Biochem. J. (1974) **138**, 63–69
Printed in Great Britain

Induction of Primary Alkylsulphatases and Metabolism of Sodium Hexan-1-yl Sulphate by *Pseudomonas* C12B

By JOHN W. FITZGERALD, KENNETH S. DODGSON* and WILLIAM J. PAYNE
Department of Microbiology, University of Georgia, Athens, Ga. 30602, *U.S.A.*

(*Received* 11 *September* 1973)

Sodium hexan-1-yl sulphate and certain related alkyl sulphate esters have been shown to serve as inducers of the formation of primary alkylsulphatases (designated as P1 and P2) in *Pseudomonas* C12B. When the organism is grown on sodium hexan-1-yl [^{35}S]sulphate as the sole source of sulphur or as the sole source of carbon and sulphur only the P2 alkylsulphatase is formed and inorganic $^{35}SO_4^{2-}$ is liberated into the media. Cell extracts contain this anion as the major ^{35}S-labelled metabolite although two unidentified labelled metabolites as well as choline *O*-[^{35}S]sulphate occur in trace quantities in some extracts. Dialysed cell extracts are capable of liberating inorganic $^{35}SO_4^{2-}$ from sodium hexan-1-yl [^{35}S]sulphate without the need to include cofactors known to be required for the bacterial degradation of *n*-alkanes. The collective results suggest that sodium hexan-1-yl sulphate can act as an inducer of P1 alkylsulphatase formation without the need for prior metabolic modification of the carbon moiety of the ester.

Some sulphate esters, e.g. potassium D-glucose 6-*O*-sulphate and choline *O*-sulphate, are hydrolysed by specific sulphatases in fungi (Lloyd *et al.*, 1968; Scott & Spencer, 1968) and the SO_4^{2-} thus liberated can be used as the source of sulphur for growth. Similarly, bacteria of the genus *Pseudomonas* utilize choline *O*-sulphate as a sulphur source via the action of choline sulphatase (choline sulphate sulphohydrolase, EC 2.1.6.a) (Takebe, 1961; Harada, 1964; Lucas *et al.*, 1972). However, instances of alteration of the carbon moiety of the ester before desulphation are known. Thus *Pseudomonas fluorescens* oxidizes potassium D-glucose 6-*O*-sulphate to D-gluconate 6-*O*-sulphate, which then serves as the source of sulphur for growth (Fitzgerald & Dodgson, 1970, 1971*b*). Moreover, when D-glucose 6-*O*-sulphate is present as the sole source of carbon and sulphur it is metabolized further to yield D-glycerate 3-*O*-sulphate, which then appears to supply the sulphur necessary for growth (Fitzgerald & Dodgson, 1971*a*).

Previous work in these laboratories (Fitzgerald & Payne, 1972) has shown that inclusion of sodium hexan-1-yl sulphate in nutrient broth elicited the formation of high activities of primary alkylsulphatase in *Pseudomonas* C12B. The aim of the present work was to induce the formation of the enzyme(s) responsible for this activity by using ^{35}S-labelled hexan-1-yl sulphate and to establish whether the ester itself serves as inducer or whether it must first be modified in some way.

* Permanent address and address for reprints: Department of Biochemistry, University College, P.O. Box 78, Cardiff CF1 1XL, U.K.

Materials and Methods

Sulphate esters

Sodium hexan-1-yl [^{35}S]sulphate (specific radioactivity 21.8μCi/mg of dry solid; The Radiochemical Centre, Amersham, Bucks., U.K.) was stored as an aqueous 12.4mM solution at −20°C. Under these conditions no decomposition due to self-irradiation occurred during a 3-month period. Potassium ethan-1-yl sulphate was prepared by a modification (Fitzgerald & Payne, 1972) of the method of Lloyd *et al.* (1961). Other primary alkyl sulphates were purchased as sodium salts from Eastman Organic Chemicals, Rochester, N.Y., U.S.A. Potassium decan-5-yl sulphate was prepared as described by Dodgson *et al.* (1974).

Bacteria and culture media

Pseudomonas C12B was maintained as described by Payne & Painter (1971). The organism was grown at 30°C with shaking in either nutrient broth or in a basal salts medium, pH 7.0 (Fitzgerald & Payne, 1972), supplemented with carbon and sulphur in the form and concentration described for each experiment. Inocula were prepared from bacteria cultured in nutrient broth for 16h at 30°C. Cell suspensions were washed once with sterile water and the size of each inoculum was adjusted so that after inoculation the medium had an initial extinction at 650nm of approx. 0.02 (Hitachi Perkin–Elmer model 139 spectrophotometer, slit-width 0.1mm, 1cm light-path). Procedures

for sterilizing complete media, preparation and dialysis of cell extracts and protein determinations were described by Fitzgerald & Payne (1972).

Assay of sulphatase activity

Release of SO_4^{2-} from 25mM solutions of alkyl sulphates by dialysed cell extracts was determined by the method of Dodgson (1961), as modified by Fitzgerald & Payne (1972). A unit of enzyme activity is defined as 1nmol of SO_4^{2-} liberated/min per mg of protein.

Determination of $^{35}SO_4^{2-}$ and choline O-[^{35}S]sulphate in culture media, resting cell supernatants and cell extracts

Bacteria were grown in 90ml of the basal salts medium or nutrient broth, supplemented with 0.55mCi of hexan-1-yl [^{35}S]sulphate, contained in 350ml Erlenmeyer flasks fitted with side-arm cuvettes. At the termination of growth (turbidimetric determination at 650nm and 420nm for coloured and uncoloured media respectively), cultures were centrifuged and clear supernatants were subjected to paper electrophoresis. The cell pellet from each culture was suspended at 4°C in 5ml of water by agitating with a Vortex mixer for 5min and centrifuged. This procedure was repeated and the clear supernatants were combined and concentrated to approx. 0.5ml by freeze-drying. The resulting concentrate is referred to hereafter as the cold-water extract.

The cold-water-extracted cell pellet from each culture was treated with 5ml of cold (4°C) 5% (w/v) trichloroacetic acid and cell debris was removed by centrifuging at the same temperature. The clear supernatant was extracted with equal volumes of diethyl ether until the pH of the aqueous phase had risen to 5.6. The ether-extracted supernatant was then concentrated to approx. 0.5ml by freeze-drying. This concentrate is designated the trichloroacetate extract.

For studies on resting cells, bacteria were grown in nutrient broth and collected by centrifuging when the culture extinction at 650nm reached approx. 0.8 (exponential-phase cells). Cell pellets were washed twice with the basal salts medium (Fitzgerald & Payne, 1972) and resuspended to an extinction of approx. 1.4 in the same medium containing 8mM-hexan-1-yl sulphate and 1.1mCi of hexan-1-yl [^{35}S]-sulphate. The cell suspension was shaken at 30°C for 6h. At time-intervals specified in the text, 0.5ml samples were centrifuged and the clear supernatants were subjected to paper electrophoresis. After 6h cells were collected by centrifugation and extracted with cold water followed by trichloroacetate as described above.

Culture supernatants, resting cell supernatants and cell extracts were analysed for the presence of $^{35}SO_4^{2-}$, choline O-[^{35}S]sulphate, hexan-1-yl [^{35}S]-sulphate and its metabolites by electrophoresis on Whatman no. 1 paper for 45min at 400V in 0.1M-sodium acetate–acetic acid buffer, pH4.5. Inorganic SO_4^{2-} moved approx. 14cm from the origin and mobilities of other components relative to SO_4^{2-} were: sodium hexan-1-yl sulphate, 0.80; metabolite I (see below), 0.52 and metabolite II, 0.33. Choline O-sulphate remained at the origin. The presence of $^{35}SO_4^{2-}$ was confirmed by coincidence of migration with authentic material for this system and by paper electrophoresis for 1.5h at 400V in 0.1M-barium acetate–acetic acid buffer, pH4.5, under which conditions SO_4^{2-} precipitated as $BaSO_4$ and remained at the origin (see Fitzgerald & Dodgson, 1971*a*). The presence or absence of choline O-[^{35}S]sulphate was determined by procedures outlined by Fitzgerald (1973).

Localization and quantitative determination of radioactive areas on papers

Radioactive areas were detected by scanning dried electrophoretograms with a Packard 7200 Radiochromatogram Scanner. The ^{35}S content of various components (expressed in some cases as a percentage of the total radioactivity present on each paper strip) was determined by measuring areas under appropriate peaks on the recording chart paper (see Dodgson *et al.*, 1961). Total radioactivity (expressed as c.p.m.) was determined by counting for 10min with the Packard TriCarb Liquid Scintillation Spectrometer with the scintillation fluid described by Phibbs & Eagon (1970).

Determination of $^{35}SO_4^{2-}$ present in extract-incubation mixtures

Dialysed extracts (1.0ml) were added to 1.0ml of 50mM-hexan-1-yl sulphate (mixture of 5mM ^{35}S-labelled and 45mM unlabelled) in 10mM-Tris–HCl buffer, pH7.5, and incubated at 30°C for 10min. The reaction was terminated by the addition of 2.0ml of trichloroacetate (4%, w/v). Precipitated protein was removed by centrifugation at 4°C and the clear supernatant was extracted with equal volumes of diethyl ether until the pH of the aqueous phase was 5.6. This phase was then concentrated to 0.5ml by freeze-drying and a 30μl sample was subjected to electrophoresis as described for culture supernatants.

Polyacrylamide-gel electrophoresis

Primary and secondary alkylsulphatase enzymes were separated and identified on polyacrylamide gels by the method of Payne & Painter (1971) as modified by Dodgson *et al.* (1974). Enzyme bands are designated P1 and P2 (primary alkylsulphatases) and S1,

S2 and S3 (secondary alkylsulphatases) as described by Dodgson *et al.* (1974).

Results

Polyacrylamide-gel electrophoresis of extracts of cells grown under different cultural conditions

Extracts of *Pseudomonas* C12B grown to the stationary phase in nutrient broth alone (condition A stationary phase, Table 1) gave enzyme bands on gel zymograms corresponding to two secondary alkylsulphatases active towards potassium decan-5-yl sulphate (the S1 and S2 enzymes, Dodgson *et al.*, 1974) and one primary alkylsulphatase active towards sodium dodecan-1-yl sulphate (the P1 enzyme). Judging from band intensity only relatively small amounts of the latter enzyme were present.

Only the S1 and S2 enzymes were present in extracts from exponential phase bacteria (condition A exponential phase, Table 1), but when hexan-1-yl sulphate (10mM) was also present in the nutrient broth the P1 enzyme also appeared in large amounts, as judged visually on the zymograms (cultural conditions B exponential phase, Table 1), suggesting that the alkyl sulphate ester was inducing the synthesis of this enzyme. Although the results are omitted from Table 1 the same P1 enzyme band appeared on zymograms when the sulphate esters of any of the following primary alcohols were substituted for hexan-1-yl sulphate in the culture medium: butan-1-ol, pentan-1-ol, octan-1-ol, decan-1-ol, dodecan-1-ol and tetradecan-1-ol. These findings were in accord with those of Fitzgerald & Payne (1972), who showed that the presence of any of these sulphate esters in the culture medium at a concentration of 10mM led to the appearance in extracts of exponential phase cells of high sulphatase activity towards hexan-1-yl sulphate. It follows that sulphatase activity towards hexan-1-yl sulphate in these particular exponential-phase extracts is attributable to the P1 enzyme. Only traces of similar enzyme activity could be detected when ethan-1-yl sulphate was present in the culture medium (Fitzgerald & Payne, 1972) and in agreement with this no P1 enzyme band was detected in the present work on zymograms of extracts of cells grown to the exponential phase under identical conditions.

Table 1. *Resolution of alkylsulphatases by polyacrylamide-gel electrophoresis of extracts of Pseudomonas C12B grown in or maintained as resting cells in different media*

Bacteria were grown in the following culture media: A, nutrient broth; B, nutrient broth containing 10mM-hexan-1-yl sulphate; C, basal salts medium containing sodium citrate (1%, w/v) and 10mM-hexan-1-yl sulphate; D, basal salts medium containing hexan-1-yl sulphate (1%, w/v); E, basal salts medium containing sodium citrate (1%, w/v) and Na_2SO_4 (1mM); F, as for E but containing hexan-1-yl sulphate (10mM); G, basal salts medium containing sodium citrate (1%, w/v), 10mM-hexan-1-yl sulphate and 1mM-hexan-1-ol; H, basal salts medium containing sodium citrate (1%, w/v), Na_2SO_4 (1mM) and hexan-1-ol (1mM). Resting cell suspensions were prepared from bacteria grown in nutrient broth to the exponential phase (see the text) and stationary phase and shaken for 4h at 30°C in basal salts medium lacking alkyl sulphate, conditions I and J respectively, and in the presence of 10mM-octan-1-yl sulphate, conditions I_1 and J_1 respectively. Presence or absence of enzyme bands on zymograms is denoted by + or − and no attempt has been made to assess intensity.

Growth conditions	Growth phase	Presence of alkylsulphatase bands on gel zymograms				
		P1	P2	S1	S2	S3
Growing cultures						
A	Exponential	−	−	+	+	−
A	Stationary	+	−	+	+	−
B	Exponential	+	−	+	+	−
B	Stationary	+	+	+	+	−
C	Stationary	−	+	−	−	−
D	Stationary	−	+	−	−	−
E	Stationary	−	−	−	−	−
F	Stationary	−	+	−	−	−
G	Stationary	−	+	−	−	−
H	Stationary	−	−	−	−	−
Resting suspensions						
I	Exponential	−	−	+	+	−
I_1	Exponential	+	+	+	+	−
J	Stationary	+	−	+	+	−
J_1	Stationary	+	+	+	+	−

When growth in the presence of nutrient broth containing hexan-1-yl sulphate was continued to the stationary phase (condition B stationary phase, Table 1), electrophoresis of extracts revealed the presence of an additional primary alkylsulphatase hydrolysing sodium dodecan-1-yl sulphate (the P2 enzyme band, Dodgson *et al.*, 1974). The formation of this additional primary alkylsulphatase by stationary phase bacteria may explain the biphasic culture age–activity pattern (determined *in vitro* by hydrolysis of hexan-1-yl sulphate) reported previously for the organism by Fitzgerald & Payne (1972). Only the sulphate esters of pentan-1-ol, hexan-1-ol and octan-1-ol of the same series of primary alkyl sulphates that was tested by those workers elicited the formation of this P2 enzyme, although only the results obtained with hexan-1-yl sulphate are recorded in Table 1.

Bacteria grown to the stationary phase in chemically defined media containing hexan-1-yl sulphate as either the sole sulphur or sole carbon and sulphur source formed one alkylsulphatase only as determined by gel electrophoresis. This was the primary alkylsulphatase designated P2 (conditions C and D, Table 1). Alkylsulphatase activity was not present if hexan-1-yl sulphate was omitted from the medium and replaced by sodium citrate and Na_2SO_4 as carbon and sulphur sources respectively (condition E), whereas the P2 enzyme was again present if hexan-1-yl sulphate was also included in that medium (condition F). There is evidence (Dodgson *et al.*, 1974) that this P2 enzyme is active towards hexan-1-yl sulphate as well as towards dodecan-1-yl sulphate and this is confirmed in the next section.

Gel electrophoresis of extracts of resting cells harvested at the exponential phase of growth and subsequently shaken in the absence of any alkyl sulphate ester (condition I, Table 1) showed the presence of the S1 and S2 enzymes and the absence of any primary alkylsulphatase. Similar extracts have previously been shown to be devoid of enzyme activity *in vitro* towards hexan-1-yl sulphate by Fitzgerald & Payne (1972). When such resting cells were shaken in the presence of 10mM-octan-1-yl sulphate (condition I_1, Table 1) extracts contained the P1 and P2 enzymes in addition to the two secondary alkylsulphatases.

When these experiments were repeated with resting cells harvested at the stationary phase of growth the S1, S2 and P1 enzymes were present in extracts of cells that had been shaken in the absence of any alkyl sulphate (condition J, Table 1). Visual examination of gel bands suggested that relatively low amounts of the P1 enzyme were present. The same three enzymes, together with the P2 enzyme, were present in extracts of similar resting cells that had been shaken with 10mM-octan-1-yl sulphate (condition J_1, Table 1). These results are in accord with the previous findings (Fitzgerald & Payne, 1972) that resting cells shaken with octan-1-yl sulphate gave extracts having high enzyme activity towards hexan-1-yl sulphate.

Influence of hexan-1-yl sulphate concentration on the formation of the P2 enzyme

Pseudomonas C12B was grown to the stationary phase in the presence of various concentrations of hexan-1-yl sulphate in the basal salts medium of Fitzgerald & Payne (1972), containing sodium citrate (1%, w/v) and Na_2SO_4 (1mM) as the principal sources of carbon and sulphur. Dialysed extracts of cells grown at concentrations of hexan-1-yl sulphate of 0.1mM or greater were capable of hydrolysing the ester (Table 2) and contained only the P2 enzyme as evidenced by gel electrophoresis (cf. condition F, Table 1). Maximum specific sulphatase activity was observed with cells that had been grown in the presence of 1mM-hexan-1-yl sulphate. These findings are consistent with those of Dodgson *et al.* (1974) that the P2 enzyme is active towards hexan-1-yl sulphate as well as towards dodecan-1-yl sulphate. Those authors also noted that induction of the formation of the secondary alkylsulphatase active towards pentan-3-yl sulphate (the S3 enzyme) by hexadecan-2-yl sulphate could only be achieved if either hexadecan-2-ol or tetradecan-2-ol was also present in the culture medium. The formation of the P2 enzyme revealed no analogous need for an alcohol, however. Table 3 shows that substitution of hexan-1-ol and Na_2SO_4 for hexan-1-yl sulphate in the culture medium gave cells that were devoid of sulphatase activity towards the latter compound (see also Table 1). When present in addition to hexan-1-yl sulphate, the alcohol had no

Table 2. *Influence of hexan-1-yl sulphate concentration on the formation of primary alkylsulphatase (P2 enzyme) by Pseudomonas C12B*

Bacteria were grown to the stationary phase in the presence of sodium hexan-1-yl sulphate in the basal salts medium containing sodium citrate (1%, w/v) and sodium sulphate (1mM) as the principal sources of carbon and sulphur. Primary alkylsulphatase activity of dialysed cell extracts was measured after incubation with 25mM-sodium hexan-1-yl sulphate at 30°C for 3min in the presence of 10mM-Tris–HCl buffer, pH7.5.

Concn. of hexan-1-yl sulphate (mM)	Primary alkylsulphatase activity (units)
0.01	0
0.10	6
0.25	31
0.50	46
0.75	82
1.00	131
5.00	128

significant effect on the amount of the P2 enzyme formed.

Metabolic fate of ^{35}S of hexan-1-yl [^{35}S]sulphate

The results presented in Table 1 show that hexan-1-yl sulphate served as an inducer for the P1 and P2 primary alkylsulphatases when *Pseudomonas* C12B was grown in nutrient broth containing that ester. The following experiments were designed to establish whether the sulphate ester itself served as an inducer or whether it had first to be metabolized in some way.

Pseudomonas C12B released large quantities of $^{35}SO_4^{2-}$ into the medium when hexan-1-yl [^{35}S]-sulphate was present but was not required for growth or cell survival. Thus after growth to the stationary phase in nutrient broth supplemented with the ^{35}S-labelled alkyl sulphate (condition A, Table 4) 56.2% of the radioactivity present in the medium could be accounted for as inorganic $^{35}SO_4^{2-}$. Under these growth conditions both the P1 and P2 primary alkylsulphatases are formed (Table 1). The only other ^{35}S-labelled component in the medium was unchanged hexan-1-yl [^{35}S]sulphate. Similar results were obtained with a resting suspension of cells harvested at exponential phase, which was shaken in the presence of the ^{35}S-labelled ester. Liberation of $^{35}SO_4^{2-}$ was apparent after 1h of shaking (Fig. 1).

When hexan-1-yl [^{35}S]sulphate was present as either the sole source of sulphur or as the sole source of carbon and sulphur a considerable proportion of the radioactivity of the medium was again accounted for as inorganic $^{35}SO_4^{2-}$ after growth to the stationary phase (conditions B and C, Table 4). Under these growth conditions only the P2 alkylsulphatase is

Table 3. *Influence of some effector compounds on the formation of primary alkylsulphatase (P2 enzyme) by Pseudomonas C12B*

Bacteria were grown to the stationary phase in the basal salts medium containing sodium citrate (1%, w/v) and the indicated effector compounds (1mM). Primary alkylsulphatase activity towards 25mM-sodium hexan-1-yl sulphate was determined. Incubation was at 30°C for 3min (60min in cases where no enzyme activity was detected) in the presence of 10mM-Tris–HCl buffer, pH 7.5.

Effector compound(s)	Primary alkylsulphatase activity (units)
Hexan-1-yl sulphate	118
Hexan-1-yl sulphate, hexan-1-ol	103
Hexan-1-yl sulphate, hexan-1-ol, Na_2SO_4	121
Hexan-1-ol, Na_2SO_4	0
Na_2SO_4	0

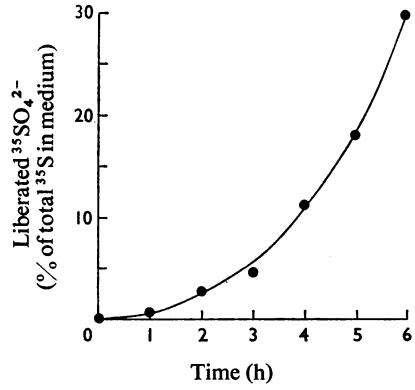

Fig. 1. *Time-course of liberation of $^{35}SO_4^{2-}$ from hexan-1-yl [^{35}S]sulphate by a resting cell suspension of Pseudomonas C12B*

Bacteria were grown in nutrient broth to the mid-exponential phase and, after washing, were shaken at 30°C in the basal salts medium containing 8mM-sodium hexan-1-yl sulphate and 0.55mCi of sodium hexan-1-yl [^{35}S]sulphate. See the text for further details.

Table 4. *Occurrence of $^{35}SO_4^{2-}$ and choline O-[^{35}S]sulphate in the culture medium after growth of Pseudomonas C12B in the presence of sodium hexan-1-yl [^{35}S]sulphate under various culture conditions*

Bacteria were grown to the stationary phase in: A, nutrient broth supplemented with 8mM-hexan-1-yl sulphate; B, basal salts medium containing sodium citrate (1%, w/v) as carbon source and 8mM-hexan-1-yl sulphate as the sole source of sulphur; C, basal salts medium containing hexan-1-yl sulphate (1%, w/v) as the sole source of carbon and sulphur. Each medium also contained 0.55mCi of hexan-1-yl [^{35}S]sulphate.

Culture condition	Recovery of ^{35}S (%)		
	As $^{35}SO_4^{2-}$	As choline O-[^{35}S]sulphate	As hexan-1-yl [^{35}S]sulphate
A	56.2	0.0	43.8
B	38.7	4.5	56.8
C	38.2	2.3	59.5

Table 5. *Occurrence of $^{35}SO_4^{2-}$, choline O-[^{35}S]sulphate and ^{35}S-labelled metabolites in extracts of Pseudomonas C12B*

Culture conditions A, B and C were as given in Table 4. Cells were also grown in nutrient broth, washed and suspended in basal salts medium containing 8 mM-hexan-1-yl sulphate and 0.55 mCi of hexan-1-yl [^{35}S]sulphate and shaken at 30°C for 6 h (resting cell suspension, condition D). Extracts of bacteria were prepared as described in the text. Metabolites I and II were not detected in cold-water extracts.

Culture conditions	Type of extract	10^{-3} × Radioactivity (c.p.m.) Total	$^{35}SO_4^{2-}$	Choline *O*-[^{35}S]sulphate	Hexan-1-yl [^{35}S]sulphate	Metabolite I	Metabolite II
A	Cold water	300	240	14.7	45.3	0	0
B		152	112	28.3	11.7	0	0
C		132	63.2	0	68.8	0	0
D		469	430	0	39.0	0	0
A	Trichloroacetate	34.3	14.1	0	1.0	19.2	0
B		8.6	0	0	0	0	8.6
C		4.1	0	0	0	4.1	0
D		121.0	50.6	0	70.4	0	0

formed. Apart from unchanged hexan-1-yl [^{35}S]-sulphate, only one other ^{35}S-labelled component could be detected in the medium and this was identified as choline *O*-[^{35}S]sulphate. *Pseudomonas* C12B is known to be able to form this compound from inorganic $^{35}SO_4^{2-}$ (Fitzgerald, 1973).

The distribution of ^{35}S in cold-water extracts and trichloroacetate extracts of cells grown under different conditions is shown in Table 5. Cold-water extracts of cells grown under conditions A and B contained inorganic $^{35}SO_4^{2-}$, unchanged hexan-1-yl [^{35}S]-sulphate and very small amounts of choline *O*-[^{35}S]-sulphate (see Fitzgerald, 1973). Similar results were obtained with cells grown under conditions C and D except that no choline *O*-[^{35}S]sulphate was detected.

Trichloroacetate extracts contained only very small amounts of radioactivity (Table 5) but such extracts from cells grown under conditions A and C contained a ^{35}S-labelled component (metabolite I, electrophoretic mobility in the sodium acetate–acetic acid buffer relative to $^{35}SO_4^{2-}$ was 0.52). Similar extracts from cells grown under condition B contained a different ^{35}S-labelled component (metabolite II, mobility relative to $^{35}SO_4^{2-}$ was 0.33). Both metabolites I and II were present in traces only and no attempts were made to identify them. It is therefore not possible to say whether they are sulphate esters derived directly or indirectly from hexan-1-yl [^{35}S]-sulphate or whether they are other ^{35}S-containing compounds derived from inorganic $^{35}SO_4^{2-}$.

Liberation of $^{35}SO_4^{2-}$ from hexan-1-yl [^{35}S]sulphate by cell extracts

Undialysed and dialysed extracts prepared from *Pseudomonas* C12B grown with hexan-1-yl sulphate as either the sole sulphur source or the sole carbon and sulphur source (Table 6, conditions B and C respectively, P2 alkylsulphatase only is formed) released $^{35}SO_4^{2-}$ as the only detectable ^{35}S-labelled product after incubation with hexan-1-yl [^{35}S]-sulphate. Similar findings were made with dialysed extracts that had been fortified with 1 mM-$MgCl_2$ and cofactors known to be required for the enzymic hydroxylation and oxidation of *n*-alkanes. It therefore seems unlikely that the extracts contain a cofactor-dependent, hydrocarbon-degrading enzyme system which might first modify the primary alkyl sulphate ester before primary alkylsulphatase induction can occur. The presence of the cofactors listed in Table 6 always resulted in a marked decrease in desulphating activity from that obtained with dialysed extracts containing no added cofactors.

Table 6. *Influence of some cofactors on the liberation of $^{35}SO_4^{2-}$ from hexan-1-yl [^{35}S]sulphate by extracts of Pseudomonas C12B*

Culture conditions B and C were as described in Table 4. Primary alkylsulphatase activity (P2 enzyme) was assayed with hexan-1-yl [^{35}S]sulphate as described in the Materials and Methods section. Cofactors were added at a concentration of 1 mM. The protein concentration of each extract was approx. 8 mg/ml.

Cofactors added	$^{35}SO_4^{2-}$ released (% of total ^{35}S in incubation mixture) Culture condition B	Culture condition C
None	74.6	67.4
NAD^+	38.8	43.0
$NADP^+$	35.9	39.1
NADH	43.2	35.3
NADPH	37.3	37.6
All	29.5	41.3

Discussion

The present work has confirmed the findings of Fitzgerald & Payne (1972) that the formation of primary alkylsulphatase in *Pseudomonas* C12B can be induced by hexan-1-yl sulphate. When the sulphate ester is present as part of a chemically defined growth medium the enzyme formed is that designated P2 by Dodgson *et al.* (1974). Apart from hexan-1-yl sulphate only the sulphate esters of pentan-1-ol, hexan-1-ol and octan-1-ol were effective as inducers of the formation of this enzyme. This relatively close inducer specificity so far as primary alkyl sulphate esters are concerned appears to contrast sharply with the wide inducer specificity for the P2 enzyme shown by secondary alkyl sulphate esters (Dodgson *et al.*, 1974).

Under those growth conditions where the P2 enzyme is the only alkylsulphatase formed, the collective evidence from studies with hexan-1-yl [^{35}S]-sulphate suggests that the sulphate ester itself serves as the inducer. Apart from hydrolysis and formation of small amounts of choline *O*-[^{35}S]sulphate from liberated inorganic $^{35}SO_4^{2-}$ (see Fitzgerald, 1973), very little metabolism of the labelled ester apparently occurs. This was a finding that was not entirely expected since a number of micro-organisms, including pseudomonads, are known to be capable of ω-oxidation of alkanes (Klug & Markovetz, 1971), and Denner *et al.* (1969), Burke (1972) and Ottery *et al.* (1970) have demonstrated that rats are capable of the ω-oxidation of primary alkylsulphate esters without loss of the ester sulphate group.

The question of the general significance of the trace quantities of metabolites I and II detected in trichloroacetate extracts of cells still remains unanswered but it seems reasonably certain that neither is directly concerned with the induction of the P2 primary alkylsulphatase. Thus both growth conditions B and C (see Table 4) produce only the P2 enzyme yet metabolite I was present in extracts of cells grown under condition C but not B, whereas metabolite II showed the converse distribution. Neither metabolite was apparently produced by resting cell suspensions that were shaken with hexan-1-yl [^{35}S]sulphate (condition D, Table 5) although such cells produced both the P1 and P2 enzymes.

For the P1 primary alkylsulphatase the inducer situation is somewhat complicated by the fact that the enzyme appears in detectable amounts when *Pseudomonas* C12B is grown to the stationary phase in nutrient broth alone. At present no method has yet been found that will allow the formation of this enzyme when the organism is grown in chemically defined media and much work remains to be done before the formation of this particular enzyme is fully understood. However, hexan-1-yl sulphate and other related sulphate esters can participate in some way in the induction of the formation of this enzyme and, once again, it appears that metabolism of the ester is not a prerequisite for the induction effect.

The results of the present study lend support to the earlier suggestions of Payne *et al.* (1965) that induction of the formation of primary alkylsulphatase in *Pseudomonas* C12B by sodium dodecan-1-yl sulphate probably is a feature of the intact ester.

We thank Mrs. Marion Andreesen and Mrs. Vivian Bergh for skilled technical assistance. The work was supported by the National Science Foundation Grant no. GU.2590.

References

Burke, B. (1972) Ph.D. Thesis, University of Wales
Denner, W. H. B., Olavesen, A. H., Powell, G. M. & Dodgson, K. S. (1969) *Biochem. J.* **111**, 43–51
Dodgson, K. S. (1961) *Biochem. J.* **78**, 312–319
Dodgson, K. S., Lloyd, A. G. & Tudball, N. (1961) *Biochem. J.* **79**, 111–117
Dodgson, K. S., Fitzgerald, J. W. & Payne, W. J. (1974) *Biochem. J.* **138**, 53–62
Fitzgerald, J. W. (1973) *Biochem. J.* **136**, 361–369
Fitzgerald, J. W. & Dodgson, K. S. (1970) *Biochem. J.* **119**, 30P
Fitzgerald, J. W. & Dodgson, K. S. (1971*a*) *Biochem. J.* **121**, 521–530
Fitzgerald, J. W. & Dodgson, K. S. (1971*b*) *Biochem. J.* **122**, 277–283
Fitzgerald, J. W. & Payne, W. J. (1972) *Microbios* **5**, 87–100
Harada, T. (1964) *Biochim. Biophys. Acta* **81**, 193–196
Klug, M. J. & Markovetz, A. J. (1971) *Advan. Microbial Physiol.* **5**, 1–43
Lloyd, A. G., Tudball, N. & Dodgson, K. S. (1961) *Biochim. Biophys. Acta* **52**, 413–419
Lloyd, A. G., Large, P. J., Davies, M., Olavesen, A. H. & Dodgson, K. S. (1968) *Biochem. J.* **108**, 393–399
Lucas, J. J., Burchiel, S. W. & Segal, I. H. (1972) *Arch. Biochem. Biophys.* **153**, 664–672
Ottery, J., Olavesen, A. H. & Dodgson, K. S. (1970) *Life Sci.* **9**, 1335–1340
Payne, W. J. & Painter, B. G. (1971) *Microbios* **3**, 199–206
Payne, W. J., Williams, J. P. & Mayberry, W. R. (1965) *Appl. Microbiol.* **13**, 698–701
Phibbs, P. V. & Eagon, R. G. (1970) *Arch. Biochem. Biophys.* **138**, 470–482
Scott, J. M. & Spencer, B. (1968) *Biochem. J.* **106**, 471–477
Takebe, I. (1961) *J. Biochem.* (*Tokyo*) **50**, 245–255