Purification and Partial Characterization of Multiple Bromoperoxidases from *Streptomyces griseus*

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The presence of multiple bromoperoxidases in extracts of *Streptomyces griseus* Tü 6 was detected. The enzyme pattern varied with the age of the culture. A haem-type bromoperoxidase (BPO 2) was always present. Additionally three nonhaem-type bromoperoxidases (BPO 1a, 1b and 3) were detected and purified to homogeneity. The *M*ₘ of non-denatured BPO 1a was 70000 ± 10000 and those of BPO 1b and 3 were 90000 ± 5000. BPO 1a and 1b were dimers with subunit *M*ₘ values of 34000 and 43000, respectively. BPO 3 was a trimer with a subunit *M*ₘ of 31000. The enzymes differed in their isoelectric points, heat stability, and *Kₘ* values. In immunodiffusion experiments BPO 1a and 3 showed partial identity with the nonhaem-type bromoperoxidase from *Streptomyces aureofaciens*. The nonhaem-type BPO 1a, 1b and 3 had neither peroxidase nor catalase activity.

**INTRODUCTION**

Halogenated organic compounds are recognized to pose a problem to the environment, because they are often toxic and cannot be degraded easily (Müller & Lingens, 1986). The production of halogenated compounds by chemical halogenation usually results in the formation of halogenated byproducts, because chemical halogenation is not very specific. Thus halogenating enzymes, which should be capable of very specific halogenation reactions, have attracted increasing attention during the last few years.

Halogenating enzymes, the so-called haloperoxidases, are produced by different organisms, such as mammals (Dumontet & Rousseau, 1983; Olsen & Little, 1984), invertebrates (Ahern et al., 1980; Deits et al., 1984), algae (Hewson & Hager, 1980; Baden & Corbett, 1980; De Boer et al., 1986a, b), fungi (Morris & Hager, 1966; Liu et al., 1987) and bacteria (Wiesner et al., 1985). The large majority of the haloperoxidases isolated from these organisms are haem-type haloperoxidases with protoporphyrin IX as the prosthetic group. These haem-type haloperoxidases are inactivated during incubation with H₂O₂ (Liu et al., 1987). As these enzymes also have peroxidase activity, oxidation products are formed in addition to the halogenated products (Corbett et al., 1980). However, nonhaem-type haloperoxidases, which have been isolated from algae (De Boer et al., 1986a, b; Itoh et al., 1985), from a fungus (Liu et al., 1987) and from bacteria (Wiesner et al., 1986; van Pée et al., 1987), have very little or no peroxidase activity and are much more stable under reaction conditions (Liu et al., 1987; Itoh et al., 1987).

Most of the halogenating enzymes so far isolated are bromoperoxidases. Only two nonhaem-type chloroperoxidases have been isolated (Wiesner et al., 1986; Liu et al., 1987). Although the bacteria from which haloperoxidases have been isolated (van Pée & Lingens, 1985a, b; Wiesner et al., 1985; van Pée et al., 1987) produce chlorinated metabolites, only bromoperoxidases could be isolated from these strains, with the exception of *Pseudomonas pyrocina*. This bacterium has a nonhaem-type chloroperoxidase and a haem-type bromoperoxidase (Wiesner et al., 1986), whereas *Streptomyces aureofaciens* produces a nonhaem-type and a haem-type bromoperoxidase (van Pée et al., 1987).

**Abbreviations:** BPO, bromoperoxidase; PMSF, phenylmethyalsulphonyl fluoride.
The two nonhaem-type bacterial haloperoxidases differ in many respects from the eukaryotic enzymes. To get more information about bacterial halogenation, we screened various Streptomyces strains, known to produce halogenated metabolites, for halogenating enzymes. In Streptomyces griseus Tü 6, which produces 6,3'-dichlorogenistein (König et al., 1977), we detected one haem-type and three nonhaem-type haloperoxidases. This was the first time that halogenating activity had been detected in crude extracts of bacteria, and also the first demonstration of multiple forms of prokaryotic haloperoxidases. Multiple forms of plant peroxidases are well known (Shannon et al., 1966; Kay & Basile, 1987), but in the case of haloperoxidases only the fungal haem-type chloroperoxidase from Caldariomyces fumago (Sae & Cunningham, 1979; Pickard & Hashimoto, 1982; Hashimoto & Pickard, 1984) and the ligninase–bromoperoxidase (Renganathan et al., 1987) from Phanerochaete chrysosporium (Leisola et al., 1987), are known to produce multiple forms. Although Kenigsdberg et al. (1987) demonstrated that the multiple forms of chloroperoxidase in C. fumago are due to post-translational modifications and are thus not real isoenzymes, little is known about the properties of these multiple forms.

Here we describe the purification and some properties of multiple forms of nonhaem-type bromoperoxidases from Streptomyces griseus Tü 6.

METHODS

**Reagents.** Hydrogen peroxide (30%, v/v) was purchased from Merck; monochlorodimedone was prepared from dimedone by chlorination with sodium hypochlorite (Hager et al., 1966); o-dianisidine (3,3'-dimethoxybenzidine) was from Sigma.

**Organism and culture conditions.** Streptomyces griseus Tü 6 was kindly supplied by Professor H. Zähner, University of Tübingen, FRG. The growth medium contained 2% (w/v) soybean flour and 2% (w/v) mannitol. The strain was grown in 1 litre Erlenmeyer flasks, containing 0-1 litre of medium, at 30°C on a rotary shaker. Cultures were harvested by centrifugation, yielding about 10 g (wet wt) l⁻¹.

**Enzyme assays.** Halogenating activity was measured according to the method of Hewson & Hager (1980) with monochlorodimedone (44 μM) as substrate in the presence of H₂O₂ (7-2 mM) and bromide (82 mM) and a suitable amount of enzyme in 0-1 mM-sodium acetate buffer (pH 5.5). The reaction was started by the addition of enzyme. The increase in monochlorodimedone absorbance at 290 nm (ε = 1.99 × 10⁴ M⁻¹ cm⁻¹) with time was recorded. One unit (1 U) of bromoperoxidase activity was defined as the formation of 1 μmol monobromomonochlorodimedone min⁻¹. The brominating activity at different temperatures was measured by preincubating the assay mixture without H₂O₂ and enzyme at the given temperature. The reaction was started by the addition of enzyme immediately after the addition of H₂O₂. The activity was measured against a blank containing the same components except enzyme.

Peroxidase activity was assayed as described by Claiborne & Fridovich (1979) with o-dianisidine (48 μM) as the substrate in the presence of 7.2 mM-H₂O₂ in 0-1 M-sodium acetate buffer (pH 5-5). The oxidation of o-dianisidine was followed at 460 nm. Catalase activity was measured photometrically at 240 nm according to Claiborne & Fridovich (1979).

**Separation and purification of the multiple bromoperoxidases.** All steps were performed at 4°C. The crude extracts were prepared by suspending one part (500 g wet wt) of cells in two parts of 500 mM-ammonium acetate buffer (pH 6-8) and disrupting the cells with a Branson sonifier J 17-A for twenty 30 s periods. The cell debris was removed by centrifugation for 30 min at 22100 g. The precipitate was resuspended in one volume of buffer and disrupted as before. Finally the extracts were combined and ammonium sulphate was added to 40% saturation. After stirring for 30 min, the precipitate was removed by centrifugation and discarded. Ammonium sulphate was added to the supernatant solution to 90% saturation and stirred for 30 min. The precipitate was collected by centrifugation, redissolved in 100 mM-ammonium acetate buffer (pH 6-8) and dialysed against 10 mM-ammonium acetate buffer (pH 6-8) for 15 h. After centrifugation, the dialysed proteins were adsorbed onto a DEAE-cellulose DE 52 column (5-6 × 8 cm), equilibrated with 50 mM-ammonium acetate buffer (pH 6-8). The sample was washed onto the column with 2 litres of this buffer, containing 0-1 mM-KCl, and eluted with a linear 600 ml gradient of 0-1-0-7 mM-KCl in 50 mM-ammonium acetate buffer (pH 6-8). Fractions (about 4 ml) were assayed for protein (A₂₈₀) and bromoperoxidase activity. Fractions containing brominating activity were pooled and dialysed against 10 mM-potassium phosphate buffer (pH 8.0). As several bromoperoxidase activities were detectable after cultivation in Erlenmeyer flasks, these were labelled according to their elution volume: BPO 1, 2 and 3. BPO 1 and 3 were further purified by heat treatment at 60°C for 15 min. After cooling the samples on ice and centrifugation, the precipitates were discarded and the supernatants were adsorbed on a Sepharose Q Fast Flow column (2.5 × 10 cm), equilibrated with 50 mM-potassium phosphate buffer (pH 8.0). BPO 1 was washed onto the column
Multiple bromoperoxidases from S. griseus

with 1 litre of the same buffer, whereas BPO 3 was washed onto the column with 0.2 m-KCl in 50 m-maleate phosphate buffer (pH 8.0). BPO 1 was eluted with a 600 ml gradient of 0-10 m-KCl in 50 m-maleate phosphate buffer (pH 8.0) and BPO 3 was eluted with a 600 ml gradient of 0-10 m-KCl in 50 m-maleate phosphate buffer (pH 8.0). Fractions were assayed for protein (A$_{280}$) and brominating activity. Pooled active fractions were dialysed against 5 litres of 10 m-sodium acetate buffer (pH 5.5).

The samples were then applied to a Sepharose Q Fast Flow column (2.5 x 10 cm) equilibrated with 50 m-maleate sodium acetate buffer (pH 5.5) and washed onto this column with 600 ml buffer. Brominating activity was eluted with a linear gradient of 0-10 m-sodium acetate buffer (pH 5.5). The pooled active fractions were dialysed against 10 m-ammonium acetate buffer (pH 6.8) and concentrated using an Amicon concentrator with a PM 10 membrane. The samples were then applied to a Sephadex G-200 column (2.5 x 10 cm), equilibrated with 100 m-sodium acetate buffer (pH 6.8).

The pooled active fractions of BPO 1 were dialysed against 5 m-maleate phosphate buffer (pH 7.0) and applied to a Bio-Gel HTP hydroxylapatite column (2.5 x 10 cm), equilibrated with 5 m-maleate phosphate buffer. The column was washed with 500 ml of this buffer and eluted with a 400 ml gradient of 5-50 m-maleate phosphate buffer (pH 7.0). The fractions were assayed for protein (A$_{280}$) and brominating activity. Active fractions were pooled and dialysed against 5 litres of 5 m-ammonium phosphate buffer (pH 6.8) and concentrated using an Amicon concentrator with a PM 10 membrane.

BPO 3 was passed through a Superose 12 Prep Grade column (HR 16/50) using the Pharmacia FPLC system and 100 m-ammonium acetate buffer (pH 6.8). The most active fractions were pooled, dialysed and concentrated as described for BPO 1.

Analysis of the purified bromoperoxidases by PAGE. A Speed Vac concentrator was used to bring the purified enzymes to a final protein concentration of about 2 mg ml$^{-1}$. Analytical PAGE under non-denaturing conditions was done in horizontal 7.5% (w/v) polyacrylamide gels (Fehrnström & Moberg, 1977) with an LKB 2177 Multiphor system. SDS-PAGE was done by the method of Laemmli (1970).

Analytical isoelectric focusing. This was done in thin-layer plates of 5% (w/v) polyacrylamide containing a 2-4% (w/v) solution of ampholines in the pH range 3.5-9.5. An LKB Multiphor system was used according to the manufacturers' instructions (Winter & Andersson, 1977). Isoelectric points were determined using the IEF calibration kit from Pharmacia (pH range 3-10-5).

Gel staining. Gels were stained for protein with Coomassie Brilliant Blue R250, for peroxidase activity with o-dianisidine, for haem iron with benzidine and for protein-bound carbohydrates with Schiff reagent, according to the methods of Barritault et al. (1976), Shannon et al. (1966), Clarke (1964) and Segrest & Jackson (1972), respectively.

Determination of $M_\text{r}$. To estimate the $M_\text{r}$ values of the native bromoperoxidases, gel filtration experiments were done with the homogeneous enzymes. The comparative studies were performed with Sephadex G-200 (2.5 x 100 cm), Sephacryl S-200 HR (2.5 x 200 cm), analytical Superose 12 (HR 10/30) and Superose 12 Prep Grade (HR 16/50).

Effect of proteases on bromoperoxidase activity. Immediately after disrupting the cells, the protease inhibitors PMSF and EDTA were added to the crude extract. With other samples this was done 1, 2 or 3 days after the preparation of the crude extracts. These were stored at room temperature in the presence of 0.05% sodium azide. The elution profiles after ion-exchange chromatography on Mono Q at pH 6.8 using the FPLC system were compared and the protein pattern and halogenating activities examined.

Immunological assays. In the Ouchterlony gel diffusion test (London et al., 1971), the BPO 1a and 3 from S. griseus were tested with an antiserum raised against the nonhaem-type bromoperoxidase from Streptomyces aureofaciens (van Pée et al., 1987).

Halogenation of pyrrolnitrin. Pyrrolnitrin was brominated as described by van Pée & Lingens (1984). For the chlorination of pyrrolnitrin, 0.1 m-sodium acetate buffer (pH 4.0) and a chloride concentration of 8-2 malert were used, as described by Wiesner et al. (1986) for the chlorination of indole. The nonhaem-type BPO 1a and 3 were used for these reactions. The reaction mixture was analysed by GC-MS as described earlier (van Pée & Lingens, 1984).

RESULTS

Enzyme pattern. After growth of S. griseus Tü 6 in Erlenmeyer flasks for 80 h, two nonhaem-type bromoperoxidases (BPO 1 and BPO 3) were detected after ion-exchange chromatography (Fig. 1). BPO 3 was the major nonhaem-type haloperoxidase present under these conditions. In addition to BPO 1 and BPO 3, a haem-type bromoperoxidase (BPO 2) was produced. This enzyme also had peroxidase and catalase activity. When cultivation was continued, the pattern of these enzymes changed. After 96 h BPO 3 activity had decreased to less than one-tenth of the
activity obtained with cells grown for 80 h, and BPO 1 had become the major nonhaem-type haloperoxidase. The amount of the haem-type BPO 2 did not change significantly.

**Effect of proteases on the protein pattern.** The overall elution profiles obtained by ion-exchange chromatography of extracts treated with protease inhibitors and of untreated extracts were very different (Fig. 2). However, the pattern of the two nonhaem-type bromoperoxidases BPO 1 and 3 did not change significantly. In extracts without inhibitors 85% of the activity of BPO 3 remained after 3 d. The activity of BPO 1 did not change at all.

**Enzyme purification.** The nonhaem-type BPO 1 was purified from cultures grown for 96 h. In the extracts obtained from these cultures, the haem-tye BPO 2 was also present. However, as BPO 2 also had peroxidase activity and could be inactivated by the addition of NaN₃, it was possible to distinguish between the enzymes. BPO 1 could be separated into BPO 1a and BPO 1b by chromatography on hydroxylapatite (Table 1). BPO 2 was inactivated and separated from BPO 1a and 1b by heat treatment, as BPO 2 was not stable above 40 °C. As the total activity of BPO 3 decreased during prolonged growth, this enzyme was purified from cultures grown for 80 h. The crude extracts also contained the haem-type BPO 2, which could be separated from BPO 3 by heat treatment at 60 °C.

**Homogeneity and M₉ determination of the subunits.** Each of the purified nonhaem-type bromoperoxidases, BPO 1a, 1b and 3, showed a single band on PAGE under non-denaturing and denaturing conditions. The M₉ values of the native enzymes were estimated to be 90000 ± 5000 (BPO 1b and BPO 3) and 70000 ± 10000 (BPO 1a). BPO 1a and 1b were dimers with subunit M₉ values of 34000 and 43000, respectively, whereas BPO 3 was a trimer with a subunit M₉ of 31000. The isoelectric points were estimated to be 4.7, 4-6 and 3-6 for BPO 1a, 1b and 3, respectively. Only BPO 2 gave positive reactions when stained for peroxidase activity, haem iron, and protein-bound carbohydrate after native PAGE.

**Temperature and pH characteristics.** Under our assay conditions, BPO 1a exhibited its highest activity at 40 °C, while BPO 3 was most active at 60 °C. The thermal stability of the bromoperoxidases was different, too: BPO 1a was totally inactivated after treatment at 60 °C.
**Multiple bromoperoxidases from S. griseus**

Fig. 2. Influence of protease inhibitors on elution profiles from ion-exchange chromatography on FPLC with Mono Q (pH 6.8). O--O, Protein; - - - M, haloperoxidase activity; ---, KCl gradient. Pattern (a) was obtained from an 80 h culture, when PMSF and EDTA were added immediately after disrupting. Adding these inhibitors 3 d after cell disruption yielded the pattern shown in (b).

for 50 min, while BPO 1b did not lose any activity under these conditions and BPO 3 retained 60% of its original activity.

The relative activity of the bromination of monochlorodimedone by BPO 1a and 3 was plotted against pH for different concentrations of H₂O₂ (1.6–54.6 mM), and Br⁻ (18–450 mM). BPO 1a showed a pH optimum of 4.5 under all conditions tested. BPO 3 also showed maximum activity at pH 4.5, except that with a low concentration of H₂O₂ (1.6 mM) the pH optimum shifted from 4.5 to 4.0.

**Kinetic properties.** The $K_m$ values of BPO 1a and 3 were calculated for the three substrates H₂O₂, Br⁻ and monochlorodimedone from Lineweaver-Burk and Hanes plots. The apparent $K_m$ values are listed in Table 2.

**Reactions catalysed by the bromoperoxidases from S. griseus.** The four bromoperoxidases detected in *S. griseus* catalysed the bromination of monochlorodimedone in the presence of H₂O₂ and Br⁻. However, none of these enzymes was able to chlorinate or fluorinate this substrate. The nonhaem-type BPO 1a, 1b and 3 did not show any peroxidase or catalase
Table 1. Summary of the purification of bromoperoxidases from S. griseus

<table>
<thead>
<tr>
<th>Purification step</th>
<th>Total protein (mg)</th>
<th>Total activity (U)</th>
<th>Specific activity (U mg⁻¹)</th>
<th>Purification (-fold)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude extract</td>
<td>124822</td>
<td>34.8</td>
<td>0.003</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>(NH₄)₂SO₄ ppt.</td>
<td>2628</td>
<td>31.5</td>
<td>0.012</td>
<td>4</td>
<td>91</td>
</tr>
<tr>
<td>DE 52, pH 6-8</td>
<td>113.2</td>
<td>7.5</td>
<td>0.067</td>
<td>22</td>
<td>22</td>
</tr>
<tr>
<td>Heat treatment</td>
<td>78.6</td>
<td>7.5</td>
<td>0.095</td>
<td>32</td>
<td>22</td>
</tr>
<tr>
<td>Sepharose Q, pH 8-0</td>
<td>34.0</td>
<td>5.3</td>
<td>0.155</td>
<td>52</td>
<td>15</td>
</tr>
<tr>
<td>Sepharose Q, pH 5-5</td>
<td>2.6</td>
<td>1.5</td>
<td>0.580</td>
<td>195</td>
<td>4</td>
</tr>
<tr>
<td>Sephadex G-200</td>
<td>0.6</td>
<td>1.5</td>
<td>2.400</td>
<td>800</td>
<td>4</td>
</tr>
<tr>
<td>Hydroxylapatite</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BPO 1b</td>
<td>0.14</td>
<td>0.23</td>
<td>1.614</td>
<td>538</td>
<td>0.7</td>
</tr>
<tr>
<td>BPO 1a</td>
<td>0.20</td>
<td>0.56</td>
<td>2.800</td>
<td>933</td>
<td>1.8</td>
</tr>
</tbody>
</table>

*BPO 1* (purified from 400 g bacteria)

<table>
<thead>
<tr>
<th>Purification step</th>
<th>Total protein (mg)</th>
<th>Total activity (U)</th>
<th>Specific activity (U mg⁻¹)</th>
<th>Purification (-fold)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude extract</td>
<td>10418</td>
<td>10.3</td>
<td>0.001</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>(NH₄)₂SO₄ ppt.</td>
<td>1341</td>
<td>10.7</td>
<td>0.008</td>
<td>8</td>
<td>104</td>
</tr>
<tr>
<td>DE 52, pH 6-8</td>
<td>126</td>
<td>2.8</td>
<td>0.024</td>
<td>24</td>
<td>27</td>
</tr>
<tr>
<td>Heat treatment</td>
<td>85</td>
<td>4.7</td>
<td>0.055</td>
<td>55</td>
<td>46</td>
</tr>
<tr>
<td>Sepharose Q, pH 8-0</td>
<td>5.8</td>
<td>2.0</td>
<td>0.350</td>
<td>350</td>
<td>19</td>
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<tr>
<td>Sepharose Q, pH 5-5</td>
<td>2.2</td>
<td>0.9</td>
<td>0.400</td>
<td>400</td>
<td>9</td>
</tr>
<tr>
<td>Sephadex G-200</td>
<td>0.5</td>
<td>0.4</td>
<td>0.800</td>
<td>800</td>
<td>4</td>
</tr>
<tr>
<td>Superose 12</td>
<td>0.3</td>
<td>0.3</td>
<td>1.107</td>
<td>1107</td>
<td>3</td>
</tr>
</tbody>
</table>

*BPO 3* (purified from 450 g bacteria)

**Table 2. Comparison of BPO 1a, BPO 1b and BPO 3 from S. griseus with the nonhaem-type bromoperoxidases of S. aureofaciens and Corallina pilulifera**

<table>
<thead>
<tr>
<th>Property</th>
<th>BPO 1a</th>
<th>BPO 1b</th>
<th>BPO 3</th>
<th>S. aureofaciens</th>
<th>C. pilulifera†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mₙ</td>
<td>70000 ± 10000</td>
<td>90000 ± 5000</td>
<td>90000 ± 5000</td>
<td>92500 ± 2500</td>
<td>790000</td>
</tr>
<tr>
<td>Subunit Mₙ</td>
<td>34000</td>
<td>43000</td>
<td>31000</td>
<td>31000</td>
<td>64000</td>
</tr>
<tr>
<td>No. of subunits</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td>pI</td>
<td>4.6</td>
<td>4.7</td>
<td>3.6</td>
<td>–</td>
<td>3.0</td>
</tr>
<tr>
<td>pH optimum</td>
<td>4.5</td>
<td>–</td>
<td>4.5</td>
<td>4.5</td>
<td>6.0</td>
</tr>
<tr>
<td>Sp. act. (U mg⁻¹)</td>
<td>1.6</td>
<td>2.8</td>
<td>1.1</td>
<td>2.3</td>
<td>26.3</td>
</tr>
<tr>
<td>Kₘ (H₂O₂) (m)</td>
<td>4.3 x 10⁻²</td>
<td>–</td>
<td>6.4 x 10⁻³</td>
<td>3.1 x 10⁻³</td>
<td>9.2 x 10⁻⁵</td>
</tr>
<tr>
<td>Kₘ (Br⁻) (m)</td>
<td>1.6 x 10⁻¹</td>
<td>–</td>
<td>9.5 x 10⁻²</td>
<td>5.9 x 10⁻²</td>
<td>1.1 x 10⁻²</td>
</tr>
<tr>
<td>Kₘ (monochlorodimedone) (m)</td>
<td>7.9 x 10⁻⁶</td>
<td>–</td>
<td>2.0 x 10⁻⁵</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

*, Not determined.
† Data from van Pée et al. (1987).

activities, whereas the haem-type BPO 2 showed these activities in addition to brominating activity.

Pyrrolnitrin was brominated, but not chlorinated, by the nonhaem-type BPO 1a and 3.

**Immunological results.** The cross-reactions were performed with antiserum raised against homogeneous nonhaem-type bromoperoxidase from Streptomyces aureofaciens. The immunological reactions between this antiserum and BPO 1a and 3 from S. griseus were examined. Both bromoperoxidases produced a spurred precipitin line (Fig. 3).
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Fig. 3. Ouchterlony gel diffusion test. The central well contained antiserum raised against bromoperoxidase from Streptomyces aureofaciens Tü 24. Wells 1 and 4 contained S. aureofaciens Tü 24 bromoperoxidase; well 2 contained BPO 3 from S. griseus Tü 6 and well 5 contained BPO 1a from S. griseus Tü 6.

DISCUSSION

Four different bromoperoxidases were detected in Streptomyces griseus Tü 6. The pattern of these bromoperoxidases varied with the age of the culture. The decrease in total activity of BPO 3 during prolonged growth was not due to its digestion by proteases present in the crude extracts, as these had no effect on the total activity of BPO 1a, 1b and 3, whereas the overall protein pattern was changed significantly. Thus BPO 1a and 1b are not artefacts, produced by digestion with proteases after preparation of the cell-free extracts. BPO 3 is probably digested in the cells during prolonged growth. The production of haem-type and nonhaem-type haloperoxidases was also detected in Pseudomonas pyrrocinia (Wiesner et al., 1986) and Streptomyces aureofaciens (van Pée et al., 1987); however, no multiple forms of the nonhaem-type haloperoxidases were detected in these strains.

BPO 3 had an $M_r$ of $90000 \pm 5000$ with a subunit $M_r$ of 31000, and is thus very similar to the nonhaem-type bromoperoxidase from S. aureofaciens, which was also reported to be a trimer (van Pée et al., 1987). The other two nonhaem-type bromoperoxidases, BPO 1a and 1b, were dimers (Table 2).

BPO 1a and 3 were the major nonhaem-type bromoperoxidases present and were thus used for further comparative studies.

The molecular and catalytic properties of the nonhaem-type bromoperoxidases from S. griseus were very similar to those of the nonhaem-type bromoperoxidase from S. aureofaciens Tü 24 (van Pée et al., 1987), but quite different from the nonhaem-type bromoperoxidase of the red alga Corallina pilulifera (Itoh et al., 1986) (Table 2). BPO 1a and 3 gave cross-reactions in immunodiffusion experiments with antiserum against S. aureofaciens bromoperoxidase; they both showed partial identity. This similarity to the enzyme from S. aureofaciens Tü 24 was also confirmed for BPO 3 by partial amino acid sequence analysis of the NH$_2$-terminal end. The sequences differed in only 5 positions out of 15 (data not shown).

Each of the bromoperoxidases purified from S. griseus differed from the others with respect to most of the properties examined. It is not clear from these results whether these enzymes are the products of the same or different genes.

None of the bromoperoxidases from S. griseus was able to catalyse the chlorination of monochlorodimedone. BPO 1a and 3 catalysed the bromination of the antibiotic pyrrolnitrin, but not its chlorination. It is not yet clear, whether these bromoperoxidases can not catalyse chlorination at all, or whether, as in the case of the nonhaem-type chloroperoxidase from Pseudomonas pyrrocinia, chlorination is much more specific than bromination (Wiesner et al., 1986).
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REFERENCES


Multiple bromoperoxidases from S. griseus