Physiological Factors Regulating Tyrosine-sulphate Sulphohydrolase Activity in *Comamonas terrigena*: Occurrence of Constitutive and Inducible Enzymes

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Tyrosine-sulphate sulphohydrolase was synthesized by actively growing cultures and by resting suspensions of *Comamonas terrigena*. Polyacrylamide gel electrophoresis revealed that bacteria exposed to exogenous tyrosine sulphate synthesized two forms of this enzyme. Only one of these was detected in extracts of bacteria which had not been exposed to the ester. Both enzymes differed from aryl-sulphate sulphohydrolase in electrophoretic mobility. The synthesis of the induced form was regulated by tyrosine as well as by most intermediates of $\text{SO}_4^{2-}$ assimilation. L-Cysteine had no effect on the synthesis of the induced form but stimulated *in vitro* activity by about 85%. These effectors did not influence either the synthesis or the activity of the other electrophoretic form which was tentatively considered as being constitutive.

**INTRODUCTION**

It is well established that ester sulphate constitutes a large proportion of the total sulphur found in soil (see, for example, Freney, 1967; Tabatabai & Bremner, 1972) but comparatively little information is available on the mechanism whereby this fraction undergoes mineralization (Fitzgerald, 1976, 1978). The widespread occurrence of tyrosine sulphate in proteins of mammalian and amphibian origin (Dodgson & Rose, 1970) coupled with observations that the ester is excreted in urine in substantial quantities (John et al., 1966; Hext et al., 1973) suggests that tyrosine sulphate could act as an important source of $\text{SO}_4^{2-}$ for the sulphur cycle in aerobic soils. Indeed, Houghton & Rose (1976) found that neutral as well as alkaline soils released substantial quantities of $^{35}\text{SO}_4^{2-}$ when incubated with either $^{35}\text{S}$-labelled tyrosine or tyrosylglycine sulphate.

Previous work demonstrated that tyrosine sulphate can act as a substrate for aryl-sulphate sulphohydrolase (EC 3.1.6.1) present in *Alcaligenes metalcaligenes* (Dodgson et al., 1959; John et al., 1966), *Pseudomonas aeruginosa* (Harada, 1964), *Klebsiella aerogenes* (Okamura et al., 1976) and in *Aspergillus oryzae*, isoenzyme I (Dodgson et al., 1959; Burns & Wynn, 1975, 1977). However, the existence of a sulphohydrolase which is specifically synthesized in response to tyrosine sulphate and which exhibits marked specificity for this particular ester has yet to be reported. In this paper, we describe the occurrence and regulatory properties of such an enzyme in *Comamonas terrigena*, a bacterium isolated from soil (Fitzgerald et al., 1975).
METHODS

Preparation of L-tyrosine O-sulphate. Chromatographically pure L-tyrosine (4 g, B.D.H.) was added gradually (over a period of 10 min) to 20 ml sulphuric acid (98%, analytical reagent grade) with constant stirring at 0 °C. The resulting syrup was poured into a mixture of crushed ice-water (500 ml) and barium hydroxide (160 g) with vigorous stirring, taking care to keep the temperature as low as possible until the solution became strongly alkaline. The precipitate of barium sulphate was removed by centrifuging and excess Ba\(^{2+}\) was removed by bubbling CO\(_2\) through the supernatant until there was no more precipitation. After filtering, the solution was evaporated to dryness in vacuo at 37 °C. The solid residue taken up in 25 ml water was filtered and passed through a small column (4 x 2.5 cm) of Dowex 50 (200 to 400 mesh; H\(^+\) form) and washed through with water, the eluate being collected in an ice-cooled beaker. The pH of the solution was then adjusted to 7 with 5% (w/v) KOH and the potassium salt of L-tyrosine O-sulphate was obtained by evaporating to dryness in vacuo at 37 °C. Recrystallization from water was achieved by dissolving the residue in approx. 5 to 10 ml boiling water made alkaline with one drop of 5% (w/v) KOH, followed by cooling quickly, filtering and drying by pressing between filter papers. A further recrystallization was performed by dissolving the product in approx. 20 ml hot alkaline water and then adding 800 ml absolute ethanol with stirring. Diethyl ether (50 to 100 ml) was also added to aid complete precipitation. After cooling, the white solid was filtered over sintered glass, dried thoroughly over P\(_2\)O\(_5\) in vacuo and stored in airtight bottles below -20 °C. The purity of the product was checked chromatographically (Dodgson et al., 1959) and spectrally (A\(_{280}\) = 278 to 295, in 0.1 M-NaOH; no absorbance above 280 nm and no specific absorbance at 250 nm).

Other sulphate esters. Dipotassium p-nitroacetate sulphate and potassium p-nitrophenyl sulphate were obtained from Sigma. Potassium salts of p-acetylphenyl sulphate, L-tyramine O-sulphate, L-serine O-sulphate and L-threonine O-sulphate were kindly provided by K. S. Dodgson, University College, Cardiff.

Culture conditions and preparation of resting suspensions. Comamonas terrigena was grown with shaking at 30 °C in a basal salts medium (BSM) containing carbon and sulphur in the form and concentration stated in Table 1. The basal salts medium (pH 7.0) contained (g l\(^{-1}\) in deionized water): KH\(_2\)PO\(_4\), 3.5; K\(_2\)HPO\(_4\), 1.5; NH\(_4\)Cl, 0.5; MgCl\(_2\).6H\(_2\)O, 0.15. Procedures for culture medium sterilization as well as other details associated with growing this bacterium were identical to those described previously for Pseudomonas C\(_9\)B (Dodgson, 1973).

For the preparation of resting suspensions, C. terrigena was grown for 24 h (onset of stationary phase, see Table 1) in BSM supplemented with sodium pyruvate (0.5%, w/v) and 0.1 mM-Na\(_2\)SO\(_4\). Bacterial pellets resulting from the centrifugation of 200 ml culture samples were resuspended in and washed once with 30 ml BSM.

To induce tyrosine-sulphate sulphohydrolase synthesis, the washed bacteria were resuspended and shaken for 4 h at 30 °C in 50 ml BSM containing 0.5 mM-tyrosine sulphate, unless otherwise indicated, in the presence or absence of additional effectors. In all cases, the results of enzyme assays on extracts of resting suspensions represent the average of two independent experiments.

Preparation of bacterial extracts and protein determinations. The protein content of resting suspensions (1 ml) was determined by the method of Lowry et al. (1951) after the bacteria had been ruptured by boiling in 5 ml 0.1 M-sodium bicarbonate. Extracts of bacteria for enzyme assay were prepared using the French pressure cell (Dodgson et al., 1974) and were dialysed at 4 °C against 410 M-Tris/HCl buffer, pH 7.5, changed six times during 24 h. These extracts were assayed for protein as described previously (Fitzgerald, 1973).

Assay for inorganic SO\(_4^{2-}\) release from tyrosine sulphate. The liberation of SO\(_4^{2-}\) from 10 mM-tyrosine sulphate in 0.01 M-Tris/HCl buffer, pH 7.5, was determined by a modification of the BaCl\(_2\)-gelatin method of Dodgson (1961). Bacterial extract (1:0 ml) was incubated at 30 °C with the ester (1:0 ml), usually for 20 min, and the reaction was terminated by adding 0.5 ml 16% (w/v) trichloroacetic acid. When determinations for SO\(_4^{2-}\) and tyrosine (see below) were carried out simultaneously the above volumes were doubled. After centrifugation, 0.5 ml BaCl\(_2\)-gelatin reagent (Dodgson, 1961) was added to 2.0 ml of the clear supernatant. After standing for 15 min, the absorbance of the suspension was read at 360 nm against a control (see below). A linear relationship existed between A\(_{280}\) and SO\(_4^{2-}\) concentrations between 0.05 and 0.5 μmol ml\(^{-1}\).

Identification of tyrosine as a product of the action of extracts of C. terrigena on tyrosine sulphate. Tyrosine was identified as follows: 3 ml of a dialysed extract (6.9 mg protein ml\(^{-1}\)) of induced bacteria were incubated for 3 h with an equal volume of 10 mM-tyrosine sulphate in 0.01 M-Tris/HCl buffer, pH 7.5. The reaction was terminated by adding 1.5 ml 16% (w/v) trichloroacetic acid and, following centrifugation, the clear supernatant was repeatedly extracted with diethyl ether until the pH of the supernatant was 5-5. The presence of tyrosine in the extracted supernatant was confirmed by chromatography (Dodgson et al., 1959) and by the observation that the supernatant acted as a substrate for mushroom tyrosinase (Sigma). Control deter-
Tyrosine-sulphate sulphohydrolase

minations (see below) carried out in parallel revealed that the occurrence of tyrosine depended on contact of the extract with the ester prior to the addition of acid and confirmed that tyrosine sulphate did not serve as a substrate for tyrosinase.

Assay for tyrosine release from tyrosine sulphate. Tyrosine liberation by bacterial extracts acting on 10 mm-tyrosine sulphate was determined by a modification of the method of Undenfriend & Cooper (1952). To 2 ml of the clear supernatant (obtained above) was added 1 ml 0·1 % (w/v) 1-nitroso-2-naphthol (Eastman Kodak) made up on the day of use in 95 % (v/v) aqueous ethanol. To this solution was added 1 ml NaNO₃ (0·5 mg ml⁻¹) in 20 % (v/v) aqueous HNO₃ (specific gravity 1·42). The final solution was immediately mixed by vortexing, incubated at 55 °C for 2 min and subsequently held on ice for 1 min. After equilibration to room temperature (2 min), the absorbance of the solution was read against a control at 518 nm (Thomas, 1944). Preliminary determinations revealed: (i) that colour development was maximal and stable, and (ii) that tyrosine sulphate (10 mm) did not undergo detectable acid-catalysed hydrolysis under these conditions. With strict adherence to this procedure, a linear relationship was obtained between A₅18 and l-tyrosine concentrations between 0·1 and 1·25 μmol ml⁻¹.

Aryl-sulphate sulphohydrolase assays and definition of enzyme unit. The activity of this enzyme toward p-nitrocatechol sulphate was determined as previously described (Fitzgerald & Cline, 1977). In all assays, control determinations were made in which extract and substrate were incubated separately and mixed immediately prior to the addition of trichloroacetic acid (tyrosine-sulphate sulphohydrolase assay) or 1 M-NaOH (aryl-sulphate sulphohydrolase assay). One enzyme unit (EU) is the amount of enzyme necessary to release 1 nmol SO₄²⁻, l-tyrosine or p-nitrocatechol, as appropriate, in 1 min.

Polyacrylamide gel electrophoresis. The experimental design for this procedure was identical to that previously described (Payne & Painter, 1971; Payne et al., 1974). Tyrosine-sulphate sulphohydrolase was localized in 6·8 % (w/v) acrylamide gels by the gel-slice technique (Fitzgerald & Laslie, 1975). Gels which exhibited identical tracking dye mobility were cut into 0·5 cm sections and each section was macerated in 2·0 ml 10 mm-tyrosine sulphate in 0·01 m-Tris/HCl buffer, pH 7·5. The mixtures were incubated at 30 °C for 6 h and 1·0 ml 16 % (w/v) trichloroacetic acid was added to terminate the reaction. After centrifugation, each clear eluate was assayed for SO₄²⁻ and tyrosine as described above. Enzyme activity is expressed in terms of the absorbance of each gel section eluate at 360 nm (SO₄²⁻ assay) or at 518 nm (tyrosine assay).

Aryl-sulphate sulphohydrolase was localized in separate gels by the above procedure except that macerated gel sections were eluted at 30 °C for 10 h with 1·0 ml 10 mm-p-nitrocatechol sulphate in 0·01 m-Tris/HCl buffer, pH 7·5, and 1·0 ml 1 M-NaOH was added to terminate the reaction. In view of the extreme dilution of total activity which results from electrophoresis and elution, extended incubation periods were chosen to facilitate detection of all enzyme species (c.f. Fitzgerald & Milazzo, 1970; Fitzgerald & Laslie, 1975). Enzyme activity is expressed in terms of the absorbance of gel section eluates at 515 nm due to the release of p-nitrocatechol from p-nitrocatechol sulphate.

RESULTS AND DISCUSSION

Synthesis of the enzyme by actively growing bacteria

Although a low basal level of desulphating activity towards tyrosine sulphate was detected in C. terrigena grown in BSM on sulphur sources other than tyrosine sulphate, the capacity to utilize the ester as a sulphur source for growth appeared to result, at least in part, from induction by tyrosine sulphate (Table 1). Thus, bacteria grown to the stationary phase in BSM containing tyrosine sulphate as the sole sulphur source exhibited about sevenfold higher desulphating activity towards this ester compared with activities detected after growth on the sulphur-containing amino acids or inorganic SO₄²⁻ (Table 1). Since nutrient broth is a meat extract/peptone preparation, the increased activity towards tyrosine sulphate of bacteria grown in this enriched medium (Table 1) may result from the presence of tyrosine sulphate in this medium. The possibility that tyrosine sulphate could serve as an inducer for the enzyme was investigated further using resting suspensions since preliminary work established that assays with a higher order of reproducibility could be expected under these conditions (Fitzgerald & Kight, 1977).

Synthesis of the enzyme by resting suspensions

Although mid-exponential phase cultures of bacteria grown on tyrosine sulphate exhibited higher enzyme activities than did stationary phase bacteria (Table 1), the age of the culture
Table 1. Tyrosine-sulphate sulphohydrolase activity of C. terrigena grown under different conditions

Bacteria were grown to the onset of the stationary phase (except where indicated) in BSM with added nutrient broth (0.8%, w/v; Difco) or in BSM with sodium pyruvate (0.5%, w/v) as the principal carbon source and the compound shown (0.1 mM) as the sole source of sulphur.

<table>
<thead>
<tr>
<th>Addition to BSM</th>
<th>Culture age</th>
<th>$A_{450}$ of culture</th>
<th>Specific activity*</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Cysteine</td>
<td>24</td>
<td>0.97</td>
<td>0.43</td>
</tr>
<tr>
<td>L-Methionine</td>
<td>24</td>
<td>0.97</td>
<td>0.57</td>
</tr>
<tr>
<td>Na$_2$SO$_4$</td>
<td>24</td>
<td>0.91</td>
<td>0.48</td>
</tr>
<tr>
<td>Na$_2$SO$_4$</td>
<td>16</td>
<td>0.33†</td>
<td>0.53</td>
</tr>
<tr>
<td>Nutrient broth</td>
<td>16</td>
<td>1.90</td>
<td>1.21</td>
</tr>
<tr>
<td>L-Tyrosine sulphate</td>
<td>24</td>
<td>0.95</td>
<td>3.47</td>
</tr>
<tr>
<td>L-Tyrosine sulphate</td>
<td>16</td>
<td>0.28†</td>
<td>4.66</td>
</tr>
</tbody>
</table>

* Bacterial extracts were assayed for the ability to release SO$_4^{2-}$ from tyrosine sulphate. All values are averages of two independent growth experiments; the experimental variation was within 15%.
† Mid-exponential phase bacteria.

employed for the preparation of resting suspensions did not influence the inducibility. Bacteria grown for 24 h in BSM containing 0.5% (w/v) sodium pyruvate and 0.1 mM Na$_2$SO$_4$ yielded the highest enzyme activity as resting suspensions in response to tyrosine sulphate and these conditions were held constant in subsequent work.

After growth under these conditions, washed stationary phase C. terrigena synthesized enzyme with desulphating activity for tyrosine sulphate in response to increasing concentrations of this ester in BSM (Fig. 1a). As expected, bacteria shaken in the absence of exogenous tyrosine sulphate exhibited a basal level of activity which remained constant for up to 6 h. In contrast, activities of the enzyme in bacteria exposed to 0.5 mM-ester increased by about sixfold during this period (Fig. 1b). No net increase in protein synthesis or $A_{420}$ was detected, indicating that the organisms were not actively dividing under these conditions. The optimal time of exposure and inducer concentration were 4 h and 0.5 mM, respectively, (Fig. 1) and these parameters were held constant in subsequent experiments.

Tyramine sulphate (0.5 mM) alone was not effective as an inducer but when added with tyrosine sulphate, the amine caused a further twofold stimulation of synthesis when resting suspensions were shaken for 4 h. No such additional increase was noted when other amino acid sulphate esters (threonine and serine sulphates) or the sulphate esters of $p$-nitrocatechol, $p$-nitrophenol and $p$-acetylphenol were tested in combination with tyrosine sulphate. These esters alone also did not act as inducers. These results suggest that, for tyramine sulphate, inducer specificity may reside primarily at the level of transport and that the decarboxylated analogue can only serve as an inducer in the presence of an effector (tyrosine sulphate) which stimulates the synthesis of a carrier. Although it is well established that tyramine can stimulate the synthesis of aryl-sulphate sulphohydrolase in Aerobacter aerogenes (Adachi et al., 1973), the unsulphated amine had no effect in the C. terrigena system when added alone or together with tyrosine sulphate.

Increased desulphating activity towards tyrosine sulphate in bacteria exposed to this ester resulted from de novo synthesis since, although the formation of the enzyme was relatively insensitive to chloramphenicol, C. terrigena exhibited low activities after exposure to inducer and either tetracycline or rifampin (Table 2). The lack of an effect by puromycin suggests that the outer membrane of C. terrigena is capable of excluding this antibiotic. Both tetracycline and rifampin reduced the activity of bacteria below the basal level observed when bacteria were shaken in the absence of tyrosine sulphate. Since neither of these compounds inhibited the desulphation of tyrosine sulphate by extracts, the results suggest that the enzyme responsible for the basal level of activity was also synthesized de novo.
Fig. 1. Effect of tyrosine sulphate concentration (a) and time of incubation (b) on the synthesis of tyrosine-sulphate sulphohydrolase by resting suspensions of C. terrigena. (a) Inorganic SO$_4^{2-}$ release from 10 mM-tyrosine sulphate by extracts of bacteria that had been incubated at 30 °C for 4 h with various concentrations of tyrosine sulphate; (b) inorganic SO$_4^{2-}$ release from 10 mM-tyrosine sulphate by extracts of bacteria that had been incubated at 30 °C for various periods in the absence of tyrosine sulphate (Δ) or in the presence of tyrosine sulphate at 0·1 mM (●), 0·5 mM (○) or 1·0 mM (■). Bacteria were grown in BSM containing 0·5% (w/v) sodium pyruvate and 0·1 mM-Na$_2$SO$_4$ for 24 h prior to resuspension for these experiments.

Table 2. Repression of tyrosine-sulphate sulphohydrolase formation by some inhibitors of protein synthesis and by rifampin

_Colomonas terrigena_ was grown in BSM containing 0·5% (w/v) sodium pyruvate and 0·1 mM-Na$_2$SO$_4$ for 24 h prior to the preparation of resting suspensions. After resuspension, the bacteria were shaken for 4 h at 30 °C in BSM containing 0·5 mM-tyrosine sulphate and the indicated effector. The specific activity of bacteria shaken in the absence of ester was 0·33.

<table>
<thead>
<tr>
<th>Effector</th>
<th>Concn (mg ml$^{-1}$)</th>
<th>Specific activity* (EU (mg protein)$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>—</td>
<td>4·25</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>0·1</td>
<td>0·08</td>
</tr>
<tr>
<td></td>
<td>0·4</td>
<td>0·02</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>0·4</td>
<td>3·49</td>
</tr>
<tr>
<td></td>
<td>2·0</td>
<td>0·61</td>
</tr>
<tr>
<td>Puromycin</td>
<td>0·5</td>
<td>4·42</td>
</tr>
<tr>
<td>Rifampin</td>
<td>0·4</td>
<td>0·08</td>
</tr>
</tbody>
</table>

* Bacterial extracts were assayed for the ability to release SO$_4^{2-}$ from tyrosine sulphate.

_Hydrolysis of tyrosine sulphate_

There is ample precedent in the literature on the desulphation of serine sulphate to suggest that SO$_4^{2-}$ liberation from an amino acid ester does not necessarily imply the action of a sulphohydrolase (Dodgson & Tudball, 1961; Tudball & Thomas, 1972; Tudball & O'Neill,
Attempts were therefore made to quantify tyrosine release from tyrosine sulphate catalysed by extracts. The release of tyrosine (determined using 1-nitroso-2-naphthol) depended on the incubation time at 30°C and was linear for up to 30 min when a freshly prepared extract of induced bacteria was mixed with 10 mM-tyrosine sulphate. Parallel assays for SO₃²⁻ revealed a similar dependence on the time of incubation but the release of the two products was not equimolar. More tyrosine than SO₃²⁻ was consistently detected and this effect became more pronounced with increasing incubation time suggesting that some SO₃²⁻ might have been utilized for biosynthesis. Since enzymes catalysing SO₃²⁻ assimilation are markedly more unstable than the sulphohydrolases (DeMeio, 1976; Fitzgerald, 1976), this experiment was repeated using the same extract which had been stored at -20°C for 1 month. Under these conditions, enzymes in C. terrigena catalysing the incorporation of SO₄²⁻ into choline sulphate were completely inactivated (Fitzgerald & Luschinski, 1977). Results obtained using the stored extract revealed an equimolar release of both products at intervals extending over a 2 h incubation period. Collectively, these results suggest that the liberation of SO₃²⁻ from tyrosine sulphate occurred as a consequence of the action of a true sulphohydrolase.

Regulatory properties of tyrosine-sulphate sulphotransferase

The widespread occurrence of tyrosine sulphate in mammalian proteins (Dodgson & Rose, 1970) suggests the possibility of its presence in bacterial protein. It was therefore possible that free tyrosine sulphate, arising from protein turnover, might serve as an endogenous inducer for the enzyme in C. terrigena, thus accounting for the basal level of activity observed with bacteria which had not been exposed to the exogenously supplied ester. During the course of this investigation, activities of bacteria examined under this condition varied by almost threefold (see Tables 2 and 3). Although the possibility that endogenous tyrosine sulphate might cause this variation could not be unequivocally ruled out, evidence for the synthesis of separate enzymes catalysing the hydrolysis of the ester by bacteria in the presence or absence of exogenous tyrosine sulphate was obtained by studying the effect of various compounds on the synthesis of the enzyme (Table 3) and by polyacrylamide gel electrophoresis (Fig. 2).

Intermediates of sulphate assimilation (except cysteine) and exogenous tyrosine (all at 1 mM) inhibited the synthesis of tyrosine-sulphate sulphotransferase in bacteria exposed to 0.5 mM-tyrosine sulphate (induced level, Table 3). With the exception of methionine, these compounds had no effect on the synthesis of the enzyme by bacteria shaken in the absence of added ester (basal level, Table 3). Methionine inhibited the synthesis of the basal level and the induced level of the enzyme by about 29 and 58%, respectively. In all cases, an increase in the inhibitor concentration to 5 mM did not reduce the induced level of tyrosine-sulphate sulphotransferase below the basal level (see effect of SO₃²⁻, Table 3).

The inclusion in BSM of an exogenous energy source in the form of either pyruvate or ATP (Table 3) inhibited the induction and stimulated the synthesis of the basal level of the enzyme. Moreover, the in vitro activity of an extract of induced bacteria was inhibited by 1 mM SO₃²⁻, S₂O₅²⁻ or by tyrosine and stimulated by SO₃²⁻, cysteine or by methionine (Table 3). These compounds (1 mM) had no effect on the activity of extracts of bacteria which had not previously been exposed to exogenous tyrosine sulphate. In every case, inhibitory and stimulatory effects on in vitro activity were completely reversed by dialysis. Thus, activities observed with bacteria after exposure to exogenous effectors were the result of effects on the synthesis of the enzyme rather than on the activity of the preformed enzyme (cf. Fitzgerald & Kight, 1977; Kight-Olliff & Fitzgerald, 1978).
Table 3. Influence of sulphur sources, tyrosine and exogenous energy sources on the in vivo synthesis and in vitro activity of tyrosine-sulphate sulphohydrolase

A resting suspension of C. terrigena was shaken at 30 °C for 4 h in BSM with or without 0·5 mM-tyrosine sulphate and with the indicated effector. The bacteria were subsequently ruptured, dialysed and extracts were assayed for the ability to release SO\(_4^{2-}\) from 10 mM-tyrosine sulphate (in vivo specific activity). Some effectors were also tested for their ability to inhibit in vitro either SO\(_4^{2-}\) or tyrosine release from tyrosine sulphate catalysed by a dialysed extract of bacteria exposed only to 0·5 mM-tyrosine sulphate during the induction period.

<table>
<thead>
<tr>
<th>Effector</th>
<th>Concentration (mM)</th>
<th>Basal level</th>
<th>Induced level</th>
<th>In vivo</th>
<th>In vitro</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0·93</td>
<td>5·23</td>
<td>5·33</td>
<td>6·66†</td>
<td></td>
</tr>
<tr>
<td>Na(_2)SO(_4)</td>
<td>1·0</td>
<td>1·08</td>
<td>3·81</td>
<td>6·17†</td>
<td></td>
</tr>
<tr>
<td>Na(_2)SO(_3)</td>
<td>5·0</td>
<td>2·68</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na(_2)S(_2)O(_3)</td>
<td>1·0</td>
<td>0·87</td>
<td>3·41</td>
<td>7·64</td>
<td></td>
</tr>
<tr>
<td>L-Cysteine</td>
<td>5·0</td>
<td>0·95</td>
<td>1·09</td>
<td>3·14</td>
<td></td>
</tr>
<tr>
<td>L-Methionine</td>
<td>1·0</td>
<td>1·15</td>
<td>5·18</td>
<td>9·84</td>
<td></td>
</tr>
<tr>
<td>L-Tyrosine</td>
<td>4·5</td>
<td>0·66</td>
<td>2·22</td>
<td>6·83</td>
<td></td>
</tr>
<tr>
<td>Sodium pyruvate</td>
<td>6·0</td>
<td>1·68</td>
<td>1·69</td>
<td>5·26</td>
<td></td>
</tr>
<tr>
<td>ATP (pH 7·0)</td>
<td>45·0</td>
<td>1·34</td>
<td>5·41</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Extracts were assayed for SO\(_4^{2-}\) release from tyrosine sulphate: basal level of activity, bacteria not exposed to tyrosine sulphate; induced level, bacteria exposed to tyrosine sulphate.
† Extracts were assayed for tyrosine release from tyrosine sulphate.

Existence of multiple enzymes and their distinction from aryl-sulphate sulphohydrolase

Assays for the release of SO\(_4^{2-}\) by gel sections eluted with tyrosine sulphate revealed the presence of two distinct peaks of activity after an extract of tyrosine sulphate-induced bacteria was subjected to polyacrylamide gel electrophoresis (Fig. 2c). Identical results were obtained when determinations for tyrosine were also carried out. When a similar approach was taken using an extract of C. terrigena not exposed to exogenous tyrosine sulphate (non-induced bacteria) determinations for SO\(_4^{2-}\) revealed a single major peak of activity (Fig. 2b) which coincided with the slower moving component evident on the electrophoretogram for induced bacteria (Fig. 2c). This latter enzyme species was also detected as the only form of the enzyme in C. terrigena grown with cysteine or methionine (Table 1). Until evidence for the endogenous generation of tyrosine sulphate is obtained (see above), this species will be considered as a constitutive form of the enzyme.

The increase in the basal level of activity observed when bacteria were exposed to exogenous tyrosine sulphate (Fig. 1b) was thus due to the sulphohydrolase with the greater anodal mobility (Fig. 2c). Only traces of activity due to this enzyme were evident on electrophoretograms of non-induced bacteria (Fig. 2b). This enzyme form was also separated from arylsulphate sulphohydrolase (Fig. 2a) which was detected in both tyrosine sulphate-induced and non-induced bacteria. Although the difference in mobility between the latter two enzymes was not great (Fig. 2a, b), this difference was reproducible. Attempts to increase resolution by altering the acrylamide concentration and/or pH were not successful.

Previous work demonstrated that resting suspensions of C. terrigena synthesized an inducible aryl-sulphate sulphohydrolase when shaken in the presence of sodium pyruvate. Although tyrosine sulphate did not serve as an inducer, extracts of bacteria induced with
Fig. 2. Localization of aryl-sulphate sulphohydrolase (a) and tyrosine-sulphate sulphohydrolase (b, c) in polyacrylamide gels. (a) $A_{415}$ values of eluates of gel sections due to release of $p$-nitrocatechol from $p$-nitrocatechol sulphate; (b, c) $A_{260}$ and $A_{415}$ values of eluates of gel sections due to release of $SO_4^{2-}$ (●) and tyrosine (○), respectively, from tyrosine sulphate. See Methods for details of assay procedures. Extracts (about 5 mg protein ml$^{-1}$) of *C. terrigena* which had previously been shaken for 4 h in BSM in the presence (a, c) or absence (b) of 0.5 mM-tyrosine sulphate were applied to gels as samples (30 µl) in 20% (w/v) sucrose. The direction of electrophoresis was towards the anode.

$p$-nitrophenyl sulphate released $SO_4^{2-}$ from tyrosine sulphate (Fitzgerald & Cline, 1977). This enzyme, however, is clearly distinct from the inducible form of tyrosine-sulphate sulphohydrolase, the synthesis of which did not require an exogenous energy source. Indeed, the inclusion of sodium pyruvate or ATP in BSM containing tyrosine sulphate inhibited the synthesis of the inducible form (Table 3).

REFERENCES


