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Bifunctional Group Participated Nitrile Group Hydrolyzing Enzyme Model Systems: Hydrolysis of the Nitrile Group of α-Aminophenylacetonitrile to Phenylglycineamide and Phenylglycine by Various Thiol Compounds

Young Bok Lee, Yang Mo Goo and Jae Keun Lee*

College of Pharmacy, Seoul National University, Seoul 151-742, and
**Department of Chemistry, Kyoungbook National University, Taegu 702-701, Korea*

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Abstract□2-Mercaptoethanol, thioglycolic acid, glutathione, 3-mercapto-1,2-propanediol and 3-mercapto-2-butanol showed catalytic activities on the hydrolysis of α-aminophenylacetonitrile to phenylglycineamide at the rate of 12.19×10^{-2}, 8.03×10^{-2}, 6.83×10^{-2}, 8.60×10^{-2} and 6.04×10^{-2} mM min^{-1}, respectively. The hydrolysis rate was faster in buffer than in water. The hydrolysis of the nitrile compound to phenylglycine was limited.

Keywords□α-Aminophenyl acetonitrile, phenylglycineamide, nitrile compound, hydrolysis rate.

Although chemically a nitrile compound is known to be hydrolyzed to an acid through an amide under strong basic or acidic condition,[1] biological hydrolysis of the nitrile is catalyzed by enzymes to proceed directly either to the corresponding acid or to the amide under much milder conditions. The enzymes involved in the hydrolysis of the nitrile group are classified into two separate groups: nitrilase[2,5] and nitrile hydratase.[3] The former is known to hydrolyze a nitrile compound to an acid directly and the latter to hydrolyze to an amide. The amide is hydrolyzed to the corresponding acid by amidase. The nitrile group-hydrolyzing enzymes were known to be inhibited by $AgNO_3$, $HgCl_2$, *p*-chloromercurybenzoate (pCMB) and *p*-chloromercuryphenylsulfonic acid (pCMPSA),[4] and the formation of an enzyme-bound thioimidate intermediate during hydrolysis of the nitrile compound was suggested.[5] For the exploration of the active site of the nitrile group-hydrolyzing enzymes, various thiol compounds were examined for the ability of hydrolyzing the nitrile group of α-aminophenylacetonitrile.

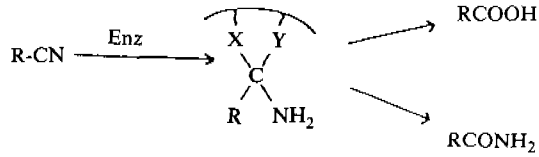
The result indicated that monofunctional thiol compounds such as ethanethiol, thiophenol and thioacetic acid could not hydrolyze the nitrile group of α-aminophenylacetonitrile at all. However, the thiol compounds having second functional group showed strong catalytic activity on the hydrolysis of the nitrile compound.[6] From these observations, we have recently proposed a new, bifunctional group-participated enzyme model system for the nitrile group-hydrolyzing enzymes.[6] At this proposal, we assumed a bifunctional group-participated tetrahedral intermediate as the intermediate formed during hydrolysis of a nitrile group by nitrile group-hydrolyzing enzymes as shown in scheme 1.

In this report we want to report general characteristics of bifunctional compounds having the catalytic activity on the hydrolysis of the nitrile group of α-aminophenylacetonitrile.

RESULTS AND DISCUSSION

We studied the microbiological production of D-phenylglycine by the hydrolysis of the nitrile group of α-aminophenylacetonitrile, and screened nitrilase in various microorganisms.[7] Also, we tried to explore the active site and the hydrolysis mechanism of nitrile group-hydrolyzing enzymes. As a part of this study, we examined many sulfur compounds that could mimic the active site of the enzymes by showing the catalytic activity on the hydrolysis of the nitrile group of α-aminophenylacetonitrile. Thus, various compounds having diverse

Scheme 1



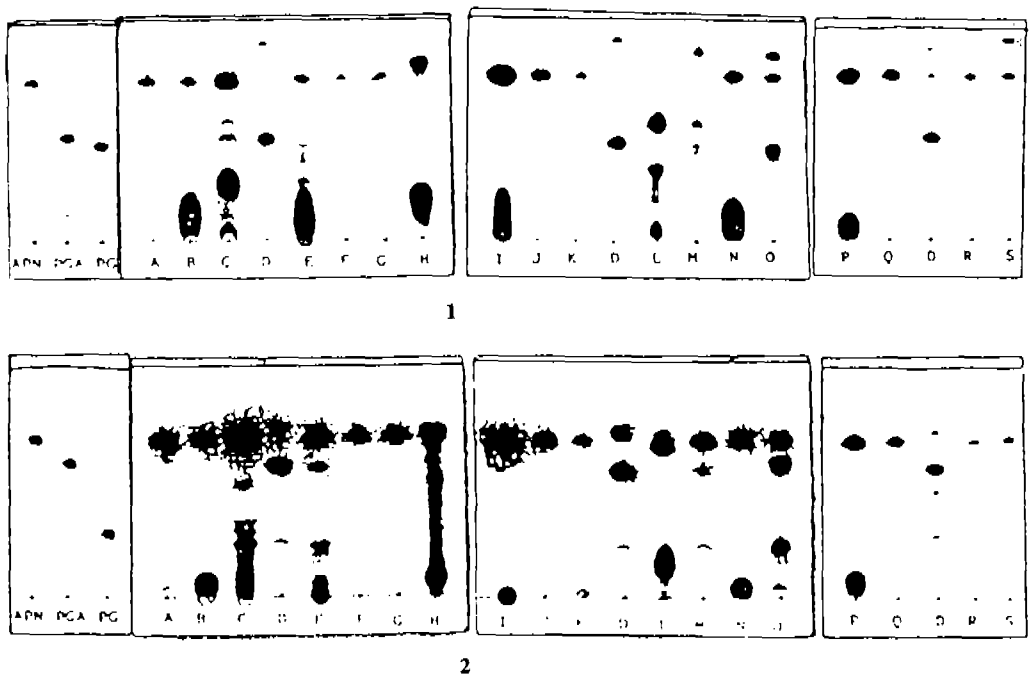


Fig. 1. TLC analysis (1, n-BuOH-AcOH-H_2O = 4:1:1; 2, i-PrOH-NH_4OH-H_2O = 20:1:1) of the products formed after 17 hrs' shaking of α-aminophenylacetonitrile with various monofunctional and bifunctional compounds.
APN: α-aminophenylacetonitrile, PGA: phenylglycineamide, PG: phenylglycine, A: TEMED, B: serine, C: cysteine-HCl, D: 2-mercaptoethanol, E: glutathione, F: glycerol, G: ethanethiol, H: ethanolamine, I: aspartic acid, J: dithiothreitol, K: ethylene glycol, L: cysteamine, M: 1,2-ethanedithiol, N: glycine, O: thioglycolic acid, P: histidine-2HCl, Q: thiophenol, R: imidazole, S: thiosalicylic acid.

functional groups were mixed with the nitrile compound in phosphate buffer and the mixtures were incubated on a rotary shaker at 28 °C. The incubated mixtures were examined by TLC at regular time intervals. The thin layer chromatograms shown in Fig. 1 were obtained by spotting 4.0 μl of the reaction mixture on silica gel plates.

The plates were developed in two different solvent systems and colorized with ninhydrin. Some of the mixtures showed the presence of phenylglycineamide and phenylglycine in the reaction mixture. The R_f values of the ninhydrin-positive spots were compared with those of phenylglycineamide and phenylglycine. To confirm the identity of the compounds formed in the reaction mixture the R_f values of two different developing solvent systems were compared.

From these studies we found that glutathione, 2-mercaptoethanol, 1,2-ethanedithiol, thioglycolic acid and cysteine could hydrolyze the nitrile compound. 2-Mercaptoethanol converted the substrate almost completely into phenylglycineamide and phenylglycine approximately in the ratio of 90:5 after 17 hrs' shaking, whereas under the same conditions glutathione showed about 20% conversion of the substrate into these products in the ratio of 1:1. Thioglycolic acid converted the substrate into these products about 80% in the ratio of 1:1. When 1,2-ethanedithiol was added in the buffer the substrate was almost completely consumed but the amounts of the hydrolyzed products were small. Cysteine led to many products, one of which was suspected to be phenylglycine. The relative ratios of the products formed were approximated by measurement of the optical density of each spot after colorization of the amine containing compound by spraying with ninhydrin (Table 1). Although several reports discussed the involvement of an enzyme-bound thioimidate during hydrolysis of a nitrile compound[5], as shown in Fig. 1 and Table 1, monofunctional thiol compounds such as ethanthiol, thiophenol and thioacetic acid did not show catalytic activity on the hydrolysis of the nitrile compound after 17 hrs' stirring at 28 °C.

Our result indicated that the thiol compounds with a second functional group such as a hydroxyl,

Table I. The results of various compounds examined for the catalytic activity on the hydrolysis of α-aminophenylacetonitrile after 17 hrs incubation at 28 °C

Compounds	*PGA	*PG
*TEMED		
Serine		
Cysteine		+ +**
Mercaptoethanol	+ + + + + +	+
Glutathione	+ +	+ +
Glycerol		
Ethanethiol		
Ethanolamine		
Aspartic acid		
Dithiothreitol		
Ethylene glycol		
Cysteamine		
1,2-Ethanedithiol	+ +	+ +
Glycine		
Thioglycolic acid	+ + +	+ + +
Histidine		
Thiophenol		
Imidazole		
Thiosalicylic acid		

*PGA: phenylglycineamide, PG: phenylglycine, TEMED: N,N,N'N'-tetramethylethylenediamine

**The relative numbers of + indicate relative amounts of phenylglycine or phenylglycineamide formed after shaking the reaction mixture for 17 hrs. The relative amounts were obtained by a densitometer for the spots produced by ninhydrin spray.

a carboxylate or a thiol group at the proper position can catalyze the hydrolysis of the nitrile compound to the amide or to the acid. The hydrolysis of the nitrile group of α-aminophenylacetonitrile catalyzed by 2-mercaptoethanol produced phenylglycineamide as the major product with very little amount of phenylglycine but its amount produced was a little variable depending on the conditions employed. However, we could not find out a condition that can increase the amount of phenylglycine. The possiblity of further hydrolysis of the amide produced to the corresponding acid was excluded since further stirring of phenylglycineamide with 2-mercaptoethanol did not produced any phenylglycine. When thioglycolic acid was used as the catalyst for the hydrolysis of the nitrile compound,

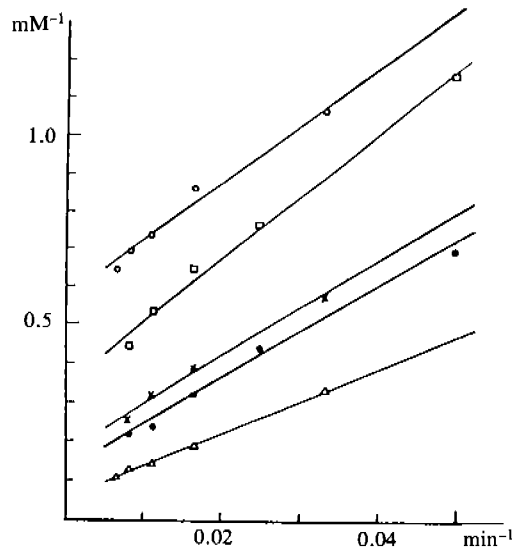


Fig. 2. Double reciprocal plot of the concentration of phenylglycineamide formed as a function of time: o, glutathione (reduced form); □, 3-mercapto-2-butanol; x, thioglycolic acid; ●, 3-mercapto-1,2-propanediol; △, 2-mercaptoethanol.

phenylglycineamide and phenylglycine were produced about equal amounts. In case of cysteine no phenylglycineamide was detected at all. Without addition of 2-mercaptoethanol or other thiol compounds α-aminophenylacetonitrile was not hydrolyzed at all.

We examined several other bifunctional thiol compounds, 3-mercapto-2-butanol, 3-mercapto-1, 2-propanediol, 2-mercaptopropionic acid, 3-mercaptopropionic acid, mercaptosuccinic acid to see the generality of the catalytic activity on the hydrolysis of the nitrile compound, but only 3-mercapto-2-butanol and 3-mercapto-1,2-propanediol catalyzed the hydrolysis of the substrate to phenylglycineamide. Other thiol-acid compounds on the incubation with the nitrile compound produced some unidentified products but none seemed to be phenylglycine or phenylglycineamide.

The relative initial rates of the hydrolysis of the nitrile group of α-aminophenylacetonitrile to phenylglylcineamide catalyzed by 2-mercaptoethanol, glutathione, thioglycolic acid, 3-mercapto-1,2-propanediol, and 3-mercapto-2-butanol are shown in Fig. 2 and Table 2. The velocities obtained from the double reciprocal plot (Fig. 2) of the concentration of phenylglycineamide formed against time (minutes) are given in Table 2. The initial velocity of 2-mercaptoethanol-catalyzed hydrolysis of the

Table II. The initial velocities for the hydrolysis of α-aminophenylacetonitrile to phenylglycineamide by thiol compounds (48.0 mM) in phosphate buffer (50 mM pH 7.1, τ½ = 1.0 with KCl) at 28°C

Compound	$V_o(\times 10^2)$ mM min^{-1}
3-mercapto-2-butanol	6.04 ± 0.20
3-mercapto-1,2-propanediol	8.60 ± 0.0034
glutathione	6.83 ± 0.055
thioglycolic acid	8.03 ± 0.0067
2-mercaptoethanol	12.19 ± 0.015

nitrile compound to phenylglycineamide was found to be increased on the concentration of the catalyst, 2-mercaptoethanol linearly. However, the catalytic mechanism of 2-mercaptoethanol seems to be more complex than that to be assumed as a simple catalyst.

As shown in Table 2, 2-mercaptoethanol hydrolyzed the nitrile compound at a rate of 12.19×10^{-2} mM min^{-1} which is 1.5-2.0 times faster than the other compounds. 3-Mercapto-1,2-propanediol, thioglycolic acid, glutathione and 3-mercapto-2-butanol showed initial velocities at 8.60×10^{-2}, 8.03×10^{-2}, 6.83×10^{-2} and 6.04×10^{-2} mM min^{-1}, respectively. The initial velocities for the 2-mercaptoethanol-catalyzed hydrolysis of α-aminophenylacetonitrile in water and in phophate buffer (pH = 6.75, 18.7 mM, the ionic strength was adjusted by adding KCl to 1.0) are found to be $(2.47 \pm 0.017) \times 10^{-2}$ and $(7.59 \pm 0.071) \times 10^{-2}$ mM min^{-1}, respectively (Fig. 3).

The 2-mercaptoethanol-catalyzed hydrolysis of the nitrile compound to the amide was about 3 times faster in the buffer (18.7 mM) than in H_2O. The linear increase of the hydrolysis rate was observed as the concentration of the buffer was increased up to 200o mM[8]. The hydrolysis reaction was found to be completely independent of the ionic strength of the medium[8]. Currently the possibility of general acid or general base catalytic mechanism on the hydrolysis of the nitrile compound to the amide by 2-mercaptoethanol was under examination. The data obtained up to now seemed to suggest that 2-mercaptoethanol acts as a general base catalyst[8].

The results given in Table 2 say that 2-mercaptoethanol is the best catalytic agent for the hydrolysis of α-aminophenylacetonitrile in our study. Also, they support the suggestion that a special geometric arrangement of the thiol group with another functional group is necessary for the catalytic activity on the hydrolysis of the nitrile group. The reaction rate was also found to be very much dependent on the pH of the medium[8].

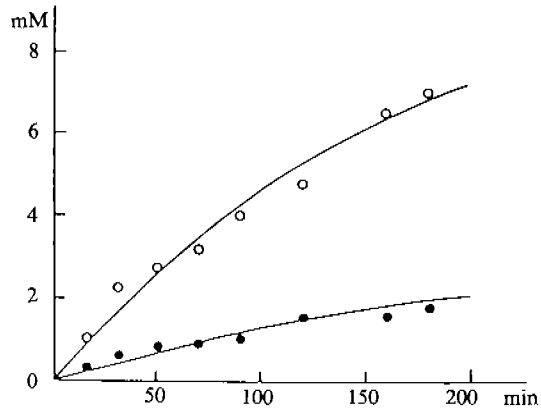


Fig. 3. The initial velocities of the hydrolysis of α-aminophenylacetonitrile (39.5 mM) to phenylglycineamide by mercaptoethanol (74.2 mM) in 18.7 mM phosphate buffer (○) (pH 6.75, τ½ = 1.0) and water (●) (τ½ = 1.0) at 28°C. The amount of phenylglycineamide formed was plotted against the reaction time.

Alkaline hydrolysis of the nitrile group of α-aminophenylacetonitrile under the same condition (28-37°C, rotary shaking) was observed in sodium hydroxide solution, only at 0.1 N or at higher concentration but below this concentration, the hydrolysis of the reaction was too slow to be detected by TLC. When the alkaline hydrolysis of α-aminophenylacetonitrile was carried out, phenylglycineamide was obtained at mild condition (0.01-0.02 N of NaOH) but when the reaction was carried out under a little stronger basic condition (above 0.02 N of NaOH) or when the reaction mixture was stirred for a little longer time, the amide produced was converted to the acid.

At strong acidic condition such as the solution of 0.1 N HCl, or CCl_3COOH, (no addition of thiol compounds) the nitrile compound was not hydrolyzed at all, by stirring or by shaking on a rotary shaker at room temperature or at 28°C. Even 2-mercaptoethanol added in the buffer (CCl_3COOH-CCl_3COONa), the pH of which adjusted below 2.0 catalyzed the hydrolysis of the nitrile compound extremely slowly. Hydrolysis of other simple nitrile compounds such as acetonitrile or benzylcyanide by 2-mercaptoethanol was not successful. The failure is probably due to the reactivity of the nitrile groups. It was reported that the hydrolysis of simple nitrile

compounds, such as alkylnitrile in sodium hydroxide solution was about 10,000 times or more slower than that of α-aminophenylacetonitrile in the same solution.

We suspected the formation of a tetrahedral intermediate as shown in Scheme 1 during hydrolysis of a nitrile catalyzed by 2-mercaptoethanol, and we presumed that two pathways were involved in the 2-mercaptoethanol-catalyzed hydrolysis of the nitrile compound; in one of the pathways, the tetrahedral intermediate seems to be decomposed directly to phenylglycineamide and in the other the tetrahedral intermediate seems to be decomposed to an imidate which is further hydrolyzed to phenylglycinamide[9].

Currently we are trying to solve all the mechanisms involved 2-mercaptoethanol-catalyzed hydrolysis of the nitrile compound looking for the confirmative evidences. Our study certainly suggests that the nitrile group-hydrolyzing enzymes should need a second functional group in addition to the thiol group for the hydrolysis of a nitrile compound either to an acid or an amide directly. The formation of either the amide or the acid seems to be dependent on the leaving abilities of the functional group participated in the formation of the tetrahedral intermediate as suggested by the hydrolysis of the nitrile compound by thioglycolic acid. At the present time the relevance of the bifunctional catalytic system to that of the nitrile group-hydrolyzing enzymes is still to be further explored.

EXPERIMENTAL

General

^{1}H NMR spectra were obtained with a JEOL NMR spectrometer (90 MHz) or a Brucker WO80SY NMR spectrometer (80 MHz), and TMS was used for internal standard. IR spectra were obtained with Beckman IR-20A and UV analysis was done with LKB ultraspec 4050 spectrometer. Perkin-Elmer 240C was used for C.H.N. analysis and Melting points were determined with a Gallenhamp melting point apparatus and are uncorrected. TLC analysis was done by using a commercial silica gel TLC plate (0.25 mm thickness, Riedel-De Haen Aktiengesellschft, Seelze-Hannover), and the amount of the density of the colorized spot was measured with Shadon Sourthern Cosmo Densitometer (Super Click Model D-101B Cosmo Co. Ltd.). The TLC plates were developed and visualized by spraying with ninhydrin solution (1.5% in acetone).

Synthesis of α-aminophenylacetonitrile

α-Aminophenylacetonitrile was prepared from benzaldehyde by cyanoamination reaction following the reported procedure[10] in 60% yield. mp. 55 °C (free form); $\lambda_{max} = 255.8$ nm ($\varepsilon = 312$ M^{-1} cm^{-1} in phosphate buffer, pH = 6.5, 25 mM, 25 °C); IR (KBr), 1720, 1580, 1400 cm^{-1}; ^{1}H-NMR (DMSO-d_6), δ 6.00 (s, 1-H) and 7.45 ppm (Ph).

Synthesis of phenylglycineamide[11]

Phenylglycineamide was prepared by hydrolysis of α-aminophenylacetonitrile with sodium hydroxide (5 N) in acetone. mp. 210 °C (decomposed); $\lambda_{max} = 254.7$ nm ($\varepsilon^{25\,°C} = 295$ $M^{-1}cm^{-1}$ in phosphate buffer, pH = 6.5, 25 mM); IR (KBr), salt form, 1720, 1480 cm^{-1}; ^{1}H-NMR (DMSO-d_6), 4.81 (s, 1H), 7.46 ppm (Ph). Anal. Calcd. for $C_8H_{10}N_2O$: C, 63.96; H, 6.71; N, 18.65. Found: C, 62.77; H, 6.66; N, 18.56.

Reaction of α-aminophenylacetonitrile with several mono- or bifunctional compounds

α-Aminophenylacetonitrile-HCl (32.9 mg, 39.2 mM) was dissolved in potassium phosphate buffer (5 m*l*, 25 mM, pH 6.5) with 74.0 mM of dithiothreitol (57.8 mg), 2-mercaptoethanol (26.3 μ*l*), ethyleneglycol (21.0 μ*l*), cysteamine (28.9 mg), 1,2-ethanedithiol(31.5 μ*l*), glycine (28.2 mg), N,N, N'N'-tetramethylethylenediamine (57.0 μ*l*), serine (39.4 mg), cystein-HCl (59.1 mg), glutathione (reduced form, 115.0 mg), glycerol (27.3 μ*l*), ethanethiol (28.0 μ*l*), ethanolamine (22.6 μ*l*), L-aspartic acid-HCl (63.6 mg), histidine-2HCl (85.5 mg), thiophenol (38.5 μ*l*), imidazole (25.5 mg), or sodium thiosalicylate (66.0 mg) and shaken on a rotary shaker (180 rpm) at 28 °C. The reaction mixtures (4.0 u*l*) were developed in two TLC plates after 17 hrs. TLC plates were developed in two different solvent systems; n-BuOH-AcOH-H_2O (4:1:1) and i-PrOH-NH_4OH (28%)-H_2O (20:1:1). After full development of the TLC plates, they were dried with air, sprayed with ninhydrin solution (1.5% in acetone) and baked at 110 °C for 10 min. The density of the color produced on each spot was read with densitometer at visible light range without any filters. Authentic samples were spotted on the same plates and calibration curves were obtained to calculate the concentration of the components produced in each reaction mixture.

Preparation of phenylglycineamide from α-aminophenylacetonitrile by catalytic hydrolysis with 2-mercaptoethanol

α-Aminophenylacetonitrile-HCl (2.0 g, 120 mM)

was dissolved in potassium phosphate buffer (100 mM, 100 m*l*) with 2-mercaptoethanol (2.1 m*l*, 250 mM) and the mixture was stirred at room temperature for 6 hr. The reaction mixture was washed with benzen (3 times with 70 m*l*) and evaporated under reduced pressure to give a residue, which was chromatographed on a column packed with Sephadex G-25 to give a white solid residue. ^{1}H-NMR, δ, 4.64 (br. NH_3^+), 4.78 (s, 1H), 7.46 (Ph) and 8.00 ppm (NH_2); IR(KBr), 1720 and 1480 cm^{-1}. The residue was dissolved in water, and the pH of the solution was adjusted to 12 by adding 1N NaOH. The solution was extracted with ethyl acetate. Evaporation of the extract gave phenylglycineamide (1.3 g, yield: 78%, mp, 210°C. decomposed); IR(KBr), 1660, 1565, 1410 cm^{-1}; $\lambda_{max} = 254$ nm ($\varepsilon = 300$ dm $mol^{-1}cm^{-1}$ in phosphate buffer, pH 6.5, 25 mM); $R_f = 0.47$ (n-BuOH-AcOH-H_2O = 4:1:1), $R_f = 0.54$ (i-PrOH-NH_4OH (28%)-H_2O = 20:1:1), $R_f = 0.51$ (n-BuOH-pyridine-H_2O = 8:2:1).

Comparison of the velocities of several bifunctional compounds on the hydrolysis of the nitrile group of α-aminophenylacetonitrile

Glutathione (reduced form, 73.8 mg, 48.0 mM), thioglycolic acid (16.9 μl, 48.0 mM), 2-mercaptoethanol (16.8 μl, 48.0 mM), 3-mercapto-2-butanol (25.5 μl, 48.0 mM) and 3-mercapto-1,2-propanediol (20.0 μl, 48.0 mM) were dissolved in 5 m*l* of phosphate buffer (50.0 mM, pH 7.1), respectively, the ionic strength of which was adjusted to 1.0 by addition of KCl. Then α-aminophenylacetonitrile-HCl (33.4 mg, 40.0 mM) was added to the solution in solid state and started to incubate on a rotary shaker (180 rpm) at 28°C. Individual reaction mixture (5.0 u*l*) was spotted on the silica gel TLC plate at certain time intervals. Authentic samples, α-aminophenylacetonitrile and phenylglycineamide were also spotted on the same TLC plates at various concentrations. The TLC plates were developed in n-BuOH-pyridine-H_2O (8:2:1) (v/v%). The developed TLC plates were dried with air and sprayed with ninhydrin (1.5% in acetone) and baked at 110°C for 10 min. The density of the color produced on each spot was read with the densitometer at visible light range. The amount of the product formed was calculated from the calibration curve obtained from the spots of authentic sample.

Comparison of the hydrolysis rate in potassium phosphate buffer and water

2-Mercaptoethanol solution (0.26 m*l*, 1426.0 mM) was added to the potassium phosphate buffer solution (3.74 m*l*, 25.0 mM) or to water (3.74 m*l*) to which α-aminophenylacetonitrile solution (0.5 m*l*, 395 mM) was added. The mixture was shaken on a rotary shaker (180 rpm) at 28°C. The reaction mixture (5.0 μl) was spotted on the TLC plates (silica gel) at time intervals with authentic samples. The TLC plates were developed and colorized by the same method and the reaction rates were obtained by the same method.

ACKNOWLEDGEMENT

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LITERATURE CITED

1. J. March, *'Advanced Organic Chemistry'*, Wiley, New York, 1985. p. 788.
2. Kuwahara, M., Yanase, H., Ishida, Y., and Kikuchi, Y.: Metabolism of aliphatic nitrile in *Fusarium solani*. *J. Ferment. Technol.* **58**, 573 (1980); Harper, D.B.: Microbial metabolism of aromatic nitriles. *Biochem. J.* **165**, 309 (1977).
3. Bui, K., Fradet, H., Arnaud, A., and Galzy, P.: A nitrile hydratase with a wide substrate spectrum produced by a *Brevibacterium* sp.. *J. Gen. Microbiol.*, **130**, 89 (1984); Asano, Y., Fujishiro, K., Tani, Y., and Yamada, H.: Aliphatic nitrile hydratase from *Athrobacter* sp. J-1, Purification and characterization. *Agric. Biol. Chem.* **46**, 1165 (1982).
4. Yanase, H., Sakai, T., and Tonomura, K.: Purification, crystallization and some properties of β-cyano-L-alanine-degrading enzyme in *Pseudomonas* sp. 13. *Agric. Biol. Chem.* **47**, 473 (1983); Asano, Y., Tachibana, M., Tani, Y., and Yamada, H.: Purification and characterization of amidase which participates in nitrile degradation. *Agric. Biol. Chem.* **46**, 1175 (1982).
5. Harper, D.B.: Fungal degradation of aromatic nitriles; Enzymology of C-N cleavage by *Fusarium solani*. *Biochem. J.*, **167**, 685 (1977); Hook, R.H., and Robinson, W.G.: Ricinine nitrilase. *J. Biol. Chem.* **239**, 4263 (1964).
6. Goo, Y.M., and Lee, Y.B.: Catalytic activity of some bifunctional compounds on the hydrolysis of a nitrile group; A new effective method for hydrolysis of α-aminophenylacetonitrile to phenylglycinamide. *J. Chem. Soc., Chem. Commun.* 1247 (1986).

7. Choi, S.Y., and Goo, Y.M.: Hydrolysis of the nitrile group in α-aminophenylacetonitrile by nitrilase; Development of a new biotechnology for stereospecific production of *S*-α-phenylglycine. *Arch. Pharm. Res.* **9**, 45 (1986); Goo, Y.M.: Nitrile group hydrolyzing enzymes in fungi, yeast, streptomycetes, and bacteria; Biochemical hydrolysis of the nitrile group in α-aminophenylacetonitrile to phenylglycine. *Korean Biochem. J.* **19**, 67 (1986).
8. Lee, Y.B.: Model studies on the nitrile group-hydrolyzing enzymes. *M.S. thesis, Seoul National University,* (1986).
9. Goo, Y.M., and Lee, Y.B.: Evidences for the formation of a tetrahedral intermediate during the hydrolysis of nitrile compounds by 2-mercaptoethanol. 1989, to be submitted.
10. Steiger, R.E.: *dl*-α-aminophenylacetic acid. *Org. Synth., Col. Vol. III,* 84 (1955).
11. Ger. Offen., 2,637,204 (*Chem. Abstr.,* **87**, 39839 (1977)).