

# Catalytic Activity of Some Bifunctional Compounds on the Hydrolysis of a Nitrile Group; a New Effective Method for Hydrolysis of $\alpha$ -Aminophenylacetonitrile to Phenylglycinamide

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Various bifunctional compounds, 2-mercaptoethanol, glutathione, cysteine, and ethane-1,2-dithiol showed catalytic activity on the hydrolysis of the nitrile group of  $\alpha$ -aminophenylacetonitrile.

Models for two typical hydrolysing enzymes, serine protease<sup>1</sup> and cysteine protease,<sup>2</sup> have been studied extensively but currently very few model studies have been performed on nitrile group-hydrolysing enzymes.<sup>3</sup> Chemically, nitriles are hydrolysed to the acid *via* the amide under acidic or basic conditions,<sup>4</sup> whereas in biological systems nitriles are hydrolysed by two distinct enzymes: nitrilase<sup>5,6</sup> and nitrile hydratase,<sup>7</sup> which lead to the acid and the amide, respectively. The types of substrate-enzyme intermediates which develop during enzymatic hydrolysis of nitriles are as yet unknown, but we assume that, since two distinct enzymes are known and one of them produces the acid directly, they form a tetrahedral complex with the substrate, decomposition of which would give either the acid or the amide as shown in Scheme 1. During investigations of the stereospecific hydrolysis of  $\alpha$ -aminophenylacetonitrile to *D*-phenylglycine with nitrilase from *Aspergillus fumigatus*,<sup>8</sup> in order to demonstrate the concept in Scheme 1, we have examined the possible catalytic activity of several bifunctional compounds on the hydrolysis of the nitrile group in  $\alpha$ -aminophenylacetonitrile and found that some cause hydrolysis very effectively. We now report our results.

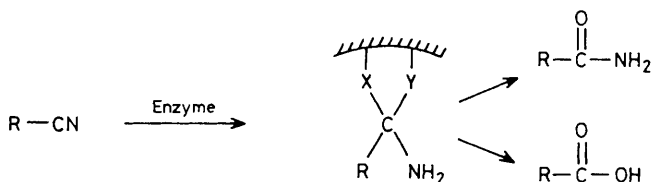
When  $\alpha$ -aminophenylacetonitrile-HCl<sup>9</sup> (32.9 mg, 40.0 mM) was dissolved in potassium phosphate buffer (5 ml, 25 mM, pH 6.5) with 14.0 mM dithiothreitol (57.8 mg), 2-mercaptoethanol (26.3  $\mu$ l), ethylene glycol (21.0  $\mu$ l), cysteamine (28.9 mg), ethane-1,2-dithiol (31.5  $\mu$ l), glycine (28.2 mg), *N,N,N',N'*-tetramethylethylenediamine (57.0  $\mu$ l), serine (39.4 mg), cysteine-HCl (59.1 mg), glutathione (reduced form, 115.0 mg), glycerol (27.3  $\mu$ l), ethanethiol (28.0  $\mu$ l), ethanalamine (26.0  $\mu$ l), *L*-aspartic acid-HCl (63.6 mg), histidine-2HCl (85.5 mg), benzenethiol (38.5  $\mu$ l), imidazole (25.5 mg), or sodium thiosalicylate (66.0 mg) and shaken on a rotary shaker (170 r.p.m.) at 28°C for 17 h, t.l.c.† showed the presence of phenylglycinamide and phenylglycine in the reactions involving ethane-1,2-dithiol, glutathione, 2-mercaptoethanol, and cysteine. 2-Mercaptoethanol converted the substrate almost completely into phenylglycinamide and phenylglycine in a ratio of 90:5, whereas under the same conditions glutathione<sup>10</sup> showed about 20% conversion into these products in a ratio of 1:1. With ethane-1,2-dithiol the substrate was almost completely consumed but the amounts of hydrolysed products were small. Cysteine led to many products, one of them being phenylglycine.

To confirm the structure of the product from 2-mercaptoethanol hydrolysis,  $\alpha$ -aminophenylacetonitrile (2.0 g, 0.012 mol) was dissolved in potassium phosphate buffer (100 mM, 100 ml) with mercaptoethanol (0.14 ml, 0.030 mol) and the mixture stirred at room temperature for 6 h. Washing with benzene, rotary evaporation, and column chromatography on Sephadex G-25 gave a major fraction,

which showed <sup>1</sup>H n.m.r. peaks at  $\delta$  4.64 (br., NH<sub>3</sub><sup>+</sup>), 4.78 (s, 1-H), 7.4 (br., Ph), and 8.0 (NH<sub>2</sub>), and i.r. bands at 1720 and 1480 cm<sup>-1</sup>. Addition of base to pH 12, extraction with ethyl acetate, and evaporation of the extract gave a crystalline residue (1.3 g, yield: 78%, m.p. 210°C; decomp.) which showed i.r. bands at 1660, 1565, and 1410 cm<sup>-1</sup> and a u.v. maximum at 254 nm ( $\epsilon$  300 dm<sup>3</sup> mol<sup>-1</sup> cm<sup>-1</sup>) in phosphate buffer (pH 6.5; 25 mM). This showed the same <sup>1</sup>H n.m.r. data and *R<sub>f</sub>* values as an authentic sample of phenylglycinamide prepared from  $\alpha$ -aminophenylacetonitrile following a literature procedure.<sup>11</sup> Although various amounts of phenylglycine were produced depending on the conditions and on the bifunctional compounds used, further hydrolysis of phenylglycinamide to phenylglycine was excluded on the basis that their ratio did not change on further stirring.

Although nitrile groups are known to be hydrolysed by strong acid or strong base catalysts,  $\alpha$ -aminophenylacetonitrile was not hydrolysed on shaking with HCl (1 M) at 28°C at all, but when it was shaken with NaOH (1 M) almost equal amounts of phenylglycine and phenylglycinamide were rapidly formed.‡ However, under neutral conditions (potassium phosphate buffer, 25 mM, pH 6.5) without addition of 2-mercaptoethanol no hydrolysed product was observed at all even after stirring for 24 h at 28°C. When hydrolysis of phenylacetonitrile or acetonitrile was examined under the same conditions with addition of 2-mercaptoethanol the reaction was extremely slow. The reaction was still slow at 100°C; after stirring for 2 h at 100°C a small amount of a hydrolysed product, an acid, was formed.§

The mechanism of hydrolysis of the nitrile group in  $\alpha$ -aminophenylacetonitrile by these bifunctional compounds is not yet known, and we presume that both functional groups participate. Several investigators suggested formation of a thioimidate intermediate during hydrolysis of a nitrile group by an enzyme,<sup>6</sup> but the monofunctional compounds benzene-thiol and ethanethiol did not show catalytic activities in our



Scheme 1

‡ For  $\alpha$ -aminopropionitrile the rate constant for base catalysed hydrolysis of the nitrile group was reported<sup>12</sup> to be  $14(\pm 1)$  dm<sup>3</sup> mol<sup>-1</sup> min<sup>-1</sup> at 39°C. In the case of the 2-mercaptoethanol catalysed hydrolysis of the nitrile group of  $\alpha$ -aminophenylacetonitrile, the rate was found to be about 1000 times slower than that of base catalysed hydrolysis of the nitrile group of  $\alpha$ -aminopropionitrile.<sup>13</sup>

§ Propionitrile was reported to show a *ca.*  $2 \times 10^4$  times slower reaction rate than  $\alpha$ -aminopropionitrile by Bejaud *et al.*<sup>12</sup>

† T.l.c. analysis was performed with authentic samples in two different solvent systems: Bu<sup>n</sup>OH-AcOH-H<sub>2</sub>O (4:1:1) and Pr<sup>i</sup>OH-NH<sub>4</sub>OH (29%)-H<sub>2</sub>O (20:1:1).

studies. At present we do not know exactly what is the requirement for the catalytic activity. It seems that a special geometric arrangement of the thiol group with another functional group such as OH or CO<sub>2</sub>H is necessary, but we do not know whether a primary thiol group or a primary hydroxy group is required. We assumed formation of a tetrahedral complex in nitrile group-hydrolysing enzymes, and the relevance of this model to the actual enzyme system is currently under investigation.

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