter & Harrison, 1950).

Electrophoresis on cellulose acetate strips followed the general method described by Kohn (1957). The enzyme solution (50 μ l.) was streaked by successive applications with a micro-constriction pipette on a cellulose acetate strip 36 $\times$ 5 cm. (Oxoid Ltd., London) in a narrow band approximately 7 cm. from the anode end. The voltage used was 300–400 V, d.c. giving a current of about 2 mA per strip, in 0.05 M-tris buffer (pH 7.6 or 8.5); electrophoresis was at room temperature. After running for approximately 8 hr the sites of enzyme activity were detected in one of three ways: (a) for PG, by inverting the moist strip on a plate of pectate agar gel (see estimation methods). After overnight incubation at 25°, the strip was removed and the plate developed with 6 N-HCl to give discrete white zones; (b) for AF, the moist strip was sprayed with an aqueous solution of *p*-nitrophenyl- α -L-arabinofuranoside (1 mg./ml.), incubated for 1 hr at 25° and laid on a filter paper moistened with saturated Na₂CO₃ solution for development of the yellow colour; (c) alternatively, for semi-quantitative estimation of AF and PG, the strip was removed and divided transversely into segments 1 cm. wide, each of which was further cut into pieces about 2 mm. across and soaked in 1 ml. 0.1 M-acetate buffer (pH 4.7) for 1 hr. Samples were then removed for enzyme assay, using the agar plate method for PG.

RESULTS

Gel filtration and column chromatography. The initial culture filtrate contained PG, AF and PTE activities. The patterns of PG, AF and macerating activity when a crude culture filtrate was applied to Sephadex G 75 and eluted with water are shown in Fig. 1.

Appropriate fractions were dialysed overnight at 4° against distilled water and applied to a column of Ecteola-cellulose. Elution with water followed by 0.1 M-NaCl resulted in the enzyme pattern shown in Fig. 2. When a crude culture filtrate was applied to a column of CM-Sephadex, followed by elution with a sodium chloride gradient, PG and AF were maximal in the early fractions. Macerating activity (due to PTE) followed in two peaks (Fig. 3 of Byrde & Fielding, 1968).

Fig. 1. *Sclerotinia fructigena*. Polygalacturonase (●), arabinofuranosidase (Δ) and macerating (○) activity of fractions from aqueous elution of Sephadex G 75 dextran gel.

Fig. 2. *S. fructigena*. Polygalacturonase (●) and arabinofuranosidase (Δ) activities of fractions from Ecteola-cellulose eluted first with water and then (from arrow) with 0.1 M-NaCl.

CM-Sephadex and Ecteola-cellulose column chromatography were used successively for partial purification of PG and AF, to give purification factors of 61 and > 24 respectively, and elimination of all but traces of the other enzymes.

For PG, CM-Sephadex was used initially, enabling the PTE component to be eliminated. When dialysed fractions high in PG and AF activity were then passed through Ecteola-cellulose, PG activity was retained by the column and could be eluted in a sharp peak with 0.1 M-NaCl. By contrast with the behaviour when fractions from Sephadex G 75 were applied to Ecteola-cellulose, AF activity was not present in the initial eluate, nor was it present in the 0.1 M-NaCl eluates of high PG activity (Table 1). The uronide concentration (McComb & McCready, 1952) was decreased from 1700 μg./ml. in the culture filtrate to 2 μg./ml. in fraction 26.

For AF purification, the chromatography was done in reverse order. With a tannic acid preparation the PG was retained on the Ecteola-cellulose. The water eluate containing 58% of the AF activity was then applied to a CM-Sephadex column which enabled the AF to be separated from the PTE (Table 2).

Unsuccessful purification methods. Several techniques conventionally used for enzyme purification proved to be unsuitable for the purification of this PG. Poor recoveries

Table 1. *Partial purification of polygalacturonase from Sclerotinia fructigena*

Fraction	Vol. (ml.)	Activity				Total activity			Sp.act. PG (units/E)	Yield (%)	Purifica- tion
		AF (μ mole/hr)	PG (u.)	PTE (ΔE_{240} /100 min. at pH 7)	AF	PG	PTE	E_{280}			
Culture filtrate	10	28.4	631	2.34	284	6310	23.4	3.14	200	100	1
Elution from CM- Sephadex (fractions 3-8)	40	2.75	100	0.01	109	4000	0.40	0.368	270	63	1.35
Elution from Ecteola- cellulose (fractions 26-30)	50	0.068	40.7	—	3.4	2035	—	0.003	12,210	31	61

—, Not detected.

Table 2. *Partial purification of arabinofuranosidase from Sclerotinia fructigena*

Fraction	Vol. (ml.)	Activity				Total activity			Sp.act. AF (units/E)	Yield (%)	Purifica- tion
		AF (μ mole/hr)	PG (u.)	PTE (ΔE_{240} /100 min. at pH 7)	AF	PG	PTE	E_{280}			
Tannic acid ppt. at 5 mg./ml. (filtered)	19	24.5	1260	0.60	465	23,940	11.4	3.5	7	100	1
Water elution from Ecteola-cellulose (fractions 14-19)	30	8.5	13	0.12	255	390	3.6	0.074	115	55	16.5
0.05 M-NaCl elution from CM-Sephadex (removal of PTE)	80	0.84	—	—	68	—	—	<0.005	>168	15	>24

—, Not detected.

(about 33 %) from the culture filtrate were obtained with acetone, ethanol or ammonium sulphate precipitation, the latter being tried at a range of pH values. Adsorption on, and elution from, pectic acid gel (Demain & Phaff, 1954) gave heavy polysaccharide contamination, whilst calcium phosphate gel, aluminium oxide and glass powder did not adsorb the enzyme. Bentonite adsorbed the activity but this could not be recovered. Of the modified celluloses, carboxymethyl cellulose gave poor adsorption of PG, by contrast with the experience of Mill & Tuttobello (1961) with the PG of *Aspergillus niger*.

DEAE-cellulose appeared less useful than Ecteola-cellulose, whilst elution from TEAE-cellulose resulted in a plateau, rather than a peak, of activity. Zone electrophoresis on starch gel (Smithies, 1955), agar gel or polyvinyl chloride gel (Kunkel & Trautman, 1959) were ineffective: the enzyme remained at the starting line. The same difficulty was encountered with paper electrophoresis, although Purr, Hottenroth, Döring & Schneider (1957) successfully separated two polygalacturonases from a commercial pectolytic enzyme by this method.

Fig. 3. *Sclerotinia fructigena*. Hydrolysis of 0.4 % solutions of sodium polypectate (■) and pectin (□) measured by the release of reducing groups at pH 4.7.

Properties of the enzymes

PG. The PG preparations used for these experiments were 0.1 M-NaCl eluates from Ecteola-cellulose showing maximum PG activity; these were dialysed overnight at 4°. Figure 3 shows the time-course of liberation of reducing groups by PG with sodium polypectate and pectin as substrates. The pattern of the curves is very similar to that reported for an endo-PG from *Aspergillus niger* by Mill & Tuttobello (1961) and shows that breakdown became very slow after 40 % hydrolysis. Polypectate was more readily attacked than pectin; this was also found by the viscosity method. The viscosity of sodium polypectate solution (0.5 %) was halved in about 1 min.

AF. The relationship between time and *p*-nitrophenol liberated from *p*-nitrophenyl- α -L-arabinofuranoside solution was linear only up to absorption values of approximately 0.1, corresponding to 15 % hydrolysis. This value was inevitably low because of the low concentration of substrate which was in short supply. By making estimations at a range of higher substrate concentrations, the Lineweaver-Burk plots obtained gave a K_m value between 0.35 and 0.36 mM in each of two experiments. This compared with values of the order of 0.65 mM for the AF of *Phytophthora palmivora* (Akinrefon, 1968).

Breakdown products. When a purified PG preparation was incubated at 25° under toluene with sodium pectate or pectin (final concentration 16 mg./ml.) no spot corresponding to D-galacturonic acid was detected after 48 hr, although two spots of lower R_F value, which were probably oligouronides were observed, even after 8 hr; these

Fig. 4. *S. fructigena*. pH/activity relationships for polygalacturonase (●) and arabinofuranosidase (Δ).

Fig. 5

Fig. 6

Fig. 5. *Sclerotinia fructigena*. pH/stability relationships for polygalacturonase (●) and arabinofuranosidase (Δ) for 20 min. exposure.

Fig. 6. *S. fructigena*. Thermal stability (20 min. exposure) of: (a) polygalacturonase in culture filtrate (●) and purified preparation (○); (b) arabinofuranosidase in culture filtrate (▲) and purified preparation (Δ).

were more pronounced when sodium pectate was the substrate. Spots corresponding to glucose were also observed.

When a purified AF preparation was similarly incubated overnight with Araban or an apple fibre preparation, monomeric arabinose was detected, but no evidence of oligomers was obtained. A trace of arabinose was also detected when pectin was used as substrate.

pH relationships. PG activity at a range of pH values was estimated by the reducing group method with sodium polypectate as substrate and acetate and phosphate buffers (final concentration 0.2 M). The results plotted in Fig. 4 were comparable with those found by Cole (1956) for *Sclerotinia fructigena* by the viscosity method with sodium polypectate as substrate, though with the peak of activity about one pH unit lower. AF activities estimated in the presence of citrate + phosphate + borate buffer are also shown in Fig. 4.

The effect of pH value on PG stability was examined over the range pH 2 to 12 by mixing 1 vol. of the appropriate citrate + phosphate + borate buffer (Teorell & Stenhagen, 1938) with 9 vol. of enzyme solution. After 20 min. at room temperature an equal volume of 0.2 M-acetate buffer (pH 4.7) was added. The decrease in PG activity assessed by the agar plate method is shown in Fig. 5, relative to untreated enzyme. The enzyme showed remarkable stability at small pH values and also up to pH 11.8. Figure 5 also shows comparable data for AF activity.

Table 3. *Sclerotinia fructigena*: molecular weight of enzyme components estimated by gel-filtration

Enzyme	Elution vol. (ml.)				Mean	Corresponding mol.wt.
	Expt 1	Expt 2	Expt 3	Expt 4		
PG	157	152	148	148	151	77,000
PG	199	205	202	193	200	38,500
AF	135	127	130	128	130	> 200,000
AF	174	177	182	173	176	53,700
AF	216	—	—	219	217	30,500

PG = Polygalacturonase. AF = α -L-Arabinofuranosidase.

Temperature stability and effect of enzyme inhibitors. Samples (2 ml.) in 20 ml. test-tubes were held at a range of temperatures for 20 min., then cooled rapidly to about 0° by immersion of the tube in acetone at -25°. Residual PG activities were estimated viscometrically, and residual AF by the usual method. Figure 6 summarizes the data for each enzyme using both crude (culture filtrate) and purified preparations.

The PG activity of each sample was also estimated by the agar plate method. The results by the two methods in three experiments showed a highly significant correlation ($P < 0.001$). The equation relating the agar-plate value (y) to the viscosity estimate (x) was:

$$y = 2.50x.$$

The inhibition of enzyme activity at 60° was not prevented by gelatin (1 mg./ml.), cysteine (10^{-3} M), ethylenediaminetetra-acetic acid (10^{-3} M) or potassium cyanide (10^{-4} M).

Tests for polyphenol oxidase activity at room temperature and at 60° were negative.

Heating to 80° for 20 min., however, resulted in a more active preparation after a further 20 min. at 60° than a control sample heated only to 60° for 20 min.

Less than 20% inhibition of PG activity in fractions eluted from Sephadex G.75 occurred in the presence of the following: KCN, 8-hydroxyquinoline, sodium diethyl-dithiocarbamate, sodium azide, iodoacetic acid, mepacrine, *o*-phenanthroline or 2,4-dinitrophenol (each at 10^{-3} M), diethyl p-nitrophenyl phosphate or di-*isopropyl* fluorophosphate (each at 10^{-4} M). Compounds were incubated for 1 hr with the enzyme before assay by the agar plate method at pH 4.7. Under these conditions, mercuric chloride inhibited activity by 50% at 10^{-3} M and by 34% at 10^{-4} M, p-chloromercuribenzoic acid by 57% at 10^{-3} M, and urea by 80% at 8 M.

When the enzyme was exposed as indicated below to proteolytic enzymes before assay, the following gave less than 20% inhibition: pepsin (0.03 mg./ml., pH 3, 37°, 30 min.), papain (0.03 mg./ml., pH 6, 37°, 30 min.), trypsin or chymotrypsin (each at 0.1 mg./ml., pH 7.5, 37°, 2 hr). Exposure to 'Pronase' (secreted by *Streptomyces griseus*) (0.1 mg. ml., pH 7.4, 30°, 24 hr) resulted in 30% loss of activity.

Molecular weight determination from Sephadex gel filtration data. One ml. of a dialysed freeze-concentrated culture filtrate of *Sclerotinia fructigena* was mixed with 1 ml. eluant and applied to a calibrated Sephadex G75 column. Molecular weight determinations (Andrews, 1964) in four experiments are summarized in Table 3. The form of AF with the smallest molecular weight was detected in only two of the four experiments.

Fig. 7. *Sclerotinia fructigena*. Migration towards cathode of polygalacturonase (●) and arabinofuranosidase (△) on electrophoretogram on cellulose acetate strips.

Electrophoresis on cellulose acetate strips. In experiments with crude culture filtrates, or various preparations from them, both enzymes migrated consistently towards the cathode. Best results were obtained by using a tannic acid preparation (10 mg./ml.); when electrophoresis was done at pH 7.6, two PG peaks were detected, one being coincident with the single peak of AF activity (Fig. 7). A similar pattern was found in three other experiments at pH 7.6 and also at pH 8.5, although the faster running PG component was generally slightly less active than the slower running one. The peak of macerating activity of the fractions coincided with the joint peak of PG and AF.

DISCUSSION

The polygalacturonase of *Sclerotinia fructigena* is apparently an endo-polygalacturonase Type I according to the classification of Demain & Phaff (1957). Its ability to cause the rapid decrease in viscosity of sodium polypectate solutions with a slow release in reducing groups, together with the absence of D-galacturonic acid in incubation mixtures, is characteristic of this type of PG. The more rapid hydrolysis of pectate than of pectin indicates that the enzyme is a polygalacturonase rather than a polymethylgalacturonase. The pH/activity relationships of the enzyme, with sodium polypectate as substrate, characterize it as being of Type I (optimum at relatively small pH value); it is in many respects similar to the endo-PG of *Aspergillus niger* described by Mill & Tuttobello (1961).

The *Sclerotinia* enzyme showed unusual stability over a wide range of pH values and was in general little affected by classical enzyme inhibitors. This is in accord with general experience with pectolytic enzymes (e.g. Husain & Dimond, 1958). The enzyme is, however, readily inactivated by several polyphenolic compounds (Byrde, Fielding & Williams, 1960; Cole & Wood, 1961) probably in a non-specific manner. It also resists breakdown by the highly versatile proteolytic enzyme from *Streptomyces griseus* (Nomoto, Narahashi & Murakami, 1960).

McClendon (1964), who used a preparation from *Sclerotinia fructigena*, showed the existence of more than one component by ion-exchange chromatography. That such components may represent polymeric forms was indicated by the present results of the molecular weight estimation with a dialysed concentrated culture filtrate, which gave peaks corresponding to molecular weights of 38,500 and 77,000. It may be significant that Swinburne & Corden (1969) observed changes in the properties of a PG secreted by *Penicillium expansum* during purification by ion-exchange chromatography which they attributed to changes in the molecular configuration of the enzyme. Two forms of the *Sclerotinia fructigena* enzyme in the tannic acid preparation were also detected on strip electrophoresis; these were not further characterized, but both carried a strong overall basic charge at pH 7.6 and pH 8.5. This basic character is likely to assist in the adsorption of the enzyme on the acidic polyuronides of the plant cell wall.

The temperature inactivation pattern of the crude *Sclerotinia fructigena* PG shows an unusual bimodal curve, reminiscent of that obtained for a proteolytic enzyme by Virtanen (1934) and with the PG's of *Botrytis cinerea* and *Erwinia aroideae* by Jarvis (1953). Virtanen ascribed these effects to complexing with other proteins. It may, however, be significant that with the purified preparation from *S. fructigena* (which, by contrast with the crude PG, was very low in uronide content) the second peak of activity at higher temperatures was much decreased. Alternatively, depolymerization by heat of a component in the crude culture filtrate into two molecules of lower polymers might be involved.

The α -L-arabinofuranosidase appears to be a typical glycosidase attacking araban by a terminal mechanism to liberate the monomer as did the arabanases from *Aspergillus* species described by Kaji, Taki, Shimazaki & Shinkai (1963). By contrast, arabanase from *Sclerotinia libertiana* liberated small quantities of oligomers in addition to monomer (Kaji *et al.* 1965). The *S. fructigena* enzyme is specific for the furanoside configuration (Byrde & Fielding, 1965), and its properties are in general similar to

those of an AF from *Phytophthora palmivora* described by Akinrefon (1968), although the K_m value is appreciably lower.

By contrast with the PG from *Sclerotinia fructigena* the AF was appreciably inactivated at high pH values and showed a normal temperature inactivation pattern. Whilst one peak of activity was detected in a tannic acid preparation by strip electrophoresis, or in a culture filtrate by gel filtration with water elution, there was evidence of two and sometimes three peaks on gel-filtration of a freeze-concentrated preparation eluted with tris buffer containing 0.1 M-KCl. Although the estimated molecular weights of the two smaller components showed no obvious numerical relationship, and that of the largest component could not be assessed with the dextran gel used, it is clear that, as with PG, the enzyme can exist in multiple forms which are readily interconverted.

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