SHORT COMMUNICATION

A Serological Study of a β-D-Fructofuranosidase from
Sphacelia sorghi McRae

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Antibodies raised against a purified β-D-fructofuranosidase from Sphacelia sorghi were cross-reacted with a purified β-D-fructofuranosidase from Claviceps purpurea. An immunodiffusion test indicated a high degree of serological identity between the S. sorghi and C. purpurea enzymes. A complement fixation test confirmed this result, the similarity in titres indicating a very close structural similarity between the enzymes.

INTRODUCTION

The genus Sphacelia comprises a diverse group of ergot fungi which are classified together on the basis of their inability to produce the stromata characteristic of the perfect sexual stage of the life cycle of species of the genus Claviceps. However, morphologically these two genera appear to be very similar; moreover, both produce alkaloids including festuclavine, chanoclavine, pyroclavine and dihydroelymoclavine (Agurell et al., 1963; Mantle, 1968).

Traditionally, fungal taxonomists have relied on morphological characters, especially the structure of the sexual fruiting bodies, to establish their classifications. Indeed, it has been suggested that these should be the only parameters for delimitation of species and higher categories (Snyder & Tousson, 1965). This reasoning has led to the seemingly incongruous classification of Sphacelia as a genus of the Fungi Imperfecti, although Claviceps is accepted as an Ascomycete. A single report (Mower et al., 1973) briefly mentioned the observation of stromata production by Sphacelia sorghi McRae. Despite the taxonomic implications of this observation, no further, more definitive, information was published.

During studies on a purified preparation of β-D-fructofuranosidase from Sphacelia sorghi (Baker, 1979; Dickerson & Baker, 1979) an antiserum was raised against the enzyme. The antiserum was tested for cross-reactivity with the equivalent enzyme from Claviceps purpurea.

METHODS

Organisms. The Sphacelia sorghi strain (R18B) was a derivative of a strain originally isolated in Nigeria from sclerotia on Sorghum vulgare (Mantle & Waight, 1968; Mantle, 1968). The Claviceps purpurea strain (29/4) was the same as that used by Dickerson et al. (1976). Basidiomycete QM806 (CMI 155,771) was from the Commonwealth Mycological Institute, Kew, Surrey.

Medium and growth conditions. The medium used to support the growth of ergot fungi was the same as that used by Dickerson et al. (1970). Sphacelia sorghi cultures were incubated at 28 °C and Claviceps purpurea at 24 °C. The medium for growing Basidiomycete QM806 contained (per litre): glucose, 10.0 g; KH₂PO₄, 2.0 g; (NH₄)₂SO₄, 1.4 g; urea, 0.3 g; MgSO₄, 7H₂O, 0.3 g; CaCl₂, 0.3 g; yeast extract, 0.1 g; FeSO₄.7H₂O, 5.0 mg; ZnSO₄, 7H₂O, 5.5 mg; MnSO₄, 4H₂O, 2.0 mg; CoCl₂.6H₂O, 2.0 mg; NaOH solution to adjust to pH 6.2; 100 ml portions were sterilized by autoclaving at 109 °C for 30 min. Basidiomycete QM806 cultures were incubated at 24 °C.

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Extraction and purification of β-D-fructofuranosidase. The β-D-fructofuranosidase from Claviceps purpurea was extracted and purified as described by Dickerson (1972). The equivalent enzyme from Sphacelia sorghi was extracted and purified as described by Dickerson & Baker (1979) to yield both glucan-associated (78% carbohydrate) and free protein preparations.

Enzyme assays. The activity of β-D-fructofuranosidase preparations was determined as described by Dickerson (1972).

Protein determination. The concentration of protein in suspensions was determined spectrophotometrically (Layne, 1957) using values corrected for optical dispersion by the method of Englander & Epstein (1957).

Isolation and purification of glucan. The extracellular glucan produced by S. sorghi in liquid culture was isolated and purified using urea as described by Dickerson & Baker (1979).

Preparation of sera. Antiserum was prepared in female New Zealand white rabbits (each about 2 kg). A sample of blood (20 ml) was withdrawn from each and allowed to clot. The supernatant serum was drawn off for use as pre-inoculation control serum.

Glucan-associated β-D-fructofuranosidase from S. sorghi (50 mg) was gently stirred for 1 h with 0.85% (w/v) NaCl (2 ml) and emulsified with an equal volume of Freund’s complete adjuvant. Intramuscular injections (0.25 ml) of the emulsion were made. After 6 weeks, a 2 mg ml⁻¹ solution of purified S. sorghi β-D-fructofuranosidase in 0.85%, (w/v) NaCl was administered by intramuscular (0.25 ml) and intravenous (0.10 ml) injections. The animals were bled after 7 d and immune serum was prepared as above.

Immunodiffusion test. Ionagar (5 ml; 5% (w/v)) in 0.2 M-sodium phosphate buffer, pH 7.0] was pipetted on to a microscope slide and allowed to set. A punch was used to cut a matrix of six wells (1 mm diam.) spaced at a distance of 5 mm apart and 5 mm from a central well (3 mm diam.). In all experiments the central well was filled with serum and each peripheral well was filled with dilutions (normally 1:10 mg ml⁻¹) of potential antigens in 0.85%, (w/v) NaCl; for the control of purified glucan, 2.0 mg ml⁻¹ was used. The loaded gels were incubated for 16 h at 20 °C in a humidifier, washed with several changes of 0.85% (w/v) NaCl, dried by blotting and stained with Coomassie Blue by the method of Uriel & Grabar (1956).

Complement fixation test. This was carried out as described by Bradstreet & Taylor (1962). The titre of the serum is defined as the greatest dilution of antiserum causing haemolysis at the optimal sensitizing concentration of the antigen; the value of the titre is expressed as the reciprocal of this dilution. Doubling dilutions of antiserum (1:40 to 1:1280) and of purified β-D-fructofuranosidase from S. sorghi (protein concentration, 0.00625 to 0.20 mg ml⁻¹) were made in 0.85% (w/v) NaCl and were used in the preliminary titration to determine the optimal sensitizing concentration of antigen; this was 0.025 mg ml⁻¹. Suspensions of the purified ergot β-D-fructofuranosidasises were prepared (protein concentration, 0.025 mg ml⁻¹) in 0.85% (w/v) NaCl and titrated against doubling dilutions of antiserum and of pre-inoculation serum (1:2 to 1:4096) made in 0.85% (w/v) NaCl. Both the complement fixation test and the immunodiffusion test were repeated several times with essentially the same results.

RESULTS

The activity of the β-D-fructofuranosidase from S. sorghi, whether glucan-associated or as the free protein, remained unchanged in the presence of antibody over a wide temperature range (20 to 40 °C) and pH range (4.0 to 7.5). Increasing the ratio of antibody to enzyme from 1:1 to 10:1, on a protein basis, also had no effect on the enzyme activity. With the conditions used for the immunodiffusion and complement fixation tests, the activity of the β-D-fructofuranosidase from C. purpurea was also unchanged in the presence of antibody.

In the immunodiffusion test, the formation of an opaque white band of precipitin, which stained with Coomassie Blue, in the agar gel between the antigen and antiserum wells was taken as a positive result. When the specific antiserum was used, all preparations of β-D-fructofuranosidase from ergot fungi yielded single sharp precipitin bands; other preparations, including the purified glucan, were negative. When β-D-fructofuranosidasises from S. sorghi and C. purpurea were placed in adjacent antigen wells the precipitin bands coalesced without spurring, indicating that the two antigens were serologically identical.

To confirm the above results, the complement fixation test was used. Quantitative results were obtained by making 1:5 dilutions of the test mixtures using 0.85% (w/v) NaCl and measuring their absorbances at 650 nm (Table 1). Defining the titre of the serum as the reciprocal of the highest dilution giving 100% fixation of complement (i.e. no lysis of erythrocytes), the β-D-fructofuranosidasises from S. sorghi and C. purpurea gave titres of
Table 1. \textit{Complement fixation test: titration of \(\beta\)-D-fructofuranosidases from \textit{S. sorghi} and \textit{C. purpurea} with antiserum raised against the \textit{S. sorghi} enzyme}

Results are absorbance measurements, made at 650 nm, of 1:5 dilutions of test mixtures in 0.85\% (w/v) NaCl. For experimental details, see Methods.

<table>
<thead>
<tr>
<th>Reciprocal dilution of serum</th>
<th>\textit{Sphacelia sorghi}</th>
<th>\textit{Claviceps purpurea}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CS*</td>
<td>AS*</td>
</tr>
<tr>
<td>2</td>
<td>0.34</td>
<td>0.34</td>
</tr>
<tr>
<td>4</td>
<td>0.35</td>
<td>0.35</td>
</tr>
<tr>
<td>8</td>
<td>0.33</td>
<td>0.34</td>
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<tr>
<td>16</td>
<td>0.15</td>
<td>0.34</td>
</tr>
<tr>
<td>32</td>
<td>0.09</td>
<td>0.33</td>
</tr>
<tr>
<td>64</td>
<td>0.06</td>
<td>0.32</td>
</tr>
<tr>
<td>128</td>
<td>0.05</td>
<td>0.32</td>
</tr>
<tr>
<td>256</td>
<td>0.05</td>
<td>0.33</td>
</tr>
<tr>
<td>512</td>
<td>0.05</td>
<td>0.21</td>
</tr>
<tr>
<td>1024†</td>
<td>0.05</td>
<td>0.07</td>
</tr>
</tbody>
</table>

* CS, Pre-inoculation control serum; AS, antiserum raised against \textit{S. sorghi} \(\beta\)-D-fructofuranosidase.
† Higher dilutions gave no further change in absorbance.

256 and 128, respectively. This difference (equal to the dilution factor) is not considered to be significant (K. A. Bettelheim, personal communication).

\textbf{DISCUSSION}

Serological studies using specific enzymes as antigens have found considerable application in the medical field where the source of the enzymes has largely been bacteria. Thus, enzymes of the \(\beta\)-ketoadipate pathway in pseudomonads (Patel \textit{et al}., 1973; Patel \& Ornston, 1976), enterobacterial asparaginases (Bascomb \& Bettelheim, 1976), invertases of oral streptococci (Burckhardt \& Guggenheim, 1976) and \(\beta\)-galactosidases from several bacteria (Guisco \textit{et al}., 1979) have all been studied using serological techniques. Little attention has been given to the use of serology in mycology, particularly as a taxonomic aid, despite the fact that it was first employed half a century ago to establish likely relationships in the Ascomycetes and in the Phycomycetes (Mez, 1929). Serological typing schemes have been devised for the identification of species of \textit{Fusarium} (Madhosingh, 1964), \textit{Phytophthora} (Burrell \textit{et al}., 1966; Merz \textit{et al}., 1969), \textit{Ceratocystis} (Amos \& Burrell, 1967) and \textit{Pythium} (Morton \& Dukes, 1967; White, 1976). In these cases, crude preparations of spores or mycelium were used as the antigen. Imamoto \textit{et al}. (1965) noted the requirement for the use of purified enzymes to raise specific antibodies to enable quantitative comparisons of homologous enzymes in crude extracts to be made. However, no attempt was made to purify a single species of antigenic protein in any of the investigations mentioned above. More recently, the specific affinities of 3-carboxymuconate cyclase from aspergillus, penicillia and other fungi for antibody raised against the purified enzyme from \textit{Aspergillus niger} have been compared (Cook \& Cain, 1977).

The immunodiffusion studies described here show that the antiserum raised against \textit{S. sorghi} \(\beta\)-D-fructofuranosidase had an affinity for \textit{C. purpurea} \(\beta\)-D-fructofuranosidase as well as the immunizing antigen. Furthermore, the coalescence of precipitin bands formed when these two antigens were in adjacent wells indicates identical serological properties suggestive of close structural similarity. Results from the complement fixation test confirmed the high degree of serological identity between the two ergot enzymes and indicated a very high degree of structural identity.

The suggestion of Wilson \& Kaplan (1964) and van Valen (1973), that relationships
between organisms could be revealed by a comparison of the structure of equivalent enzymes, has received support from the present serological studies on β-D-fructofuranosidases which have helped to clarify the taxonomic relationship between *C. purpurea* and *S. sorghi*. Further support for the relationship may be obtained from morphological studies (Mantle, 1968; Mower *et al*., 1973) and biochemical studies such as analysis of DNA base pairing (De Ley, 1968; Dutta & Ojha, 1972); moreover, alkaloid analysis (Mantle & Waight, 1968) has helped to confirm the similarity of the two ergot fungi, although dihydroergosine is unique to *S. sorghi*.

A wider application of the serological approach used here might assist in the resolution of similar problems in the field of mycology; for example, more than one hundred types of ergot fungi have been described (Bové, 1970) but the number of species this figure represents is unknown.

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**REFERENCES**


