Acylation Activity of the Wide Spectrum Amidase of
Brevibacterium sp. R312

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The wide spectrum amidase from Brevibacterium sp. R312, which can hydrolyse many amides to the corresponding acids, was shown to transfer the acyl groups of amides, acids and esters to hydroxylamine. The transfer rates of these reactions in cytoplasmic fractions were measured and compared. The $K_m$ and $V_{max}$ were determined for different substrates in the presence of hydroxylamine. The enzyme was also shown to transfer the acyl group of the amide analogue $N$-methylacetamide to hydroxylamide and that of acetamide to the hydroxylamine analogue methylhydroxylamine.

INTRODUCTION

Brevibacterium sp. R312 is able to transform different nitriles to the corresponding acids, with an intermediary amide step (Jallageas et al., 1978a). Two enzymes with broad specificity are involved in this transformation. A nitrile hydratase first hydrates the nitriles to the corresponding amides (Arnaud et al., 1977; Bui et al., 1984a). Subsequently an amidase hydrolyses the amides to the corresponding acids (Jallageas et al., 1978; Maestracci et al., 1984).

Besides having amide hydrolysing activity, the amidase of Brevibacterium sp. R312, in common with other microbial amidases (Clarke, 1970; Thalenfeld & Grossowicz, 1976; Miller & Gray, 1982; Asano et al., 1982), is also an acyltransferase (Thiéry et al., 1986b). It has the following activities: amide hydrolase, amide transferase, acid transferase and ester transferase (Table 1). It seemed interesting to us to study the activity ratios and the affinities of the enzyme for different substrates involving transferase activity.

METHODS

Bacterial strain. The wild-type Brevibacterium sp. R312 was described by Arnaud et al. (1976a, b).

Culture conditions. The basal minimum medium (MM) used had the following composition (g l$^{-1}$): KH$_2$PO$_4$, 1.2; Na$_2$HPO$_4$.12H$_2$O, 1.95; K$_2$HPO$_4$, 0.98; CaCl$_2$.2H$_2$O, 0.012; ZnCl$_2$, 0.0012; FeSO$_4$.7H$_2$O, 0.0012; MnSO$_4$.4H$_2$O, 0.0012. This medium was adjusted to pH 7.0 and sterilized by autoclaving. Filter-sterilized solutions of MgSO$_4$.7H$_2$O and thiamin chlorhydrate (Thiéry et al., 1986a) were added to give concentrations of 0.5 g l$^{-1}$ and 0.002 g l$^{-1}$, respectively. Glucose and (NH$_4$)$_2$SO$_4$ were added to give final concentrations of 10 and 5 g l$^{-1}$, respectively. The non-substrate inducer N-methylacetamide was added to induce the amidase (Maestracci et al., 1984). The cultures were grown aerobically in 5 l Erlenmeyer flasks filled to one-tenth of their volume and incubated at 28 °C with shaking.

Preparation of extracts. Disruption of bacteria by grinding and preparation of extracts were done as described by Jallageas et al. (1978a). $K_m$ values were determined in supernatants obtained by centrifugation of crude extracts at 180000 g for 90 min.

Enzyme assays. Amide hydrolase was assayed using a GLC apparatus to monitor the disappearance of the substrate (amide) as described by Jallageas et al. (1978b) and Bui et al. (1984b). Acylation transferase was assayed using the method developed by Brammar & Clarke (1964), based on the colorimetric determination of the resulting hydroxamic acids which yield red-brown complexes with Fe(III). The reaction medium was of the following
Table 1. *Comparison of relative reaction rates of hydrolysis and transfer*

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Relative specific activity (%) *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amide hydrolase CH₃–CONH₂ + H₂O → CH₃COOH + NH₃</td>
<td>3.0</td>
</tr>
<tr>
<td>Amide transferase CH₃–CONH₂ + NH₂OH → CH₃–CONHOH + NH₃</td>
<td>100</td>
</tr>
<tr>
<td>Acid transferase CH₃–COOH + NH₂OH → CH₃–CONHOH + H₂O</td>
<td>8.9</td>
</tr>
<tr>
<td>Ester transferase CH₃–COO–CH₂CH₃ + NH₂OH → CH₃–CONHOH + CH₂CH₂OH</td>
<td>4.4</td>
</tr>
</tbody>
</table>

* The 100% value corresponds to an activity of 35 μmol min⁻¹ (mg protein)⁻¹.

Table 2. *Amide, acid and ester transferase activity: determination of Kₘ and relative Vₘₘₐₓ for different substrates*

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Relative Vₘₘₐₓ (%) *</th>
<th>Kₘ (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amides</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetamide</td>
<td>100</td>
<td>31</td>
</tr>
<tr>
<td>Propionamide</td>
<td>28</td>
<td>88</td>
</tr>
<tr>
<td>Acrylamide</td>
<td>49</td>
<td>93</td>
</tr>
<tr>
<td>Acids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetate</td>
<td>8.9</td>
<td>83</td>
</tr>
<tr>
<td>Propionate</td>
<td>111</td>
<td>628</td>
</tr>
<tr>
<td>Acrylate</td>
<td>3.3</td>
<td>88</td>
</tr>
<tr>
<td>Esters</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>4.4</td>
<td>18</td>
</tr>
<tr>
<td>Ethyl propionate</td>
<td>13.5</td>
<td>16</td>
</tr>
<tr>
<td>Ethyl acrylate</td>
<td>14.7</td>
<td>218</td>
</tr>
</tbody>
</table>

* The 100% value corresponds to an activity of 35 μmol min⁻¹ (mg protein)⁻¹.

RESULTS AND DISCUSSION

Comparison of the relative rates of hydrolysis and transfer reactions

Transfer reactions were assayed under conditions of hydroxylamine saturation (500 mM). The transfer of the acyl group of acetamide to hydroxylamine was about 30-fold faster than the hydrolysis of acetamide to ammonium acetate (Table 1), and 10- and 20-fold faster than the transfer of the acyl group of acetate and ethyl acetate. It appears that in the presence of hydroxylamine, the amidase would convert acetamide to acetoxyhydroxamic acid rather than to ammonium acetate.

Amide transferase activity

This study was assayed with acetamide, propionamide and acrylamide (Table 2). The rate of reaction was greatest with acetamide. The order of the relative rates of hydrolysis of these amides (propionamide > acrylamide > acetamide) (Maestracci et al., 1984) was the reverse of the transfer reaction rates of the acyl groups to hydroxylamine, as was also observed for the amidase of *Pseudomonas aeruginosa* (Clarke, 1970). Acetamide was also the substrate for which the enzyme had the highest affinity in the hydrolysis reaction (Kₘ = 2.3, 12 and 43 mM for acetamide, acrylamide and propionamide, respectively).
An amidase from Brevibacterium sp.

Acid transferase activity

This was assayed with acetate, propionate and acrylate (sodium salts resulting from the neutralization of the corresponding acids with NaOH) (Table 2). The rate of reaction was greatest with propionate but the amidase had the lowest affinity for this compound. The highest transfer rate with *P. aeruginosa* was with acetate (Clarke, 1970).

Ester transferase activity

This was assayed with ethyl acetate, ethyl propionate and ethyl acrylate (Table 2). The rate of reaction was greatest with ethyl acrylate but the enzyme had the lowest affinity for this substrate. The highest transfer rate with *P. aeruginosa* was with ethyl acetate (Clarke, 1970).

Effect of amide and hydroxylamine analogues on acyltransferase activity

The action of amidase in the presence of hydroxylamine was assayed with the amide analogues thioacetamide, acetamidine, N-methylacetamide and *N*,*N*-dimethylacetamide. In the case of thioacetamide no conclusion could be reached since spontaneous decomposition occurred. Of the other compounds, only *N*-methylacetamide was converted to acetoxyhydroxamic acid by the amidase. However, the transfer rate of the acyl group of this amide analogue to hydroxylamine was 180-fold slower than for acetamide. Also, the affinity of the enzyme for acetamide (\(K_m = 31 \text{ mM}\)) was greater than for *N*-methylacetamide (\(K_m = 62 \text{ mM}\)).

Hydrolysis of *N*-methylacetamide by the amidase of *Brevibacterium* sp. R312 was not observed by Maestracci *et al.* (1984). It is thus surprising to find that in the presence of hydroxylamine, this enzyme converts *N*-methylacetamide to acetoxyhydroxamic acid. It is presumed that there was hydrolysis of *N*-methylacetamide, although the reaction might have occurred at such a slow rate that it was not detected by the GLC technique used.

Finally, the enzyme was shown to transfer the acyl group of acetamide to the hydroxylamine analogue methylhydroxylamine. However, the rate of reaction was three-fold slower than with hydroxylamine.

Conclusions

The amidase of *Brevibacterium* sp. R312 is known to hydrolyse many amides (Maestracci *et al.*, 1984). We have now shown that it also has transferase activity. It is able to catalyse the transfer of acyl groups from amides, organic acids and esters to hydroxylamine. However, the non-acyl compound acetamidine was not a substrate. The enzyme is thus an acyltransferase. As the hydrolysis reaction of amides can be considered a transfer reaction of their acyl groups to water, the enzyme should be classed as an acyltransferase (EC 2.3.1) instead of an acylamide amidohydrolase (amidase) (EC 3.5.1.4).

REFERENCES


