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Specificity of P2 Primary Alkylsulphohydrolase Induction in the Detergent-Degrading Bacterium *Pseudomonas* C12B

EFFECTS OF ALKANESULPHONATES, ALKYL SULPHATES AND OTHER RELATED COMPOUNDS

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Primary alkanesulphonates were shown to serve as non-metabolizable (gratuitous) inducers of the P2 primary alkylsulphohydrolase enzyme in resting cell suspensions of *Pseudomonas* C12B. The effects of increasing concentrations of inducer on the production of enzyme were complex and suggestive of a multiphasic phenomenon. However, it was possible to determine $K_{inducer}$ constants (analogous to K_m or K_i) for alkanesulphonates of chain length from C_7 to C_{12}. These decreased with increasing chain length in a manner characteristic of an homologous series. Primary alkyl sulphates also served as good inducers of alkylsulphohydrolase, but valid kinetic values could not be obtained because these esters are good substrates for the enzyme and are therefore appreciably hydrolysed during the induction period. Small amounts of enzyme were also produced when cyprinol sulphate, dodecyltriethoxy sulphate ($C_{12}H_{23}$–[O–CH_2–CH_2]$_3$–O–$SO_3^-Na^+$), Crag herbicide and some secondary alkyl sulphates were tested as inducers.

The first detailed reports on the occurrence and inducibility of primary alkylsulphohydrolase were made by Payne and his colleagues working with a bacterium (designated *Pseudomonas* C12B) isolated from detergent-enriched soil (Payne & Feisal, 1963; Williams & Payne, 1964; Payne *et al.*, 1965). Subsequent work (Payne & Painter, 1971; Fitzgerald *et al.*, 1974) revealed that two forms of the enzyme, distinguishable by electrophoresis, were synthesized when the bacterium was grown in enriched media containing a primary alkyl sulphate ester. These two forms were designated as P1 and P2 by Dodgson *et al.* (1974).

Although some studies on the mechanisms regulating the induction of primary alkylsulphohydrolase in this and related bacteria have been made (Fitzgerald & Payne, 1972*b*; Fitzgerald & Kight, 1977; Kight-Olliff & Fitzgerald, 1978), little attention has been paid to the precise specificity of the induction process. Earlier work revealed that sodium dodecyl sulphate could serve as an inducer (Williams & Payne, 1964), and Fitzgerald & Payne (1972*a*) tested a number of other primary alkyl sulphates and found the highest activities were obtained after exposure of the bacterium to hexyl sulphate or octyl sulphate. Induction depends on the presence of the intact ester, for, although bacteria exhibiting P2 primary alkylsulphohydrolase activity were able to hydrolyse hexyl sulphate (Fitzgerald, 1974), the hydrolysis products did not act as inducers, and induction occurred without prior metabolism of the carbon moiety of the intact ester (Fitzgerald *et al.*, 1974). Despite these findings, the lack of non-metabolizable (gratuitous) inducers has imposed a limitation on interpretations that could be made with regard to inducer specificity and effectiveness (see Fitzgerald & Payne, 1972*a*).

In the present study, factors influencing the induction of the P2 primary alkylsulphohydrolase were investigated in cells previously depleted of the constitutive S1 and S2 secondary alkylsulphohydrolases (Fitzgerald & Laslie, 1974, 1975) and harvested at a point in the culture cycle at which the P1 primary alkylsulphohydrolase was either absent or present in traces only. The use of a series of alkanesulphonates as potential inducers enabled a more reliable interpretation of results relating to the length of the alkyl chain and inducer activity, as these alkyl sulphate analogues were not metabolized by *Pseudomonas* C12B under the experimental conditions employed.

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Materials and Methods

Sulphate esters and sulphonates

Unless otherwise indicated, primary and secondary alkyl sulphate esters were prepared by the method of Dodgson *et al.* (1974). The sulphate esters of heptan-4-ol, nonan-5-ol, D- and L-octan-2-ols and DL-decan-2-ol (racemic mixture) were prepared as potassium salts by the pyridine/SO_3 procedure of Matcham & Dodgson (1977). Sodium salts of cyprinol sulphate and dodecyltriethoxy sulphate ($C_{12}H_{23}$–[O–CH_2–CH_2]$_3$–O–$SO_3^-Na^+$) were gifts from Professor G. A. D. Haslewood and Dr. D. Howes respectively. Crag herbicide [sodium 2-(2,4-dichlorophenoxy)ethyl sulphate] was obtained from Union Carbide and Carbon Corp., New York, NY, U.S.A. All primary alkanesulphonates were purchased as sodium salts from Cambrian Chemicals, Croydon, Surrey, U.K., except for hexanesulphonate and heptanesulphonate, which came from Eastman-Kodak, Rochester, NY, U.S.A., and K and K Laboratories, Plainview, NY, U.S.A., respectively. Sodium [1-^{14}C]undecanesulphonate (approx. specific radioactivity 7.2μCi/mg) was a gift from Dr. A. H. Olavesen of this Department.

Preparation and use of resting cell suspensions for inducer specificity studies

Pseudomonas C12B was grown with shaking at 30°C in 1-litre Erlenmeyer flasks containing nutrient broth (Difco Laboratories, Detroit, MI, U.S.A.) (400ml/flask), supplemented with 2.5% (w/v) trisodium citrate (final pH of medium was 7.45). Under these conditions the cells lost about 90% of their complement of the constitutive S1 and S2 secondary alkylsulphohydrolases (see Fitzgerald & Laslie, 1974, 1975). Other procedures associated with growing and maintaining this bacterium have been described (Fitzgerald *et al.*, 1974). Because the P1 primary alkylsulphohydrolase is synthesized principally by stationary-phase cultures of cells grown in nutrient broth (Fitzgerald & Payne, 1972*a*; Fitzgerald *et al.*, 1974), cultures were pooled and cells from 200ml portions were harvested when cultures reached an A_{650} spectrophotometric reading of between 0.4 and 0.5 (after about 26h), so as to minimize the occurrence of this enzyme in resting cells (see Fitzgerald *et al.*, 1974).

Each cell pellet was washed once by resuspension in 20ml of a buffered (pH7.0) basal salts medium containing KH_2PO_4 (1.5g), K_2HPO_4 (3.5g), NH_4Cl (0.5g), NaCl (0.5g) and $MgCl_2$,6H_2O (0.15g) per litre of water. The washed cells were subsequently resuspended in 10ml of the same basal salts medium containing potential inducer at concentrations specified in the text. The suspensions were then shaken at 30°C for 5h. Control determinations revealed that no increase in A_{420} of cell suspensions occurred during the shaking period. Other controls in which cells were shaken in the absence of added inducer enabled an assessment to be made of any primary alkylsulphohydrolase activity synthesized under those circumstances. Zymography (see below) was used to confirm that only the P2 enzyme was produced during the induction period. Finally, in order to correct for the variation in specific activities of the P2 enzyme that were produced under standard conditions in different experiments, a cell suspension was always shaken with 4mM-potassium hexyl sulphate in order to provide a standard control to which the results obtained with other inducers could be quantitatively related.

Preparation, dialysis and assay of cell extracts

At time intervals specified in the text, the induction of P2 alkylsulphohydrolase synthesis was terminated by the rapid freezing of each cell suspension. Cell extracts were subsequently prepared by passing the thawed suspensions (three times) through a chilled French pressure cell. Extracts were clarified by centrifuging at 25000$g_{av.}$ and 4°C on an MSE High Speed 18 centrifuge for 1h, and each supernatant was dialysed for 24h (when an alkyl sulphate was present as inducer) or for 48h (when an alkanesulphonate was present) against several changes of 0.01M-Tris/HCl buffer, pH8.3.

Assay of sulphohydrolase activity and protein. Dialysed extracts were assayed for primary alkylsulphohydrolase activity by the procedure of Dodgson *et al.* (1974). Potassium hexyl sulphate was used as assay substrate at a final concentration of 18mM in the presence of 0.1M-Tris/HCl buffer, pH8.3. Incubation was at 30°C. These conditions are optimal for the P2 primary alkylsulphohydrolase (Cloves *et al.*, 1980). A unit of enzyme activity is defined as the amount required to release 1μmol of SO_4^{2-}/h, and specific activity as units/mg of protein. Protein was determined by the method of Lowry *et al.* (1951), with bovine serum albumin (fraction V) as standard.

Zymography. Cell extracts were routinely examined for the presence of the P1 and P2 primary and the S1, S2 and S3 secondary alkylsulphohydrolase enzymes by the polyacrylamide-gel zymogram technique of Payne & Painter (1971) as modified by Payne *et al.* (1974).

Determination of critical micelle concentrations

The minimum concentration at which micelles are found (critical micelle concentration) was determined for a series of primary alkyl sulphates and alkanesulphonates by measuring the change in the absorption spectrum of Rhodamine 6G in the presence of each compound, by the procedure of Carey & Small (1969). Determinations were made (in the absence of

bacteria) at 30°C on appropriate concentrations of each compound (see Table 1) dissolved in the basal salts medium containing 1.25μM-Rhodamine 6G (BDH Chemicals, Poole, Dorset, U.K.). Preliminary determinations revealed that primary alkyl sulphates and alkanesulphonates of carbon chain length less than C_9 and C_{10} respectively did not form micelles at concentrations up to 20mM. The critical micelle concentrations for compounds of greater chain length (up to C_{14}) are listed in Table 1. The reliability of these data was assessed by plotting the logarithm of the critical micelle concentration for each compound against carbon chain length, as suggested by Shinoda *et al.* (1963). Linear relationships, characteristic of homologous series, were obtained from both the primary alkyl sulphate series and the alkanesulphonate series (results not shown).

Results

Preliminary studies of inducer specificity

In preliminary studies, maximum yields of the P2 alkylsulphohydrolase were detected when resting cells of *Pseudomonas* C12B were shaken at 30°C for 5h in the basal salts medium containing 4mM-potassium hexyl sulphate [the sulphate ester previously used to induce the synthesis of this enzyme (see Dodgson *et al.*, 1974; Fitzgerald *et al.*, 1974; Fitzgerald, 1974)]. Subsequently, resting suspensions were shaken for 5h at 30°C with 4mM concentrations of C_2–C_{14} primary alkyl sulphates, C_4–C_{14} secondary 2-yl alkyl sulphates and C_4–C_{12} primary alkanesulphonates. The results of these experiments (Fig. 1) showed that the induction of this enzyme was dependent on both the nature and the position of the sulphur-containing moiety. Thus maximum specific activities of the P2 enzyme were detected in cells after prior exposure to the primary alkyl sulphates, and trace quantities only of the enzyme were synthesized in response to detergents in which the ester sulphate group is located at C-2. Fig. 1 also shows that compounds in which the C–O–SO_3 structure is replaced by the C–SO_3 structure (the primary alkanesulphonates) could also serve as inducers. The specificity for the induction of the P2 enzyme is thus qualitatively similar to that previously noted for the synthesis of primary alkylsulphohydrolase by *Pseudomonas aeruginosa* (Fitzgerald & Kight, 1977). However, the marked inability of the C_{10} and C_{11} primary alkyl sulphate esters to serve as inducers in *Pseudomonas* C12B (Fig. 1) was unexpected and suggested that the ability (or inability) of a particular detergent to form micelles at a concentration of 4mM might be important in this system. This does not appear to be the case, as undecyl sulphate (at a concentration of 4mM), but not decyl sulphate, will be present as micelles (Table 1), yet neither ester served as an inducer at that concentration. Furthermore, maximum enzyme specific activities were found in cells exposed to dodecyl sulphate at a concentration sufficient to ensure that almost the whole of the detergent would be present in micellar form.

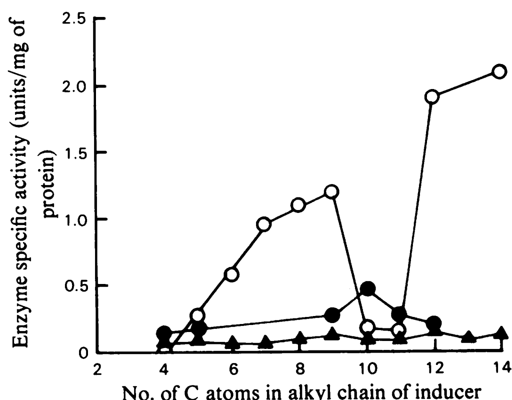

Fig. 1. *Relationship between carbon chain length of potential inducer and the synthesis of the P2 alkylsulphohydrolase by Pseudomonas C12B*

Resting-cell suspensions were exposed to 4mM concentrations of inducer as described in the text. ○, Primary alkyl sulphates; ●, alkanesulphonates; ▲, secondary 2-yl alkyl sulphates.

Table 1. *Critical micelle concentrations for some primary alkyl sulphates and alkanesulphonates in the presence of basal salts medium*

Experimental details are given in the text. Alkyl sulphates were present as potassium salts and alkanesulphonates as sodium salts.

Compound	Critical micelle concentration (mM)
Nonyl sulphate	10.50
Decyl sulphate	6.16
Undecyl sulphate	2.20
Dodecyl sulphate	0.47
Tetradecyl sulphate	0.10
Decanesulphonate	9.80
Undecanesulphonate	3.07
Dodecanesulphonate	1.06
Tridecanesulphonate	0.41
Tetradecanesulphonate	0.20

Induction by primary alkyl sulphates at optimum concentrations

The failure of 4mM concentrations of C_{10} and C_{11} primary alkyl sulphates to induce the synthesis of the P2 alkylsulphohydrolase was investigated further by shaking resting cells for 5h at 30°C with different

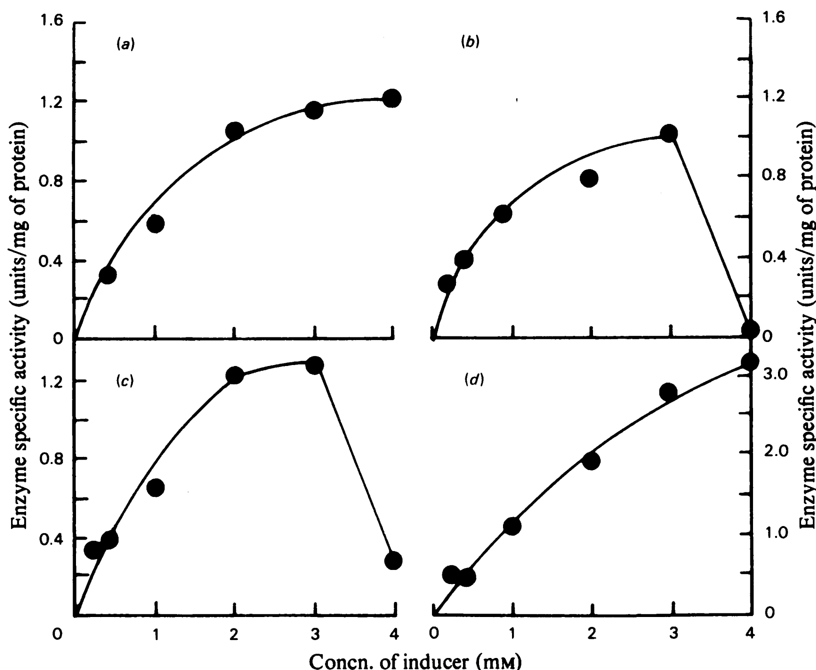

Fig. 2. *Influence of primary alkyl sulphate concentration on the synthesis of the P2 primary alkylsulphohydrolase by Pseudomonas C12B*

Experimental details are given in the text. Results are expressed in relation to that obtained with 4mM-potassium hexyl sulphate. (*a*) Nonyl sulphate; (*b*) decyl sulphate; (*c*) undecyl sulphate; (*d*) dodecyl sulphate.

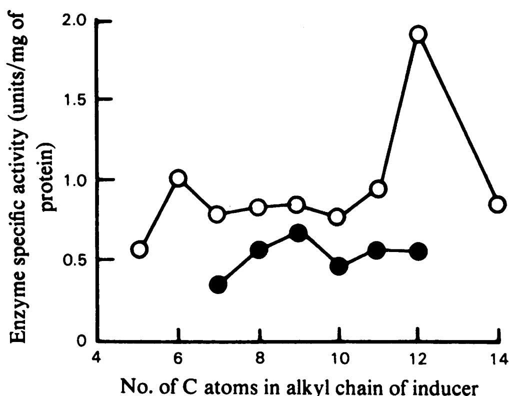

Fig. 3. *Relationship between carbon chain length and the synthesis of the P2 alkylsulphohydrolase after exposure of Pseudomonas C12B to optimum concentrations of inducer*

Experimental details are given in the text. ○, Primary alkyl sulphates; ●, alkanesulphonates.

concentrations of these and other primary alkyl sulphates. Fig. 2 shows that, in contrast with the results obtained for the C_9 and C_{12} esters, maximum induction of the enzyme occurred at 3mM concentrations of the C_{10} and C_{11} alkyl sulphates. Moreover, a pronounced inhibition of induction resulted when the concentration of the latter esters was increased to 4mM. Although no explanation of this effect is yet available, no inhibition of induction by excess inducer was observed with the C_9 or the C_{12} esters (Fig. 2) or when the sulphate esters of pentanol, hexanol, heptanol, octanol or tetradecanol were employed at concentrations up to 4mM (results not shown). Further, in separate experiments in which resting cells were exposed for 5h to optimum concentrations of each primary alkyl sulphate, a plot of P2 alkylsulphohydrolase activity against carbon chain length (Fig. 3) now gave a single peak of activity only (cf. Fig. 1), indicating that maximum synthesis of enzyme occurred with the C_{12} detergent dodecyl sulphate.

Induction by primary alkanesulphonates at optimum concentrations

The possibility that inhibition of induction of the P2 enzyme might also occur when primary alkanesulphonates were present in excess was examined by shaking resting-cell suspensions as before with various concentrations of these compounds. With two exceptions (hexanesulphonate and heptane

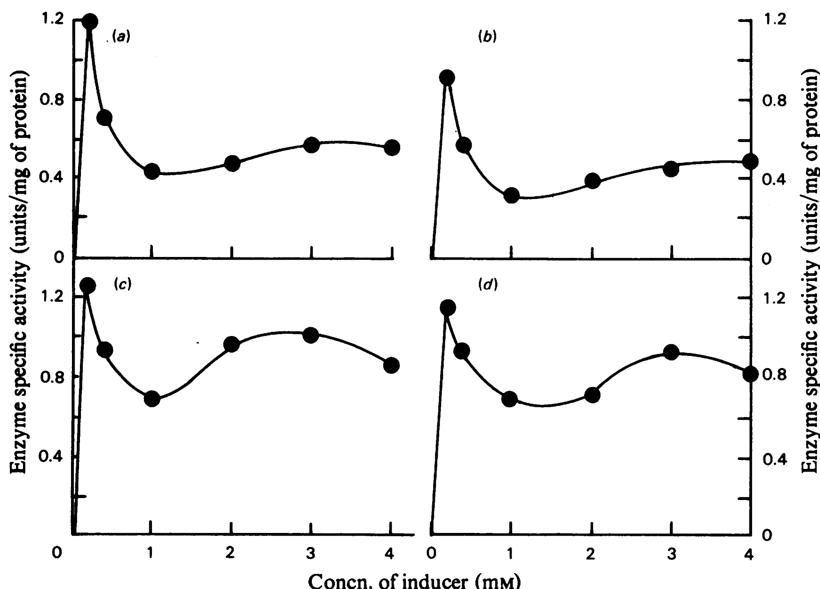

Fig. 4. *Examples of the influence of alkanesulphonate concentration* (0–4 mM) *on the synthesis of the P2 primary alkylsulphohydrolase by Pseudomonas C12B*

Experimental details are given in the text. Results are expressed in relation to that obtained with 4 mM-potassium hexyl sulphate. (*a*) Nonanesulphonate; (*b*) decanesulphonate; (*c*) undecanesulphonate; (*d*) dodecanesulphonate.

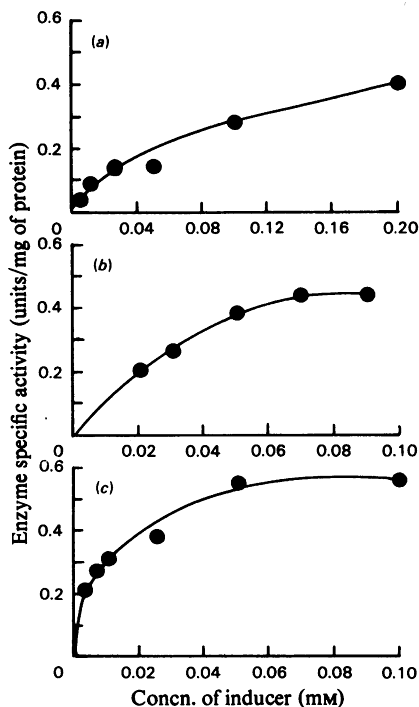

Fig. 5. *Examples of the influence of alkanesulphonate concentration* (0–0.2 mM) *on the synthesis of the P2 primary alkylsulphohydrolase by Pseudomonas C12B*

Experimental details are given in the text. Results are expressed in relation to that obtained with 4 mM-potassium hexyl sulphate. (*a*) Heptanesulphonate; (*b*) octanesulphonate; (*c*) nonanesulphonate.

sulphonate) a pronounced inhibitory effect was noted when concentrations exceeded 0.2 mM, and this was generally followed by some restoration of induction as the concentration was increased further (see Fig. 4 for some examples). In these particular studies it proved impossible to study the effects of alkanesulphonates of chain length greater than C_{12} because even prolonged dialysis of the cell extracts after enzyme induction failed to remove all the detergent. Because alkanesulphonates serve as competitive inhibitors for the P2 alkylsulphohydrolase (Cloves *et al.*, 1980), particular care had to be taken that such inhibition was not complicating the experimental observations. This was established for compounds of chain length up to C_{12} by assaying a standard sample of the P2 enzyme in the presence of either a boiled sample of the extract of the cells exposed to inducer

or of 0.01 M-Tris/HCl buffer. These controls established that dialysis of extracts for 48 h successfully decreased the concentrations of C_6–C_{12} alkanesulphonates to non-inhibitory values.

The plots of enzyme induction against inducer concentration (Fig. 4) suggested that two distinct Michaelis–Menten-type relationships might be operating, the first of these (at low inducer concentrations) being subjected to inhibition by excess inducer. Accordingly, the induction experiments with the alkanesulphonates were repeated with much lower inducer concentrations (0–0.2 mM) than before. In all cases a relationship was observed between enzyme production and inducer concentration that was analogous to the Michaelis–Menten relationship between enzyme activity and substrate concentration (see Fig. 5 for some examples). Double-reciprocal plots allowed an apparent $K_{inducer}$ value (analogous to K_m) to be calculated for each inducer, and these values decreased as the inducer chain length increased. An approximately linear relationship was observed when the logarithms of the values obtained were plotted against the numbers of carbons in the alkyl chains, behaviour characteristic of a homologous series (Fig. 6).

In a separate experiment, pooled resting cells were exposed to optimum concentrations of each alkanesulphonate, as deduced from the data shown (for some examples) in Fig. 5. Induction (assessed in terms of specific activity) was maximal at a chain length of C_9 (Fig. 3).

Metabolic instability of primary alkyl sulphates

It did not escape notice that the primary alkyl sulphate esters, as well as serving as inducers for the P2 enzyme, were also substrates for this enzyme (Cloves *et al.*, 1980). Moreover, previous work (Fitzgerald *et al.*, 1974) had shown that, when resting suspensions of *Pseudomonas* C12B were shaken for 6 h with 8 mM-potassium hexyl [^{35}S]sulphate, about 30% of the label was released into the medium as $^{35}SO_4^{2-}$. In the present study, potassium octyl sulphate [an effective inducer of the P2 enzyme (Fig. 1) and a better enzyme substrate than hexyl sulphate (Cloves *et al.*, 1980)] was readily hydrolysed during exposure to resting cells for 5 h. Hydrolysis was complete at initial concentrations of 0.2 and 0.4 mM and then steadily decreased as the concentration was further increased, until 64% of the ester had been hydrolysed when the initial concentration was 4 mM. The requirement for relatively high concentrations (3–4 mM) of this and other alkyl sulphate esters for maximal induction (e.g. Fig. 2) may therefore probably be related (at least to some degree) to the loss of the inducer through hydrolysis.

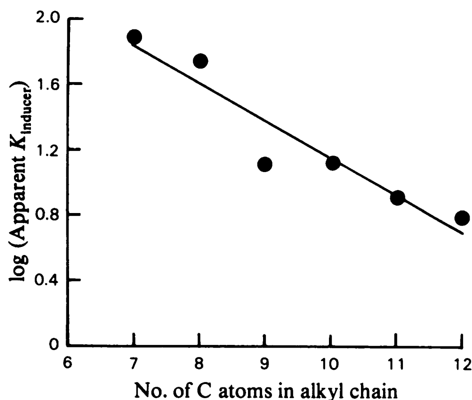

Fig. 6. *Semi-logarithmic relationship between the carbon chain length and the apparent $K_{inducer}$ values for alkanesulphonates*

Metabolic stability of primary alkanesulphonates

The observation that full induction of the P2 enzyme occurred, in most instances, at sulphonate concentrations below 0.2 mM (Fig. 5) suggested that these compounds might not be metabolized by resting cells of *Pseudomonas* C12B and would thus be more satisfactory effectors for induction studies. However, a previous report (Fitzgerald *et al.*, 1977) that actively growing cultures of this bacterium could utilize octanesulphonate as a sulphur source made it essential to determine whether resting cells of *Pseudomonas* C12B could modify alkanesulphonates in any way. This possibility was examined by shaking resting cell suspensions at 30°C in basal salts medium containing [1-^{14}C]undecanesulphonate (1.44 μCi in 20 μl) and unlabelled undecanesulphonate (at concentrations of 0.1, 1.0 and 3.0 mM). After 5 h the cells were collected by centrifuging and the supernatants were kept at −10°C until required. Cells were resuspended in the basal salts medium and ruptured. After removal of cell debris by centrifuging, samples of the clear cell extracts and the original supernatants were subjected to t.l.c. in chloroform/methanol/water (65:25:4, by vol.) by the procedure used by Taylor (1975) to separate mammalian metabolites of this alkanesulphonate. A single radioactive component was detected (radioautography on Industrex D X-ray film; Kodak, London, U.K.) on chromatograms of each cell extract and supernatant, irrespective of the time of exposure to the film. The mobility of the component was identical with that of authentic undecanesulphonate and its intensity on radioautograms was considerably increased if [1-^{14}C]-undecanesulphonate was added to each cell extract and supernatant before chromatography.

The relative distribution of radioactivity between cell extracts and supernatants was assessed by counting the radioactivities of suitable portions with a Packard Tri-Carb liquid-scintillation spectrometer,

Table 2. *Influence of various compounds on the synthesis of the P2 primary alkylsulphohydrolase*

Experimental details are given in the text. All results are expressed relative to that obtained for hexyl sulphate, which is taken as 1.0.

Effector	Concentration (mM)	P2 alkylsulphohydrolase activity (units)
Hexyl sulphate	4	1.0
D-Octan-2-yl sulphate	1	0.079
	4	0
L-Octan-2-yl sulphate	1	0.047
	4	0
DL-Decan-2-yl sulphate	1	0
	4	0
Heptan-4-yl sulphate	1	0
	4	0.165
Nonan-5-yl sulphate	1	0.131
	4	0.289
Octanol	4	0
Octanol plus SO_4^{2-}	4	0
Decanol	4	0
Decanol plus SO_4^{2-}	4	0
Dodecanol	4	0
Dodecanol plus SO_4^{2-}	4	0
Sodium octanoate	1	0.057
	4	0.037
Sodium stearate	1	0.061
	4	0
Cyprinol sulphate	1	0.013
	4	0.267
Crag herbicide	3	0.353
Dodecyltriethoxy sulphate	3	0.270

with a scintillation fluid containing 2.5g of 2,5-diphenyloxazole/litre of toluene/2-methoxyethanol (4:1, v/v). Further counts of radioactivity were made after the addition of an internal standard of [1-^{14}C]undecanesulphonate. In all cases complete recoveries of radioactivity were obtained (approx. 90% in the supernatants and 10% in the cell extracts), establishing that no ^{14}C had been lost as $^{14}CO_2$ as a result of the metabolism of the detergent.

Induction of the P2 primary alkylsulphohydrolase by other compounds

The finding that primary alkanesulphonates induced the synthesis of an enzyme that hydrolyses primary alkyl sulphates suggested that other compounds with related structural features might serve as inducers of the enzyme. Table 2 presents the results of experiments with other potential inducers. The fatty acids sodium octanoate and sodium stearate both served as weak inducers, but no such activity was seen with any of the primary or secondary alcohols tested, either in the presence or absence of inorganic sulphate. With the exception of DL-decan-2-yl sulphate, the secondary alkyl sulphates tested showed weak inducer activity. Cyprinol sulphate (the bile alcohol sulphate produced by several species of fish), Crag herbicide (see Vlitos, 1953) and the detergent sodium dodecyltriethoxy sulphate also served as inducers of the P2 enzyme. All three compounds possess a primary alkyl sulphate ester group. In further experiments with Crag herbicide and dodecyltriethoxy sulphate a Michaelis–Menten type of relationship was observed between the amount of enzyme produced and the concentration of inducer. Inhibition of induction by excess inducer occurred in both cases. It seems probable that a further search would reveal other compounds capable of inducing the P2 primary alkylsulphohydrolase.

Discussion

The present work establishes that the induction of the P2 primary alkylsulphohydrolase of *Pseudomonas* C12B is not restricted to simple primary alkyl sulphates. Surprisingly, however, the earlier findings (Dodgson *et al.*, 1974) that secondary alkyl sulphates caused good induction of the enzyme when present during growth of the organism on nutrient broth did not find a parallel in the present experiments with resting-cell suspensions. Only small amounts of P2 enzyme activity could generally be detected after shaking such suspensions with various secondary alkyl sulphates. Furthermore, it is unlikely that failure to induce the enzyme in quantity could be attributed to inhibition with excess inducer (as noted with the alkanesulphonates and some primary alkyl sulphates), since both D- and L-forms of octan-2-yl sulphate and DL-decan-2-yl sulphate caused little or no induction when present at 1mM concentrations only. The reasons for the contrasting results of experiments with resting cells and those in which secondary alkyl sulphates were present at all times during culture on nutrient broth therefore remain obscure for the moment.

The manipulative and other practical difficulties accompanying the type of experiments described in the present paper are considerable. Nevertheless it proved possible to obtain important quantitative results, particularly with the alkanesulphonates. The decrease in $K_{inducer}$ values with increase in chain length suggests that an important feature of inducer specificity resides in the alkyl chain. This is supported by the finding that other compounds possessing alkyl chains (e.g. primary alkyl sulphates, dodecyltriethoxy sulphate and cyprinol sulphate) can serve as inducers of the enzyme. However, the facts that long-chain primary alcohols did not induce the enzyme and that alkanecarboxylic acids and secondary 2-yl alkyl sulphates were relatively ineffective inducers imply that an important degree of specificity also resides in the presence, nature and position of the anionic component of the potential inducer. Indeed, in spite

of the fact that octyl sulphate was almost completely hydrolysed by resting cells during the 5h shaking period, the sulphate ester was still a significantly better inducer than any of the alkanesulphonates tested. This suggests that relatively small amounts of intact ester are needed for induction and that the sulphate ester group is conferring an important degree of specificity on the induction process.

The inducer concentration–specific enzyme activity curves obtained with the alkanesulphonates were particularly interesting (see Fig. 4) because of their apparent multi-phasic character. At the present time one can only speculate on the reasons for these unusual curves, but a possible explanation might be that they largely reflect the involvement of two different transport mechanisms rather than some multi-phasic phenomenon at gene level. The Michaelis–Menten relationship obtained at low inducer concentrations and the inhibitory phase that followed could be interpreted as reflecting a transport system that becomes severely inhibited as the inducer concentration is increased. The restoration of induction when inducer concentration is increased still further might then reflect the operation of a second transport system that exhibits relatively low affinity for alkanesulphonates. The ability of more than one system to be involved in the transport of material into cells is not uncommon. Thus *Saccharomyces cerevisiae* possesses two systems that can transport SO_4^{2-} ions (Breton & Surdin-Kerjan, 1977), one of which has high affinity for SO_4^{2-} ($K_m = 5\,\mu$M) and the other a much lower affinity ($K_m = 350\,\mu$M). Other dual transport systems exist, for example, for the transport of L-methionine in *Escherichia coli* (Kadner, 1974) and for the transport of D- or L- alanine in *Bacillus subtilis* (Clark & Young, 1974). The possibility that membrane damage resulting from lipid solubilization is reflected in any part of the multi-phasic curves obtained in the present work cannot be disregarded, but seems unlikely, since the phenomenon is seen with some inducers (e.g. octanesulphonate) at concentrations well below those at which detergent micelles could be expected to form (see Table 1). No studies on the mechanisms of transport of alkyl sulphates or alkanesulphonates into bacterial cells appear to have been reported, and it seems essential to undertake such studies with the alkanesulphonates before further progress can be made in the interpretation of the multi-phasic induction curves obtained in the present work.

Finally, the ability of cyprinol sulphate (from carp bile) to serve as an inducer of the P2 alkylsulphohydrolase raises the possibility that several other naturally occurring compounds may also be active. For example, the phytoflagellates *Ochromonas danica* and *Ochromonas malhamensis*, as well as *Chlorella pyrenoidosa*, produce and release into the growth medium considerable quantities of long-chain alkyl sulphates. In detailed studies on *O. danica* by Haines and his colleagues (for review, see Haines, 1971), several different chlorinated and non-chlorinated disulphate esters (containing sulphated primary and secondary alcohol groups) have been characterized. Similarly a number of different disulphated long-chain alcohols (containing both primary and secondary alkyl sulphate ester groups) have shown to be present (Liem & Laur, 1976) in a variety of brown seaweeds (Fucacae species). Sulphoconjugation of primary alcohols by mammals can also occur, both *in vivo* (Boström & Vestermark, 1960) and *in vitro* (Spencer, 1960), and a similar conjugation mechanism has been recognized in the toad (*Xenopus laevis*) that is not identical with that involved in the formation of bile alcohol sulphates (Scully *et al.*, 1970; Scully, 1971). Furthermore, Haines (1971) has drawn attention to other reports in the literature that may indicate the existence of other alkyl sulphates of biological origin, and time may reveal that such compounds are not uncommon. It may be, therefore, that soil bacteria, including *Pseudomonas* C12B, may have encountered naturally occurring inducers of the P2 enzyme from time immemorial and not merely since the advent of synthetic alkyl sulphate detergents. The ability of such organisms to produce alkylsulphohydrolase enzymes is therefore perhaps not unduly surprising.

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