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Production of *R*-(−)-Ketoprofen from an Amide Compound by *Comamonas acidovorans* KPO-2771-4

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***R*-(−)-2-(3′-Benzoylphenyl)propionic acid [*R*-(−)-ketoprofen] was produced from racemic 2-(3′-benzoylphenyl)propionamide (keto-amide) by the isolated bacterial strain *Comamonas acidovorans* KPO-2771-4. Sodium fumarate as the carbon source and 2-azacyclononanone or isobutyronitrile as the enhancer in the culture medium were effective for bacterial growth and the enhancement of *R*-(−)-ketoprofen-producing activity. *R*-(−)-Ketoprofen produced from the keto-amide by resting cells was present in 99% enantiomeric exess. *C. acidovorans* KPO-2771-4 has an *R*-enantioselective amidase for keto-amide because the purified amidase from the bacterium hydrolyzed keto-amide, producing optically pure *R*-ketoprofen and ammonia.**

Optically active 2-arylpropionic acids are useful, active substances pharmaceutically, especially the nonsteroidal anti-inflammatory drugs "profens." Although the various profen enantiomers have different biological activities, most (except naproxen) are used as racemic compounds because there are few industrial processes for producing the enantiomers. There is an increasing demand for these enantiomers because they are more effective for therapy (6). The *R*-enantiomer of 2-(3′-benzoylphenyl)propionic acid (*R*-ketoprofen) also has been developed from racemic ketoprofen as a chiral drug (24). We therefore investigated a new process for producing optically active 2-arylalcanoic acids from racemic 2-arylalcanenitriles or 2-arylalcanoic acid amides by the action of microorganisms (22, 23). Other groups have proposed the production of *S*-2-arylpropionic acids from 2-arylpropionamides by enantioselective *S*-amidases (3, 8) but not a process for producing *R*-ketoprofen from ketoprofen's amide or nitrile compound.

We report here the screening of microorganisms that enantiometrically hydrolyze racemic 2-(3′-benzoylphenyl)propion amide (keto-amide) to produce *R*-(−)-2-(3′-benzoylphenyl) propionic acid [*R*-(−)-ketoprofen] (Fig. 1). One of them, *Comamonas acidovorans* KPO-2771-4, was studied in detail with regard to its activities and its enzyme.

MATERIALS AND METHODS

Chemicals. *R*,*S*-2-(3′-Benzoylphenyl)propionic acid (*R*,*S*-ketoprofen) was purchased from Sigma-Aldrich Japan K. K. *R*,*S*-2-(3′-Benzoylphenyl)propionitrile (keto-nitrile) was purchased from Hamari Chemicals Ltd. (Yonezawa, Japan). *R*,*S*-2-(3′-Benzoylphenyl)propionamide (*R*,*S*-keto-amide) was synthesized by the reaction of the keto-nitrile and concentrated HCl (1:1) at 25°C for 17 h, the organic layer being recovered after ethyl acetate extraction at pH 11 to remove the ketoprofen (86% yield). The analytical findings matched those reported previously (19). *R*-Keto-amide was synthesized from racemic keto-amide and *Rhodococcus maris* BP-479-9 (containing *S*-amidase) in a reaction mixture containing 15% methanol and 10 mM potassium phosphate (pH 7.8), at 28°C for 29 h. It was recovered by the removal of the *S*-ketoprofen produced by dichloromethane extraction at pH 11 (46% yield). The analytical data matched those reported previously (19).

Peptone, yeast extract D-3, and agar were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). DEAE-cellulose (DE-52) was from Whatman BioSystems Ltd., and Sephacryl S-400 and Phenyl Sepharose CL-4B were from Pharmacia Fine Chemical Co., Ltd. All the other chemicals used were obtained from commercial sources.

Media. The minimal medium for the isolation of the *R*-keto-amide-utilizing microorganisms contained (per liter) 4 g of glucose, 1 g of KH_2PO_4, 1 g of K_2HPO_4, 1 g of NaCl, 0.2 g of $MgSO_4 \cdot 7H_2O$, 0.03 g of $FeSO_4 \cdot 7H_2O$, 1 mg of biotin, 1 mg of thiamine hydrochloride, and 1 g of *R*-keto-amide. The medium was adjusted to pH 7.0 with NaOH and then autoclaved. The minimal-medium plates were prepared by adding 18 g of agar to 1 liter of medium.

The rich medium used for the multiplication of cells contained (per liter) 10 g of glucose, 10 g of yeast extract D-3, 5 g of peptone, 2 g of K_2HPO_4, 0.2 g of $MgSO_4 \cdot 7H_2O$, 0.03 g of $FeSO_4 \cdot 7H_2O$, 1 g of NaCl, and 1 g of ε-caprolactam (pH 6.8).

Isolation of bacteria. The bacteria used were isolated from soil samples by an enrichment culture technique. A 1:10 dilution of each sample was made with sterile water, and the suspension was incubated at 30°C for 1 h. One-hundred-microliter samples of the suspension were inoculated into sterile Pyrex test tubes that contained 5 ml of minimal medium, after which the tubes were incubated for 3 to 10 days at 30°C until they became turbid. This procedure was performed sequentially three to five times, and then turbid samples were streaked onto minimal-medium plates to isolate single colonies.

Screening for *R*-ketoprofen-producing strains. Microorganisms were grown aerobically at 28°C for 1 to 2 days in rich medium. The cells then were centrifuged and suspended in 0.1 M potassium phosphate buffer (pH 8.0). The reaction mixture used to screen the *R*-ketoprofen-producing strains contained 100 μmol of potassium phosphate buffer (pH 8.0), 5 μmol of keto-amide as the substrate, 0.05 ml of methanol, and the cells (final optical density at 610 nm, 5 to 100) in a total volume of 1.0 ml. The reaction was carried out at 30°C for 5 h with reciprocal shaking (250 strokes per minute) and then terminated by centrifugation at 18,000 × *g* for 10 min. The amount of ketoprofen in the supernatant was determined as described below.

Identification of the microorganisms. The bacteria were identified by using the classification given in *The Prokaryotes* (2) and *Bergey's Manual of Systematic Bacteriology* (15).

Isolation of *R*-ketoprofen. *C. acidovorans* KPO-2771-4 was cultured at 30°C for 20 h in medium that contained (per liter) 10 g of sodium fumarate, 10 g of yeast extract D-3, 5 g of peptone, 2 g of K_2HPO_4, 0.2 g of $MgSO_4 \cdot 7H_2O$, 30 mg of $FeSO_4 \cdot 7H_2O$, 1 g of NaCl, and 1 g of isobutyronitrile (pH 6.8). The cells (2.7 g [dry weight]) were collected by centrifugation and suspended in 1,000 ml of 0.1 M potassium phosphate buffer (pH 8.0). A 500-mg portion of the keto-amide dissolved in 5 ml of methanol was added to 95 ml of the cell suspension, and this mixture was incubated at 30°C with stirring (150 rpm). The cells were removed by centrifugation after 40 h. The pH was adjusted to 8.4 with 2 N NaOH, and the supernatant was washed with 100 ml of ethyl acetate. After the pH of the water layer was adjusted to 3.0 with 6 N HCl, the desired product was extracted with 100 ml of ethyl acetate. The extract was concentrated under reduced pressure and yielded 232 mg of crystals, which were recrystallized from H_2O (220 mg).

Analytical methods. The amounts of ketoprofen and keto-amide were assayed by analytical high-pressure liquid chromatography (Tosoh CCPM system equipped with a NovaPak Phenyl column [Waters Ltd.]) with the solvent system 0.05 M KH_2PO_4 (pH 3.5)–acetonitrile (6:4, vol/vol) at a flow rate of 1.0 ml/min. The A_{254} was measured. The respective retention times for keto-amide, ketoprofen, and keto-nitrile were 1.4, 2.2, and 4.4 min. Alternatively, the ammonia formed was measured by the method of Fawcett and Scott (9).

The optical purity of ketoprofen was determined by direct analysis of the

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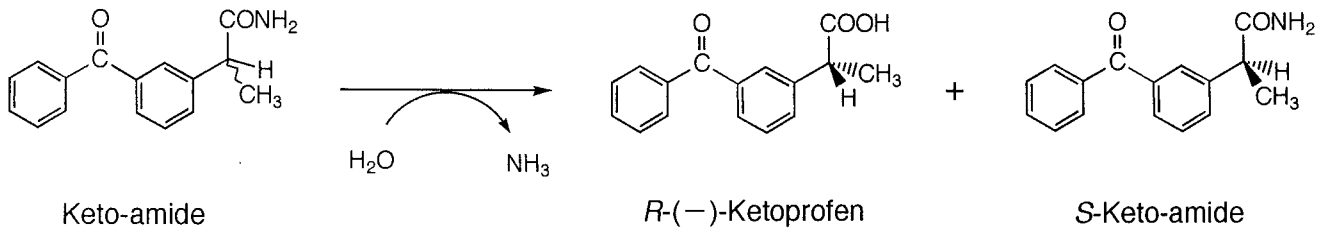

FIG. 1. Enzymatic conversion of racemic keto-amide to *R*-(−)-ketoprofen.

enantiomers. The reaction solution was adjusted to pH 3.0 with 1 N HCl. The ketoprofen produced was extracted with dichloromethane. The solution was assayed by high-pressure liquid chromatography on an Optipak-TA column (Waters Ltd.) with the solvent system hexane-isopropanol-acetic acid (92:8:0.3) at a flow rate of 1.0 ml/min. The respective retention times for *R*-(−)- and *S*-(+)-ketoprofen were 14.6 and 19.5 min. The A_{254} was measured.

The optical purity of keto-amide was determined by direct analysis of the enantiomers. The reaction solution was assayed by high-pressure liquid chromatography on a CHIRALCEL OD-R column (Daiceru Inc., Tokyo, Japan) with a solvent of acetonitrile-H_2O (2:8) at a flow rate of 1.5 ml/min. The respective retention times for the *S*- and *R*-keto-amide were 42.6 and 49.0 min. The A_{220} was measured.

Enzyme assay. Keto-amide-hydrolyzing activity (amidase) was assayed in a reaction mixture (0.5 ml) containing 2.5 μmol of keto-amide, 0.025 ml of methanol, 0.5 mg of bovine serum albumin, 25 mmol of potassium phosphate buffer (pH 8.0), and an appropriate amount of the enzyme. The reaction took place at 30°C for 30 min and was stopped by an addition of 1.5 ml of methanol. The amount of ketoprofen formed was measured by high-pressure liquid chromatography as described above. One unit of enzyme activity was defined as the amount of enzyme that catalyzed the formation of 1 μmol of ketoprofen per min in the reaction mixture. The protein concentration was determined by the method of Lowry et al. (16) with bovine serum albumin as the standard.

Purification of the enzyme that hydrolyzes keto-amide. *C. acidovorans* KPO-2771-4 was cultured as described in "Isolation of *R*-ketoprofen." All the purification procedures were done at 0 to 5°C. The buffer solution used throughout was 0.03 to 0.05 M potassium phosphate buffer (pH 8.0) containing 0.1 mM EDTA and 5 mM dithiothreitol.

Step 1. Preparation of cell extract. Washed cells from 2 liters of culture broth were suspended in 103 ml of 0.03 M buffer and then disrupted in a Sonic 300 Dismembrator (Artex Systems Co.) run at maximum amplitude for 30 min. Cell debris was removed by centrifugation for 20 min at 15,000 × *g*.

Step 2. Ammonium sulfate fractionation. Saturated ammonium sulfate solution in 0.05 M buffer was added to the enzyme solution to a concentration of 30%. After the mixture had been stirred for 1 h, the precipitate formed was removed by centrifugation for 20 min at 15,000 × *g* and discarded. The ammonium sulfate concentration was increased to 70% saturation, and the mixture was again stirred for 1 h. The resulting precipitate was collected by centrifugation for 20 min at 15,000 × *g*, after which it was dissolved in 50 ml of 0.05 M buffer and dialyzed overnight against the same buffer.

Step 3. Ultracentrifugation. The enzyme solution was centrifuged at 100,000 × *g* for 2 h at 3°C with an RP50-2 rotor and a 55p-72 Himac centrifuge (Hitachi Co., Tokyo, Japan). The supernatant solution was collected.

Step 4. DEAE-cellulose column chromatography. The enzyme solution was applied to a DEAE-cellulose column (2.5 by 41 cm) equilibrated with 0.05 M buffer. After the column had been washed with buffer containing 0.1 M NaCl, it was flushed with a linear gradient of NaCl in the same buffer (0.1 to 0.5 M; 1,000 ml). The enzyme was eluted with buffer containing 0.22 M NaCl. The active fractions obtained were combined.

Step 5. Phenyl Sepharose CL-4B column chromatography. The enzyme solution was applied to a Phenyl Sepharose CL-4B column (1.7 by 22 cm) equilibrated with 0.05 M buffer. After the column had been washed with buffer containing 20% ethylene glycol (100 ml), it was flushed with a linear gradient of ethylene glycol in the same buffer (20 to 60%; 350 ml). The enzyme was eluted with buffer containing 38% ethylene glycol. The active fractions obtained were combined.

Step 6. Second DEAE-cellulose column chromatography. The enzyme solution was applied to a DEAE-cellulose column (1.7 by 22 cm) equilibrated with 0.05 M buffer, and the column was flushed with a linear gradient of NaCl in the same buffer (0 to 0.5 M; 300 ml). The enzyme was eluted with buffer containing 0.2 M NaCl. The active fractions were combined and then were concentrated by filtration on a YM10 membrane in a Diaflo Cell 8050 (Amicon Co., Danvers, Mass.).

Step 7. Sephacryl S-400 column chromatography. The concentrated solution was applied to a Sephacryl S-400 column (1.4 by 74 cm) equilibrated with 0.05 M buffer. The rate of elution was kept at 6 ml/h. The active fractions (140- to 168-ml fractions) were combined.

Analytical methods for the amidase. Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis was done with 4 to 12% polyacrylamide slab gels by using a Tris-glycine buffer system (21). The molecular weight of the subunit of the enzyme was calculated from the relative mobilities of standard proteins.

RESULTS

Screening of microorganisms. The ability to produce *R*-(−)-ketoprofen from keto-amide was examined for 54 strains isolated during screening for the production of *S*-(+)-arylpropionic acids from their corresponding amides (22) and for 350 type culture strains obtained from the American Type Culture Collection, Rockville, Md., and the Institute for Fermentation, Osaka, Japan. Forty-three distinct strains had ketoprofen-producing activity. The optical purities of the products coincided with the *S*-(+)-conformation for all the microorganisms. The same ability was examined for 563 strains isolated from 150 different soil samples. Fifty-nine strains had this activity. Results for representative strains are shown in Table 1. The optical purity of the product varied with the microorganism used. Of the active bacteria, KPO-2771-4 had both the highest level of activity and enantioselectivity in the reaction that produced the *R*-(−)-ketoprofen from racemic keto-amide.

A taxonomic study of strain KPO-2771-4 was done. The strain was gram negative and aerobic and had rod-shaped cells (0.6 to 1.3 by 1.4 to 2.7 μm). There was swimming motility, but no spores formed. The growth temperature was 5 to 37°C. The organism was oxidase and catalase positive. Tests for reduction of nitrate and nitrite were negative. The organism was negative for acid formation from glucose but positive for acid formation from fructose. Tests for arginine dihydrolase and gelatin hydrolysis were negative, whereas tests for lipase (Tween 80 hydrolysis) and cytochrome oxidase were positive. The organism did not use glucose, arabinose, mannose, mannitol, *N*-acetylglucosamine, maltose, or caprate as a carbon source but did use gluconate, acetate, adipate, malate, citrate, and phenyl acetate. The organism was positive for alkalization with allantoin or tartarate. On the basis of these results, KPO-2771-4 is considered to belong to *C. acidovorans.*

TABLE 1. Production of *R*-(−)-ketoprofen from racemic keto-amide by selected bacteria and optical purity of the product

Strain	Amt of *R*-(−)-ketoprofen produced from keto-amide[a]
HP-1913	0.46 (72.4)
PP-638	1.19 (64.6)
KPO-2001	20.0 (0.1)
KPO-2404-7	0.96 (97.4)
KPO-2519-2	0.89 (98.7)
KPO-2771-4	115 (99.9)

[a] Micromoles of ketoprofen produced per milligram of cells (dry weight) per hour. Numbers in parentheses are the optical purities of the ketoprofen produced, calculated as $[R/(S + R)] \times 100$, where *R* and *S* are the amounts of the *R* and *S* enantiomers, respectively.

TABLE 2. Purification of the amidase from *C. acidovorans* KPO-2771-4[a]

Step	Amt of total activity (U)	Amt of total protein (mg)	Sp act (U/mg)	Yield (%)
Cell extract	25.2	809	0.0311	100
$(NH_4)_2SO_4$ fractionation	21.2	391	0.0542	84.1
Ultracentrifugation	19.1	311	0.0614	75.8
DEAE-cellulose	20.5	41.4	0.500	81.3
Phenyl Sepharose CL-4B	15.5	4.35	3.56	61.5
DEAE-cellulose	10.9	1.13	9.69	43.3
Sephacryl S-400	8.60	0.743	11.5	34.1

[a] Enzyme activity was determined as described in Materials and Methods.

Effect of carbon source on growth and enzyme activity of *C. acidovorans* KPO-2771-4. Because *C. acidovorans* KPO-2771-4 does not use typical sugars such as glucose, various carbon sources such as ethanol, glycerine, acetate, actamide, citrate, and fumarate were tested for their effects on its growth and enzyme activity in rich medium without glucose. Sodium fumarate gave the best growth. Addition of the carbon sources did not greatly alter the enzyme activity or optical purity of the *R*-(−)-ketoprofen produced.

Effect of inducers on growth and enzyme activity. The effects of inducers on growth and enzyme activity were tested in rich medium with sodium fumarate as the carbon source and without ε-caprolactam (standard conditions). Additions of *n*-butyramide, *R*-keto-amide, and ammonium chloride were repressive to some extent (57, 79, and 56%, respectively, of the standard activity). ε-Caprolactam and ε-caprolactone did not influence the activity. The enzyme activity present constitutively in the cells was enhanced by 2-azacyclononanone and isobutyronitrile (143 and 147%, respectively). The optical purity of *R*-(−)-ketoprofen was not affected by the compound added. We therefore selected rich medium with 0.1% (wt/vol) isobutyronitrile as the best enhancer.

Identification of *R*-(−)-ketoprofen. The *R*-(−)-ketoprofen formed from keto-amide by resting cells of *C. acidovorans* KPO-2771-4 was isolated and crystallized as described in Materials and Methods. The nuclear magnetic resonance and infrared spectra of the product coincided with those reported previously (13). The melting point was 74 to 76°C. The specific rotation ($[\alpha]_D^{20}$) was −56.7° (c = 0.76, CH_2Cl_2). Comparison of this value with the previously reported one (−57.1°) (4) shows that the optical purity of the *R*-(−)-ketoprofen formed was in 99% enantiomeric excess, which agrees well with the results of the high-pressure liquid chromatography analysis.

Purification of the enzyme responsible for ketoprofen production. We purified the enzymes responsible for ketoprofen production and studied their characteristics in order to investigate the mechanism for the production of *R*-(−)-ketoprofen from racemic keto-amide. The amidase in *C. acidovorans* KPO-2771-4 was purified 370-fold by the procedures described in Materials and Methods (summarized in Table 2). The purified enzyme gave only one protein band during SDS-polyacrylamide gel electrophoresis and a symmetrical protein peak during gel filtration by column chromatography, indicating that it was purified to homogeneity. The molecular weight of the subunit was estimated to be 54,000 ± 2,000 by SDS-polyacrylamide gel electrophoresis as described in Materials and Methods.

Time course of the *R*-(−)-ketoprofen-producing reaction. The time course of *R*-(−)-ketoprofen production from racemic keto-amide was studied by using the purified enzyme. The amount of keto-amide was reduced rapidly to 50% of the initial concentration, after which the reaction appeared to stop (Fig. 2). Identical molar concentrations of *R*-(−)-ketoprofen and ammonia were produced in a manner corresponding to that for the decrease in the amount of keto-amide, which is evidence that the reaction was catalyzed by an amidase. No detectable amount of *S*-(+)-ketoprofen was found, and all the *S*-keto-amide remained throughout the reaction. These findings show that this enzyme is an *R*-enantioselective amidase for keto-amide.

DISCUSSION

No activity for the production of *R*-(−)-ketoprofen from the racemic keto-amide has been detected in many of the culture strains isolated during screening for the production of *S*-(+)-arylpropionic acids from their corresponding amides, nor has it been detected in many of the type culture strains. *R*-Amidase activity was not common in *R*-keto-amide-utilizing microorganisms isolated from soil samples. Only three strains produced *R*-(−)-ketoprofen from racemic keto-amide, indicating that the *S*-amidases and nonenantioselective amidases for keto-amide are widespread in microorganisms, whereas the *R*-amidase for keto-amide is rare.

Of the three active strains, KPO-2771-4 had both extremely high activity and *R*-enantioselectivity and was shown to belong to *C. acidovorans*. The other two strains, KPO-2404-7 and KPO-2519-2, were identified as belonging to the genera *Cur-*

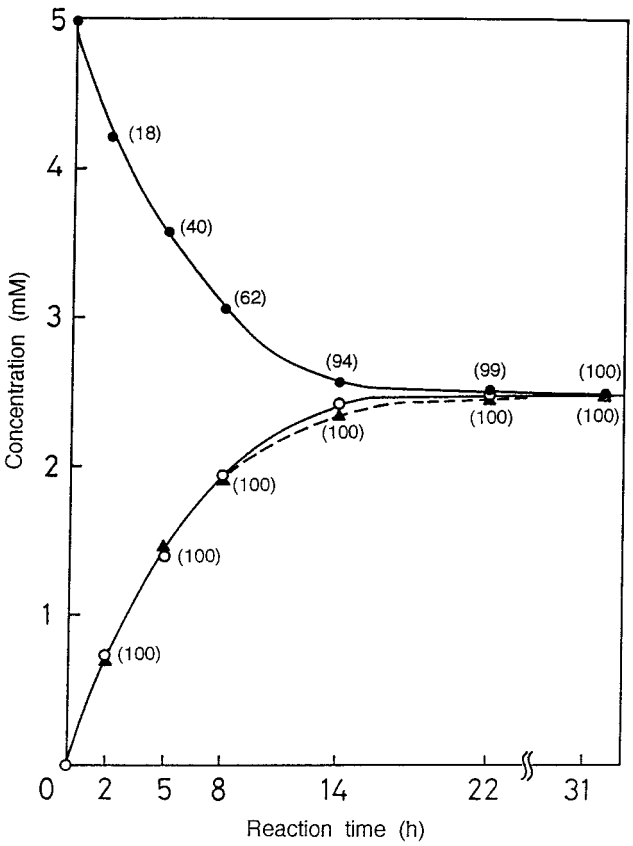

FIG. 2. Time course of *R*-(−)-ketoprofen production from keto-amide by the purified amidase from *C. acidovorans* KPO-2771-4. The reaction mixture (5 ml), containing 25 μmol of keto-amide, 250 μl of methanol, 5 mg of bovine serum albumin, 250 mmol of potassium phosphate buffer (pH 8.0), and 0.07 U of the amidase, was incubated with shaking at 30°C for 0, 2, 5, 8, 14, 22, and 31 h. The metabolites were analyzed as described in Materials and Methods. Symbols: ●, keto-amide; ○, *R*-(−)-ketoprofen; ▲, ammonia. Numbers in parentheses indicate the optical purities (% enantiomeric excess) of *S*-keto-amide remaining and *R*-(−)-ketoprofen produced, respectively.

tobacterium and *Arthrobacter*, respectively (data not shown). *C. acidovorans* KPO-2771-4 does not have nitrile-hydrolyzing enzymes (nitrilase and nitrile hydratase), unlike *Alcaligenes faecalis* ATCC 8750 (22), *Corynebacterium* sp. strain C5 (20), and *Arthrobacter* sp. strain J1 (1), which have both nitrilase and amidase or both nitrile hydratase and amidase.

The amidase of *C. acidovorans* KPO-2771-4 was purified from a cell extract, 370-fold in a yield of 34.1%, which is evidence that amidase corresponding to 0.27% of the soluble proteins was produced in cells grown under experimental conditions in which sodium fumarate was the carbon source and isobutyronitrile was the enhancer.

This *R*-enantioselective amidase was present constitutively in cells grown in the rich medium. In many microorganisms, amidases are induced by alkylamide compounds (7, 10, 11, 14). In *C. acidovorans* KPO-2771-4, however, amidase expression was repressed by the amides *n*-butylamide and *R*-keto-amide, as well as by ammonium ions. This repression by amides is considered dependent on ammonium ions released by the amides in the cells. In contrast, the alicyclic amide 2-azacyclononanone enhanced the expression of amidase, whereas the shorter-chain, alicyclic amide ε-caprolactam did not. This differs from *Rhodococcus butanica* amidase induced with ε-caprolactam (12). On the basis of these results, we concluded that the expression of the amidase from *C. acidovorans* KPO-2771-4 is affected by ammonium ions and amide compounds.

The expression of the amidase from *C. acidovorans* KPO-2771-4, however, was enhanced when isobutyronitrile was added to the medium. Induction of acetonitrile has been reported for *Arthrobacter* sp. strain J1 amidase (1), but in that case, induction of both nitrile hydratase and amidase is essential for metabolizing acetonitrile and for growth. *C. acidovorans* KPO-2771-4 does not have nitrile hydratase. The reason for this change in expression needs to be clarified by further research on the genetic expression mechanism of this amidase.

The subunit molecular weight of the amidase is 54,000 ± 2,000. The amidase is larger than the amidases from *Pseudomonas aeruginosa* (33,000 to 35,000) (5), *Brevibacterium* sp. strain R312 (43,000) (7), *Arthrobacter* sp. strain J1 (39,000) (1), and *Rhodococcus* spp. (44,500 to 49,000) (17, 18).

The stoichiometric production of optically pure *R*-(−)-ketoprofen and ammonia, in a manner that corresponds to the decrease in the amount of keto-amide and in the time course of *R*-(−)-ketoprofen production from racemic keto-amide by using the purified enzyme, shows that this amidase has high-level enantioselectivity for *R*-keto-amide, which is evidence that *C. acidovorans* KPO-2771-4 amidase differs from the *S*-enantioselective amidases of *Brevibacterium* sp. strain R312 and a *Rhodococcus* sp. strain (17), *Rhodococcus equi* TG328 (10), a *Rhodococcus* sp. (strain SP 361) (3), and *Rhodococcus* sp. strain C3 II (8). Ours therefore is the first report of the production of *R*-arylpropionic acid from the corresponding racemic amide by using *R*-enantioselective amidase. The enzyme's properties and amino acid or base sequences will be reported elsewhere.

REFERENCES

1. **Asano, Y., M. Tachibana, Y. Tani, and H. Yamada.** 1982. Purification and characterization of amidase which participates in nitrile degradation. Agric. Biol. Chem. **46:**1175–1181.
2. **Balows, A., H. G. Trüper, M. Dworkin, W. Harder, and K. H. Schleifer (ed.).** 1992. The prokaryotes, 2nd ed. Springer-Verlag Co., Berlin.
3. **Beard, T., M. A. Cohen, J. S. Parratt, N. J. Turner, J. Crosby, and J. Moilliot.** 1993. Stereoselective hydrolysis of nitriles and amides under mild conditions using a whole cell catalyst. Tetrahedron Asymmetry **4:**1085–1104.
4. **Blazevic, N., M. Zinic, T. Kovac, V. Sunjic, and F. Kajfez.** 1975. Identification methods for (+ −)-alpha-(3-benzoylphenyl)propionic acid (Ketoprofen) and its enantiomers based on physicochemical properties. Acta Pharm. Jugosl. **25:**155–164.
5. **Brown, P. B., M. J. Smith, P. H. Clarke, and M. A. Rosemeyer.** 1973. The subunit structure of the aliphatic amidase from *Pseudomonas aeruginosa*. Eur. J. Biochem. **34:**177–187.
6. **Caldwell, J., A. J. Hutt, and S. Fournel-Gigleux.** 1988. The metabolic chiral inversion and dispositional enantioselectivity of the 2-arylpropionic acids and their biological consequences. Biochem. Pharmacol. **37:**105–114.
7. **Chion, C. K. N. C. K., R. Duran, A. Arnaud, and P. Galzy.** 1991. N-terminal amino acid sequence of *Brevibacterium* sp. R312 wide-spectrum amidase. Appl. Microbiol. Biotechnol. **36:**205–207.
8. **Effenberger, F., and J. Böhme.** 1994. Enzyme-catalyzed enantioselective hydrolysis of racemic naproxen nitrile. Bioorg. Med. Chem. **7:**715–721.
9. **Fawcett, J. K., and J. E. Scott.** 1960. A rapid and precise method for the determination of urea. J. Clin. Pathol. **13:**156–159.
10. **Gilligan, T., H. Yamada, and T. Nagasawa.** 1993. Production of *S*-(+)-2-phenylpropionic acid from (*R,S*)-2-phenylpropionitrile by the combination of nitrile hydratase and stereoselective amidase in *Rhodococcus equi* TG328. Appl. Microbiol. Biotechnol. **39:**720–725.
11. **Joeres, U., and M. R. Kula.** 1994. Screening for a novel enzyme hydrolyzing L-carnitine amide. Appl. Microbiol. Biotechnol. **40:**599–605.
12. **Kakeya, H., N. Sakai, T. Sugai, and H. Ohta.** 1991. Microbial hydrolysis as a potent method for the preparation of optically active nitriles, amides and carboxylic acids. Tetrahedron Lett. **32:**1343–1346.
13. **Kawai, N., N. Kato, Y. Hamada, and T. Shioiri.** 1983. New methods and reagents in organic synthesis. 35. A new synthesis of some non-steroidal anti-inflammatory agents with the 2-arylpropionic acid skeleton by the use of diphenyl phosphorazidate (DPPA) as a 1,3-dipole. Chem. Pharm. Bull. **31:** 3139–3148.
14. **Kobayashi, M., H. Komeda, T. Nagasawa, H. Yamada, and S. Shimizu.** 1993. Occurrence of amidases in the industrial microbe *Rhodococcus rhodochrous* J1. Biosci. Biotechnol. Biochem. **57:**1949–1950.
15. **Krieg, N. R., and J. G. Holt (ed.).** 1984. Bergey's manual of systematic bacteriology, vol. 1. Williams & Wilkins Co., Baltimore.
16. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193:**265–275.
17. **Mayaux, J.-F., E. Cerbelaud, F. Soubrier, P. Yeh, F. Blanche, and D. Pétré.** 1991. Purification, cloning, and primary structure of a new enantiomer-selective amidase from a *Rhodococcus* strain: structural evidence for a conserved genetic coupling with nitrile hydratase. J. Bacteriol. **173:**6694–6704.
18. **Nawaz, M. S., A. A. Khan, J. E. Seng, J. E. Leakey, P. H. Siitonen, and C. E. Cerniglia.** 1994. Purification and characterization of an amidase from an acrylamide-degrading *Rhodococcus* sp. Appl. Environ. Microbiol. **60:**3343–3348.
19. **Spickett, R. G. W., V. Armando, P. Jose, M. Jacinto, M. Miguel, and D. J. Roberts.** 1976. Anti-inflammatory and related pharmacological properties of amides of substituted aryl alkanoic acids. Eur. J. Med. Chem. Chim. Ther. **11:**7–12.
20. **Tani, Y., M. Kurihara, and H. Nishise.** 1985. Characterization of nitrile hydratase and amidase, which are responsible for the conversion of dinitriles to mononitriles, from *Corynebacterium* sp. Agric. Biol. Chem. **53:**3151–3158.
21. **Weber, K., and M. Osborn.** 1969. The reliability of molecular weight determinations by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. J. Biol. Chem. **244:**4406–4412.
22. **Yamamoto, K., K. Oishi, I. Fujimatsu, and K.-I. Komatsu.** 1991. Production of R-(−)-mandelic acid from mandelonitrile by *Alcaligenes faecalis* ATCC 8750. Appl. Environ. Microbiol. **57:**3028–3032.
23. **Yamamoto, K., Y. Ueno, K. Otsubo, K. Kawakami, and K.-I. Komatsu.** 1990. Production of *S*-(+)-ibuprofen from a nitrile compound by *Acinetobacter* sp. strain AK226. Appl. Environ. Microbiol. **56:**3125–3129.
24. **Young, J. W., N. M. Gray, and W. J. Wechter.** September 1993. Pharmaceutical compositions containing optical pure R(−) ketoprofen. WO patent 9317677 A1.