Utilization of Aliphatic Amides and Nitriles by *Nocardia rhodochrous* LL100-21

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A wide range of nitriles and amides act as carbon and/or nitrogen sources for *Nocardia rhodochrous* LL100-21. Growth on a number of aliphatic nitriles or their corresponding amides resulted in the coinduction of both acetonitrile hydratase and acetamidase activities. In each case the specific activity of acetonitrile hydratase was higher than that of acetamidase. The substrate and inducer specificities were determined for the two enzymes and found to be quite distinct. Propionitrile, although an excellent inducer of both enzymes, was only slowly hydrolysed by acetonitrile hydratase. Conversely acrylonitrile was a good enzyme substrate but had a complete lack of effect as an inducer. Butyramide and methacrylamide acted as poor substrates for acetamidase but induced high levels of both acetonitrile hydratase and acetamidase. Dinitriles of long chain length, lactonitrile and iodoacetamide were inhibitory to growth and enzyme activity.

INTRODUCTION

Aliphatic nitriles are catabolized in two stages, via conversion to the corresponding amide and then to the acid plus ammonia by a nitrile hydratase and an amidase (acylamide amidohydrolase; EC 3.5.1.4) respectively (Arnaud *et al.*, 1976*a, b*, 1977; Jallageas *et al.*, 1978; DiGeronimo & Antoine, 1976; Asano *et al.*, 1980). Benzonitrile and related aromatic nitriles (Harper, 1976, 1977*a, b*) and a heterocyclic nitrile (Robinson & Hook, 1964; Hook & Robinson, 1964) are converted directly to the corresponding acids and ammonia via a nitrilase (nitrile aminohydrolase; EC 3.5.5.1) with little formation of free amide and no separate requirement for amidase activity for production of acid.

Brevibacterium strain R312 utilizes aliphatic nitriles as sources of nitrogen for growth by using a constitutive nitrile hydratase possessing a wide substrate spectrum (Arnaud *et al.*, 1976*a, b*, 1977) and a non-specific amidase (Jallageas *et al.*, 1978). *Pseudomonas aeruginosa* and several other micro-organisms, although unable to catabolize aliphatic nitriles, form amidases that only utilize a small range of aliphatic amides (Clarke, 1980). *Arthrobacter* sp. J-1 utilizes aliphatic nitriles via an inducible nitrile hydratase and amidase (Asano *et al.*, 1982). *Nocardia rhodochrous* LL100-21 can grow on a range of aliphatic nitriles and amides, as well as benzonitrile and benzamide as carbon and/or nitrogen sources (DiGeronimo & Antoine, 1976; Collins & Knowles, 1983). Studies of the enzyme activities suggest that the aliphatic nitriles and amides, and benzonitrile and benzamide are utilized by separate enzyme systems, which are inducible (Collins & Knowles, 1983).

In the present study we have investigated the ability of a wide range of nitriles and amides to act as inducers, substrates and inhibitors of the aliphatic nitrile hydratase and amidase enzymes of *N. rhodochrous* LL100-21. Although these enzymes act on several nitriles and amides respectively, we have termed them, for convenience, acetonitrile hydratase and acetamidase.

METHODS

Organism and growth conditions. *Nocardia rhodochrous* strain LL100-21 was grown on a minimal medium consisting of M-9 salts (Miller, 1972) with ammonium salts omitted, plus 1 ml trace metals l^{-1} (Bauchop & Eldsen, 1960) and a range of nitriles, amides and acids as carbon and nitrogen, carbon or nitrogen sources supplied at a concentration of 20, 6 or 2 mM. Where indicated, the medium was supplemented with 10 mM-succinate as the source of carbon and/or 10 or 2 mM- NH_4Cl as the source of nitrogen. A 2% (v/v) inoculum of a 24 h stationary phase culture grown on the same carbon and nitrogen source as the experimental culture was used to inoculate the experimental flasks. Growth was in either 100 ml medium in 250 ml conical flasks or 500 ml medium in 2-l conical flasks. Incubation was at 30 °C in an orbital shaker (250 r.p.m.).

Bacterial growth was measured turbidimetrically at 600 nm in an SP 500 spectrophotometer (Pye Unicam), using 1 ml cuvettes.

Harvesting and preparation of cell-free extracts. Bacteria were harvested in the mid-exponential phase (OD_{600} 0.3 to 0.8) by centrifugation at 23 000 g for 10 min at 4 °C, washed twice in 100 mM- KH_2PO_4/K_2HPO_4 buffer (pH 7.0) and resuspended in fresh buffer to about one-twentieth of the original culture volume. Bacteria were disrupted by sonication (MSE sonicator, 150 W, 6×15 s, 0 °C) and centrifuged at 10 000 g for 10 min at 4 °C to give a cell-free extract.

Determination of acetonitrile, acetamide, acetate and ammonia. Samples of growth medium (2 ml) from cultures containing acetonitrile or acetamide were centrifuged for 1 min in a Microfuge (Beckman Instruments). A 100 μ l sample of the supernatant was mixed with 25 μ l 25 mM-propionic acid (as an internal standard) in 250 mM-HCl and a 2 μ l sample was injected into a model 204 gas chromatograph (Pye Unicam) equipped with a flame ionization detector and linked to a model CDPI integrator. A glass column (1.5 m $\times$ 4 mm i.d.) was packed with Porapak Q (Waters Associates), mesh 80–100. The detector was at 250 °C, the column at 190 °C and the injector at 210 °C. The carrier was N_2 at 50 ml min^{-1} . Quantitative measurements were obtained by comparison with a standard curve of known concentrations of the test compounds by their peak areas relative to that of the propionic acid internal standard. For ammonia analysis, 100 μ l of the sample was mixed with 0.5 ml nitroprusside reagent and 0.5 ml alkaline hypochlorite reagent was added (Fawcett & Scott, 1960). After mixing, the solution was incubated at 35 °C for 15 min and the absorbance measured at 570 nm. An NH_4Cl solution was used as a standard.

Determination of nitrile hydratase and amidase activities. Acetamidase activity was routinely measured by colorimetric assay of the ammonia produced. In cases where amides were present which interfered with the colorimetric assay, activity was measured by GLC analysis of the acetate produced. Because acetamidase activity was lower than acetonitrile hydratase activity, the latter was measured by the sum of the initial rates of acetamide and acetate formation. The reaction mixture (5 to 10 ml) at 30 °C contained 100 mM- KH_2PO_4/K_2HPO_4 buffer (pH 7.0), 20 mM- or 6 mM-nitrile or amide as substrate and bacteria or cell-free extract. The reaction was initiated by addition of substrate. For GLC analysis of the reaction products of nitrile hydratase and amidase, 100 μ l samples of the reaction mixture were removed at intervals up to 30 min and added to 25 μ l 25 mM-propionic acid in 250 mM-HCl. When intact bacteria were used they were removed by centrifugation for 1 min in a microfuge before assay of reaction products. The samples were stored at –20 °C until required and then assayed by GLC as described above, except that when amides were the substrate the column was at 210 °C and the injector at 230 °C. Ammonia was assayed as described above.

Other assays. Protein in cell-free extracts was measured by the modified biuret method of Gornall *et al.* (1949). Where intact bacteria were used, activity was related to dry weight, obtained by drying 1 ml samples for 15 min in a model 7910 microwave oven (Philips).

A standard curve relating optical density to dry weight was constructed.

Chemicals. Acetamide, acetonitrile, benzamide, benzoic acid and formamide were obtained from Sigma. Benzonitrile and acetic acid were obtained from BDH. Acetamide was recrystallized from hot ethanol. Wherever possible, all other reagents were of analytical grade. Glass-distilled water and acid-washed glassware were used throughout.

RESULTS

The ability of *Nocardia rhodochrous* LL100-21 to grow on an extensive range of nitriles, amides and acids, when they were supplied as the sole carbon and nitrogen, carbon or nitrogen sources, is shown in Tables 1, 2 and 3. Additionally thioacetamide, thiourea, methylcarbamate, glycineamide, cyanoacetamide and nicotinamide could only be used as the sole source of nitrogen.

It is clear that a range of simple aliphatic nitriles and amides could act as carbon and nitrogen sources, confirming and extending the data of Collins & Knowles (1983). However, the 1-carbon compounds cyanide and formamide did not act as carbon sources although formamide served as

Table 1. Comparison of nitriles as substrates, inducers and inhibitors of *N. rhodochrous* LL100-21 acetonitrile hydratase

Bacteria were grown on M-9 salts with ammonium salts omitted, plus trace metals. Growth substrates were added as indicated. To study inhibition of growth, 10 mM-succinate and 10 mM-NH₄Cl were added as carbon and nitrogen sources.

Nitrile	Growth substrate*		Growth inhibitor†	Enzyme substrate‡	Enzyme inducer‡		Percentage enzyme inhibition
	C+N	N			Nitrile hydratase	Amidase	
KCN	—	—	ND	—	ND	ND	ND
Acetonitrile	+++	+++	—	++	++	++	—
Propionitrile	+++	+++	—	+	+++	+++	—
Butyronitrile	+++	+++	—	+	++	++	—
Isobutyronitrile	++	++	—	—	++	++	—
Valeronitrile	+++	+++	—	+	+	+	ND
Acrylonitrile	—	++ LC	+++	++	—	—	ND
Methacrylonitrile	—	+++	—	+	—	—	ND
Malononitrile	—	—	+++	+	ND	ND	ND
Succinonitrile	—	+	—	+	—	—	ND
Glutaronitrile	—	+	—	—	—	—	50
Adiponitrile	—	+	—	—	—	—	100
Fumaronitrile	—	—	+++	—	ND	ND	68
Lactonitrile	—	—	+++	—	ND	ND	100
Phenylacetoneitrile	—	++ LC	ND	ND	—	—	59
Benzonitrile	++ LC	++ LC	+++	—	—	—	74

ND, Not determined.

* + + +, OD₆₀₀ > 1.0 within 24 h; + +, OD₆₀₀ > 1.0 within 72 h; +, OD₆₀₀ > 0.05 but < 1.0 within 72 h; LC, only supported growth when supplied at a concentration of less than 6 mM; —, no growth.

† —, No inhibition, OD₆₀₀ > 1.0 within 24 h; + + +, no growth.

‡ + + +, + +, +, —, Relative activities (cf. Tables 4 and 5).

a nitrogen source. The unsaturated compounds acrylamide, methacrylamide, acrylonitrile and methacrylonitrile only supported growth as sources of nitrogen. The dinitriles succinonitrile and glutaronitrile only served as poor nitrogen sources, whereas fumaronitrile and malononitrile would not support growth when supplied as either carbon or nitrogen sources. Amides derived from dinitriles were only able to support growth when present as the nitrogen source, as were N-substituted amides. Substitution with a sulphur group, as in thioacetamide, resulted in a loss of ability to act as a carbon source and the addition of a phenyl group (phenylacetamide), an iodo group (iodoacetamide) or a hydroxyl group (lactonitrile) prevented the compound from serving as a carbon or nitrogen source.

Some nitriles, amides or acids were added at 30 mM to a medium containing 10 mM-succinate and 10 mM-NH₄Cl as the sources of carbon and nitrogen respectively. Under these conditions, some of them, including those which could be utilized as a sole carbon or nitrogen source, inhibited growth (Tables 1, 2 and 3).

When acetamide was used as the sole source of carbon and nitrogen, it was utilized via conversion to ammonia and acetate over a period of 12 h during growth (Fig. 1a) (cf. Collins & Knowles, 1983). Under these conditions synthesis of both acetonitrile hydratase and acetamidase began almost immediately and ceased once there was no acetamide left in the medium (Fig. 1b). Growth terminated on depletion of acetate. In contrast, growth on acetonitrile resulted in the disappearance of substrate within 6 h after inoculation with the concomitant production of acetamide, which was utilized by conversion to acetate and ammonia during exponential growth (Fig. 2a). The specific activity of acetonitrile hydratase

Table 2. *Comparison of amides as substrates, inducers and inhibitors of N. rhodochrous LL100-21 acetamidase*

Bacteria were grown on M-9 salts with ammonium salts omitted, plus trace metals. Growth substrates were added as indicated. To study inhibition of growth, 10 mM-succinate and 10 mM-NH₄Cl were added as carbon and nitrogen sources.

Amide	Growth substrate*		Growth inhibitor†	Enzyme substrate‡	Enzyme inducer‡		Percentage enzyme inhibition
	C+N	N			Nitrile hydratase	Amidase	
Formamide	—	+++	ND	+	ND	+	ND
Acetamide	+++	+++	—	++	++	++	—
Propionamide	+++	+++	—	+++	++	+	—
Butyramide	+++	+++	—	+	+	++	—
Isobutyramide	+++	+++	—	+	++	++	—
Acrylamide	—	++ LC	+++	+++	+	+	ND
Methacrylamide	—	+++	—	+	++	+++	ND
Malonamide	—	+	—	ND	ND	ND	ND
Succinamide	—	+	—	ND	ND	ND	ND
Adipamide	—	+	—	ND	ND	ND	ND
Lactamide	—	+++	ND	ND	ND	ND	ND
Urea	—	+++	ND	+	ND	+	ND
N-Methylformamide	—	+	—	ND	ND	—	—
		SG					
N-Ethylformamide	—	+	—	ND	ND	—	7
		SG					
N-Methylacetamide	—	+	—	ND	ND	—	—
N-Ethylacetamide	—	+	—	ND	ND	+	—
N,N'-Dimethylacetamide	—	+	—	ND	ND	—	16
Iodoacetamide	—	—	+++	ND	ND	ND	68
Phenylacetamide	—	—	ND	ND	ND	ND	ND
Benzamide	++ LC	++ LC	—	—	—	—	ND

ND, Not determined.

* + + +, OD₆₀₀ > 1.0 within 24 h; ++, OD₆₀₀ > 1.0 within 72 h; +, OD₆₀₀ > 0.05 but < 1.0 within 72 h; LC, only supported growth when supplied at a concentration of less than 6 mM; SG, slow growth.

† —, No inhibition, OD₆₀₀ > 1.0 within 24 h; + + +, no growth.

‡ + + +, ++, +, —, Relative activities (cf. Tables 4 and 5).

and acetamidase increased when acetamide appeared in the medium until it had been completely converted to acetate and ammonia, whereupon the activity of both enzymes disappeared rapidly (Fig. 2*b*). Exponential growth did not commence until the acetonitrile had been depleted from the medium, and growth terminated on depletion of acetate. During growth on either acetamide or acetonitrile, acetonitrile hydratase and acetamidase activities of intact bacteria exhibited similar patterns of induction, but the specific activity of acetamidase was approximately one-tenth that of acetonitrile hydratase.

In cell-free extracts, acetamidase activity had a broad pH optimum at 7.0 with 92% of maximal activity at pH 6.5 and 7.5. In contrast, acetonitrile hydratase had a sharp pH optimum at 7.0 with only 70% maximal activity at pH 6.5 and 7.5.

To study inducer specificity, extracts were prepared from bacteria grown on the nitriles and amides listed in Table 4 at a concentration of 20 mM or 6 mM and acetonitrile hydratase and acetamidase activities were measured with either 20 mM-acetonitrile or 20 mM-acetamide as substrate. All the aliphatic nitriles and amides that were effective inducers coinduced both enzymes, the specific activity of acetonitrile hydratase always being higher than that of acetamidase. Where an amide acted as an inducer of enzyme activity so did its corresponding nitrile, with the exception of the unsaturated amide acrylamide, and its methylated derivative,

Fig. 1. Growth of *N. rhodochrous* on 20 mM-acetamide. Samples (2 ml) were aseptically removed from the cultures and (a) the growth measured as OD_{600} (□). The ammonia concentration in the medium (■) was measured colorimetrically, and the acetamide (○) and acetate (△) concentrations by GLC. (b) Acetonitrile hydratase (▲) and acetamidase (▽) activities were measured in intact cells at various stages of growth as described in Methods using 20 mM-acetonitrile and 20 mM-acetamide respectively as enzyme substrates.

Table 3. Comparison of acids as growth substrates and inhibitors

Bacteria were grown on M-9 salts with ammonium salts omitted, plus trace metals. Acids were added as growth substrates, as indicated, with 2 mM- NH_4Cl . To study the effect of acids as growth inhibitors, 10 mM-succinate and 10 mM- NH_4Cl were added as carbon and nitrogen sources.

Acid	Growth substrate*	Growth inhibitor†
Acetate	+++	—
Propionate	+++	—
Butyrate	+++	—
Isobutyrate	+++	—
Valerate	++	+
	LC	
Acrylate	—	+++
Methacrylate	—	+
Malonate	—	+++
Succinate	+++	—
Glutarate	++	+++
	LC	
Fumarate	+	+++
	LC, SG	
Lactate	+++	—
Phenylacetate	—	+++
Benzoate	++	+++
	LC	

* + + +, $OD_{600} > 1.0$ within 24 h; + +, $OD_{600} > 1.0$ within 72 h; +, $OD_{600} > 0.05$ but < 1.0 within 72 h; LC, only supported growth when supplied at a concentration of less than 6 mM; SG, slow growth; —, no growth.

† —, No inhibition, $OD_{600} > 1.0$ within 24 h; +, $OD_{600} > 0.05$ but < 1.0 within 72 h; + + +, no growth.

methacrylamide, of which the corresponding nitriles acrylonitrile and methacrylonitrile were non-inducers.

The substrate specificities of acetonitrile hydratase and acetamidase were studied using cell-free extracts prepared from harvested bacteria that had been grown to the mid-exponential phase on acetonitrile or acetamide respectively (Table 5). Acetonitrile and acrylonitrile were

Fig. 2. Growth of *N. rhodochrous* on 20 mM-acetonitrile. (a) Growth was measured as OD₆₀₀ (□). The ammonia concentration in the medium (■) was measured colorimetrically and the acetonitrile (●), acetamide (○) and acetate (△) concentrations by GLC. (b) Acetonitrile hydratase (▲) and acetamidase (▽) activities were measured in intact cells using 20 mM-acetonitrile or 20 mM-acetamide as enzyme substrates.

Table 4. Induction of enzymes exhibiting acetonitrile hydratase and acetamidase activities by growth on nitriles and amides

Enzyme activities were measured at 30 °C in cell-free extracts derived from bacteria that had been harvested in the mid-exponential growth phase (OD₆₀₀ 0.4 to 0.7). Acetonitrile hydratase activity was measured by the initial rate of formation of acetamide plus acetate, and acetamidase by the initial rate of formation of ammonia, using 20 mM-acetonitrile and 20 mM-acetamide as enzyme substrates, respectively. Enzyme activities, determined in six assays, are given in μmol min⁻¹ (mg protein)⁻¹.

	Growth concn (mM)	Nitrile hydratase activity	Amidase activity	Growth substrate	Growth concn (mM)	Nitrile hydratase activity	Amidase activity
Acetonitrile	20	2.42	0.22	Acetamide	20	2.82	0.26
Propionitrile	20	6.42	0.43	Propionamide	20	1.06	0.11
Butyronitrile	20	1.02	0.24	Butyramide	20	0.74	0.26
Isobutyronitrile	20	1.28	0.20	Isobutyramide	20	1.61	0.16
Valeronitrile	20	0.28	0.05	Acrylamide	20	0.42	0.08
Acrylonitrile	6	0	0.01	Methacrylamide	20	1.79	0.64
Methacrylonitrile	20	0	0	Benzamide	6	0	0
Benzonitrile	6	0	0				

rapidly hydrolysed by acetonitrile hydratase, whereas longer-chain saturated nitriles were only slowly hydrolysed. The branched isomer isobutyronitrile was not a substrate and the addition of a methyl group, as in methacrylonitrile, decreased the rate of hydrolysis. Substitution by a hydroxyl group, as in lactonitrile, caused a loss of ability to act as an enzyme substrate. Of the dinitriles studied, only the saturated 3- and 4-carbon compounds acted as substrates (Table 5).

Propionamide and acrylamide were excellent substrates for acetamidase, being hydrolysed two to three times faster than acetamide (Table 5). Although butyramide was as good an inducer as acetamide (Table 4), it was only slowly hydrolysed by acetamidase (Table 5).

The substrate specificities of the amidase activity induced by growth on the various acetamide analogues were also examined (Table 6). Although different levels of enzyme activity were induced during growth on the acetamide analogues, the patterns of the relative activities were similar to that for acetamidase, regardless of the growth substrate (Table 6), suggesting that in general the analogues were inducing the same enzyme. However, the high value obtained for the relative enzyme activity using formamide as the enzyme substrate after growth on formamide (156%, see Table 6) suggests the induction of a specific formamidase. Similarly, growth on urea appeared to induce a urease.

Table 5. *Substrate specificity of acetonitrile hydratase and acetamidase induced by growth on acetonitrile and acetamide*

A range of nitriles and amides, supplied at a concentration of 20 mM, were examined as substrates for acetonitrile hydratase and acetamidase of extracts of bacteria induced by growth on 20 mM-acetonitrile and 20 mM-acetamide respectively. The numbers in parentheses are the specific activities [$\mu\text{mol min}^{-1} (\text{mg protein})^{-1}$] determined in six assays.

Acetonitrile hydratase		Acetamidase	
Substrate	Percentage activity	Substrate	Percentage activity
KCN	0	Formamide	12.1
Acetonitrile	100.0 (2.42)	Acetamide	100.0 (0.26)
Propionitrile	3.5	Propionamide	277.0
Butyronitrile	1.2	Butyramide	3.9
Isobutyronitrile	0	Isobutyramide	1.4
Valeronitrile	4.2	Acrylamide	195.0
Acrylonitrile	67.1	Methacrylamide	1.6
Methacrylonitrile	10.4	Benzamide	0
Malononitrile	9.6	Urea	1.2
Succinonitrile	10.4		
Glutaronitrile	0		
Adiponitrile	0		
Fumaronitrile	0		
Lactonitrile	0		
Benzonitrile	0		

Table 6. *Substrate specificity of acetamidase induced by growth on various amides*

The substrate specificity of acetamidase activity in cell-free extracts prepared from harvested bacteria grown on various acetamide analogues as carbon and/or nitrogen sources was examined. The amidase activities relative to that of acetamide (100%) are given for each growth condition. The numbers in parentheses are the specific activities [$\mu\text{mol ammonia formed min}^{-1} (\text{mg protein})^{-1}$] determined in six assays; ND, not determined.

Amide present during growth	Acetamide	Percentage hydrolysis of various substrates relative to that of acetamide						
		Propion-amide	Butyr-amide	Isobutyr-amide	Acryl-amide	Form-amide	Urea	Benzamide
Acetamide	100 (0.26)	277.0	3.9	1.4	195.0	12.1	1.2	0
Propionamide	100 (0.11)	302.0	1.6	1.2	260.0	13.6	1.1	0
Butyramide	100 (0.26)	277.0	4.1	2.1	196.0	13.3	1.4	0
Isobutyramide	100 (0.16)	235.0	2.8	2.0	183.0	10.7	2.5	0
Acrylamide	100 (0.08)	275.0	2.8	4.4	238.0	14.6	17.3	0
Formamide	100 (0.01)	238.0	ND	ND	251.0	156.0	ND	ND
Urea	100 (0.01)	187.0	ND	ND	140.0	ND	706.0	ND
Benzamide	0	0	ND	ND	0	ND	ND	0

The aliphatic acetamidase induced by growth on the various acetamide analogues was unable to hydrolyse benzamide, confirming that benzamide is utilized by a separate enzyme system (Collins & Knowles, 1983). Although present in intact cells, benzamidase activity could not be detected in cell-free extracts of bacteria grown on benzamide, as shown previously (Collins & Knowles, 1983); there was a lack of activity over a wide pH range (5.6–8.0). This could be due to lability of the enzyme or a requirement for a cofactor, as yet undetermined.

Several amides and nitriles (at 50 mM) were tested as possible inhibitors of acetamidase or acetonitrile hydratase activity by using cell-free extracts prepared from bacteria grown on acetamide or acetonitrile and with either acetamide or acetonitrile (5 mM) as the substrate for the assays. Many non-substrate amides and nitriles acted as enzyme inhibitors (Tables 1 and 2).

DISCUSSION

N. rhodochrous LL100-21 has been shown to utilize a range of aliphatic nitriles and amides as carbon and/or nitrogen sources via a nitrile hydratase and an amidase, both of which are inducible.

The ability of simple aliphatic amides to coinduce the hydratase and amidase enzymes at levels comparable to those resulting from growth on the corresponding nitriles, with the exception of propionitrile and propionamide, suggests that biosynthesis of these two enzymes is closely related, and strongly supports the hypothesis of Collins & Knowles (1983) that they were formed from structural genes utilizing the same regulatory apparatus with the amide as the most likely inducer.

Clearly inducer and substrate specificities of the acetonitrile hydratase and acetamidase are quite distinct. Acetamide and propionamide, which were good substrates for acetamidase, were also good inducers of both the nitrile hydratase and amidase. However, butyramide, isobutyramide and methacrylamide were poor substrates but induced high levels of both enzymes, which could account for the good growth achieved on these compounds. Similarly, butyronitrile and isobutyronitrile acted as good inducers even though butyronitrile was only slowly hydrolysed by acetonitrile hydratase and enzyme activity with isobutyronitrile as substrate could not be detected. Propionitrile was an excellent inducer of both enzymes, the level of induction being two to three times that by acetonitrile.

Chain length appeared to be important in substrates for nitrile hydratase and amidase. Thus, acetonitrile was the best substrate for the hydratase enzyme, while propionitrile and butyronitrile with their increasing number of carbon atoms were hydrolysed progressively more slowly. In 4-carbon compounds the presence of the branched methyl group (i.e. in isobutyronitrile) prevented function as a substrate, while the presence of an unsaturated double bond (as in methacrylonitrile) enhanced the rate of hydration. However, acrylonitrile, of shorter chain length, was hydrolysed some seven times faster than methacrylonitrile.

Propionamide was the substrate most rapidly hydrolysed by acetamidase, amides of both longer and shorter chain length being hydrolysed far more slowly. The other 3-carbon amide, acrylamide, was also an extremely good substrate for the enzyme, but again, the derivative with a branched methyl group (methacrylamide) was a relatively poor substrate.

The presence of two nitrile groups in those compounds with a chain length of five carbons or more resulted in the ability to act as severe enzyme inhibitors. Inhibition of growth and amidase activity by iodoacetamide could be due to its known reaction with thiol groups rather than to its structural resemblance to acetamide.

The wide range of aliphatic amides that act as enzyme substrates for the inducible acetamidase of *N. rhodochrous* contrasts with the narrower range described for the amidase of *Pseudomonas aeruginosa* (Clarke, 1970). The relative substrate and inducer specificities also differ in the two bacteria, and no non-substrate inducers of *N. rhodochrous* acetamidase were found.

The inducible enzymes allowed *N. rhodochrous* to grow on a number of nitriles as the sole carbon and nitrogen source but the substrate spectrum was narrow in comparison to those of the inducible nitrile hydratase of *Arthrobacter* sp. J-1 (Asano *et al.*, 1980, 1982), and the constitutive enzyme of *Brevibacterium* sp. R312 (Arnaud *et al.*, 1977).

As yet, too little is known about the molecular properties of the amidases and, in particular, the nitrile hydratases of micro-organisms to explain these differences in specificities. Their clarification awaits purification and characterization of the enzymes involved.

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REFERENCES

- ARNAUD, A., GALZY, P. & JALLAGEAS, J.-C. (1976*a*). Amidase activity of some bacteria. *Folia microbiologica (Praha)* **21**, 178–184.
- ARNAUD, A., GALZY, P. & JALLAGEAS, J.-C. (1976*b*). Etude de l'activité nitrilasique de quelques bactéries. *Revue des fermentations et des industries alimentaires* **31**, 39–44.
- ARNAUD, A., GALZY, P. & JALLAGEAS, J.-C. (1977). Etude de l'acétonitrilase d'une souche de *Brevibacterium*. *Agricultural and Biological Chemistry* **41**, 2183–2191.
- ASANO, Y., TANI, Y. & YAMADA, H. (1980). A new enzyme 'nitrile hydratase' which degrades acetonitrile in combination with amidase. *Agricultural and Biological Chemistry* **44**, 2251–2252.
- ASANO, Y., FUJISHIRO, K., TANI, Y. & YAMADA, H. (1982). Aliphatic nitrile hydratase from *Arthrobacter* sp. J-1. Purification and characterisation. *Agricultural and Biological Chemistry* **46**, 1165–1174.
- BAUCHOP, T. & ELSDEN, S. R. (1960). The growth of micro-organisms in relation to their energy supply. *Journal of General Microbiology* **23**, 457–469.
- CLARKE, P. H. (1970). The aliphatic amidases of *Pseudomonas aeruginosa*. *Advances in Microbial Physiology* **4**, 179–222.
- CLARKE, P. H. (1980). The utilization of amides by microorganisms. In *Microorganisms and Nitrogen Sources*, pp. 537–562. Edited by J. W. Payne. New York: John Wiley & Sons.
- COLLINS, P. A. & KNOWLES, C. J. (1983). The utilization of nitriles and amides by *Nocardia rhodochrous*. *Journal of General Microbiology* **129**, 711–718.
- DIGERONIMO, M. J. & ANTOINE, A. D. (1976). Metabolism of acetonitrile and propionitrile by *Nocardia rhodochrous* LL100-21. *Applied and Environmental Microbiology* **31**, 900–906.
- FAWCETT, J. K. & SCOTT, J. E. (1960). A rapid and precise method for the determination of urea. *Journal of Clinical Pathology* **13**, 156–159.
- GORNALL, A. G., BARDAWILL, C. J. & DAVID, M. M. (1949). Determination of serum proteins by the biuret reaction. *Journal of Biological Chemistry* **177**, 751–766.
- HARPER, D. B. (1976). Purification and properties of an unusual nitrilase from *Nocardia* NCIB 11216. *Biochemical Society Transactions* **4**, 502–504.
- HARPER, D. B. (1977*a*). Microbial metabolism of aromatic nitriles. Enzymology of C–N cleavage by *Nocardia* sp. (*Rhodochrous* group) NCIB 11216. *Biochemical Journal* **165**, 309–319.
- HARPER, D. B. (1977*b*). Fungal degradation of aromatic nitriles. Enzymology of C–N cleavage by *Fusarium solani*. *Biochemical Journal* **167**, 685–692.
- HOOK, R. H. & ROBINSON, W. G. (1964). Ricinine nitrilase. II. Purification and properties. *Journal of Biological Chemistry* **239**, 4263–4267.
- JALLAGEAS, J.-C., ARNAUD, A. & GALZY, P. (1978). Etude l'acetamidase d'une souche de *Brevibacterium*. *Journal of General and Applied Microbiology* **24**, 103–114.
- MILLER, J. H. (1972). *Experiments in Molecular Genetics*, p. 431. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
- ROBINSON, W. G. & HOOK, R. H. (1964). Ricinine nitrilase. I. Reaction product and substrate specificity. *Journal of Biological Chemistry* **239**, 4257–4262.