JPP Journal of Pharmacy and Pharmacology

JPP 2002, 54: 205–212
© 2002 The Authors
Received May 30, 2001
Accepted September 7, 2001
ISSN 0022-3573

# Tissue distribution of and species differences in deacetylation of *N*-acetyl-L-cysteine and immunohistochemical localization of acylase I in the primate kidney

Aiko Yamauchi, Nobuhiko Ueda, Sayuri Hanafusa, Eri Yamashita, Masaru Kihara and Shinsaku Naito

**Abstract**

Species differences in the biotransformation of *N*-acetyl-L-cysteine (NAC) have been investigated to evaluate the usefulness of NAC as a constituent in parenteral nutrition solutions in place of cysteine. The activity of NAC-deacetylating enzyme (acylase) was measured in various tissues of different species (rat, rabbit, dog, monkey, and man). Acylase activity was highest in the kidney in all species studied. Enzyme activity in the liver was 10 %–22 % of that in the kidney in the rat, rabbit, monkey, and man, but almost no hepatic activity was seen in the dog. NAC-deacetylating activity was very low in other organs. The tissue distribution of acylase I was determined by Western blotting and an immunohistochemical method employing specific antibody against porcine acylase I (EC 3.5.1.14). The immunoblotting study showed a 46-kDa protein band corresponding to porcine acylase I in the kidney of all species. In liver cytosol, 46 kDa and/or 29 kDa bands were observed in the rat, rabbit, monkey, and man, but not in the dog. In the immunohistochemical study, positive staining with anti-acylase I antibody was observed clearly in the renal proximal tubules in the monkey and man. These results suggested that the kidney and liver were the main organs responsible for the biotransformation of NAC to cysteine in mammals other than the dog.

## Introduction

It is widely recognised that the composition of amino acid solutions used in total parenteral nutrition must be optimized to match the amino acid content of dietary protein. The amino acid solutions used in parenteral nutrition usually contain adequate amounts of the essential amino acids. Cysteine (L-cysteine) is a nonessential amino acid because it can easily be synthesized from L-methionine via the transulfuration pathway, which occurs largely in the liver of healthy organisms (Cooper 1983). However, under certain physiological and pathophysiological conditions, such as those seen in premature infants (Gaull et al 1972) or patients with liver cirrhosis (Sturman et al 1970) or homocystinuria (Gaull et al 1974), the enzymatic conversion of methionine to cysteine is known to be ineffective. Although the provision of exogenous cysteine to such patients is therefore important, commercially available amino acid solutions for total parenteral nutrition generally do not contain cysteine due to its limited solubility and instability.

Department of Pharmaceutical Information Science, Graduate School of Pharmacy, The University of Tokushima, 1-78, Sho-machi, Tokushima 770-8505, Japan

Aiko Yamauchi, Sayuri Hanafusa, Eri Yamashita, Masaru Kihara

Department of Drug Safety Evaluation, Naruto Research Institute, Otsuka Pharmaceutical Factory, Inc., 772 Tateiwa, Muya-cho, Naruto, Tokushima 772-8601, Japan

Nobuhiko Ueda, Shinsaku Naito

**Correspondence:** A. Yamauchi, Department of Pharmaceutical Information Science, Graduate School of Pharmacy, The University of Tokushima, 1-78, Sho-machi, Tokushima 770-8505, Japan. E-mail: aiko@ph2.tokushima-u.ac.jp

**Acknowledgements:** The authors wish to express their thanks to Biomedical Research Institute, HAB Discussion Group for providing the human tissue samples used in the study.

*N*-Acetyl-L-cysteine (NAC), an amino acid derivative, may be a reasonable alternative to cysteine because NAC is more stable, less toxic, and more readily soluble than cysteine (Bonanomi & Gazzaniga 1980). Therefore, our intention is to use NAC as a constituent of amino acid injection solutions in place of cysteine. Neuhäuser et al (1986) found that NAC was superior to methionine as a source of cysteine during four-week parenteral nutrition in the rat. However, Magnusson et al (1989) reported that the usefulness of NAC as a cysteine precursor was not apparent in healthy volunteers who received NAC (5 g) by 4-h intravenous infusion, since no increase in the plasma concentration of cysteine was observed. This disparity may be attributable to differences in NAC biotransformation between the two species studied.

The deacetylation of *N*-acetyl-L-amino acids is catalysed by several aminoacylases (Anders & Dekant 1994) such as acylase I (*N*-acyl-L-amino acid amidohydrolase) (EC 3.5.1.14) (Bruns & Schulze 1962), acylase II (aspartoacylase) (EC 3.5.1.15) (Goldstein 1976), and acylase III, which preferentially catalyses the *N*-deacetylation of *N*-acyl aromatic amino acids (Bruns & Schulze 1962; Endo 1978; Suzuki & Tateishi 1981). It is known that NAC is hydrolysed by cytosolic acylase I (Sheffner et al 1966; Sjödin et al 1989; Uttamsingh et al 1998). However, species differences in and the tissue distribution of acylase I have not been fully clarified.

This study has been conducted to determine the activity of cytosolic NAC deacetylase using post-column-derivatization high-performance liquid chromatography (HPLC) and to identify the presence of acylase I using Western blot analysis in various organs of several mammalian species. In addition, the immunohistochemical localization of acylase I in the kidney of the monkey and man was investigated.

## Materials and Methods

### Materials

NAC, cysteine, and acylase I from porcine kidney (Grade I) were obtained from Sigma Chemical Co. (St Louis, MO). Dithiothreitol, hydrogen hexachloroplatinate (IV) hexahydrate, potassium iodide, complete Freund's adjuvant, horseradish peroxidase-coupled goat anti-rabbit IgG antibody, and biotinylated goat anti-rabbit lgG antibody were purchased from Wako Pure Chemical Industries, Ltd (Osaka, Japan). Fluorescein isothiocyanate (FITC)-labelled streptavidine was purchased from DAKO Japan Co., Ltd (Kyoto, Japan). Sodium 1-octanesulfonate was purchased from Tokyo Kasei Kogyo Co., Ltd (Tokyo, Japan), and the Peroxidase Stain Kit for immunoblotting and blocking solution in Tris-buffered saline were purchased from Nakalai Tesque, Inc. (Kyoto, Japan). All other chemicals and reagents used were of analytical reagent grade. For reversed-phase HPLC, a STD-ODS II column (4.0-mm internal diameter × 150 mm) was purchased from Shinwa Chemical Industries, Ltd (Kyoto, Japan). A PEEK tube for post-column derivatization was purchased from Shimadzu Corporation (Kyoto, Japan).

### HPLC apparatus

The HPLC system used consisted of two 880-PU HPLC pumps, an 860-CO column oven, an 801-SC system controller, an 875-UV UV/vis detector (Jasco International Co., Ltd, Tokyo, Japan), a D-2000 chromato-integrator (Hitachi, Ltd, Tokyo, Japan), and an AS-8000 auto sampler (Tosoh Co., Tokyo, Japan).

### Animals and tissue preparations

The study was approved by the Animal Care Committee of the University of Tokushima and followed the Guidelines for the Care and Use of Laboratory Animals.

Male Wistar rats (7-weeks old) and New Zealand White rabbits (2.9–3.5 kg) were obtained from Japan SLC Co. (Hamamatsu, Japan) and were housed and maintained at the Biological Resources Laboratory, Faculty of Pharmaceutical Sciences, University of Tokushima at Tokushima, Japan. All animals received a standard pellet diet before being killed. Unfasted rats were killed by decapitation, and various tissues (kidney, liver, pancreas, brain, lung, small intestinal mucosa, and heart) were removed, minced, and homogenized in 3 vols 0.25 M sucrose containing 1 mM EDTA and 10 mM Tris-HCl (pH 7.4, 4°C) using a Teflon–glass homogenizer. The homogenate was centrifuged at 9000 *g* for 20 min at 4°C and the supernatant was then further centrifuged at 100 000 *g* for 60 min at 4°C. The final supernatant was used as the cytosol fraction. Cytosol fractions from rabbit kidney and liver were prepared in the same manner. The cytosol fractions from the kidney, liver, pancreas, brain, lung, small intestinal mucosa, and heart of Nosan beagle dogs (7–12-weeks old, 8.0–11.0 kg) and Cynomolgus monkeys (3–4-years old, 3.0–4.0 kg) were obtained from The Life Science Tsukuba Labs of Nemoto & Co., Ltd (Ibaragi, Japan). For immunohistochemical studies, kidney tissue was obtained from the same source. Procedures involving animals and their care were conducted in conformity with the guidelines

of The Life Science Tsukuba Labs of Nemoto & Co., Ltd that followed the Guide for the Care and Use of Laboratory Animals (National Research Council, USA). Human kidney and liver cytosol fractions and kidney tissue samples were supplied by the National Disease Research Interchange through Biomedical Research Institute, HAB Discussion Group (Chiba, Japan). The review committee of the Biomedical Research Institute approved the protocol for this study.

Protein was measured using BCA (bicinchonic acid) Protein Assay Reagent (Pierce Chemical Co., IL) with bovine serum albumin as the standard.

### Enzyme assay procedure

The activity of NAC-deacetylating enzyme (acylase I) was measured in various tissue cytosol fractions of different species (rat, rabbit, dog, monkey, and man). NAC-deacetylating activity was assayed by measuring the formation of cysteine. The cytosol was placed on an NAP-10 desalting column (Amersham Pharmacia Biotech Japan, Tokyo, Japan), which was previously equilibrated with borate buffer (0.2 M, pH 8.0). Small compounds such as cysteine and sulfur-containing compounds were thus removed from the protein fraction. This fraction was used as the enzyme preparation. The enzyme (0.05–1.0 mg protein) was incubated in a volume of 1 mL at 37°C for 10 min with 4 mM NAC in 0.2 M borate buffer (pH 8.0). The reaction was terminated by the addition of 5.5 M perchloric acid containing 10 mM dithiothreitol (100 $\mu$L). The mixture was allowed to stand for 10 min in an ice-bath and then centrifuged at 13000 *g* for 5 min. To a sample of the supernatant (800 $\mu$L) was added 120 $\mu$L 50% phosphoric acid and 190 $\mu$L 3 M potassium hydrogen carbonate containing 20 mM EDTA. After centrifuging at 2000 *g* for 5 min, 500 $\mu$L of the supernatant (pH 1.5) was taken and further centrifuged at 10000 *g* for 5 min for HPLC analysis.

### Post-column-derivatization HPLC

Cysteine produced by the enzyme reaction was measured using post-column-derivatization HPLC (Kawano et al 1997; Harada et al 2001). A 50-$\mu$L portion of the sample was analysed on a reversed-phase HPLC column (STD-ODS II), which was eluted isocratically at 0.6 mL min$^{-1}$ with an eluent consisting of 0.1 M sodium phosphate buffer (pH 2.2) containing 3 mM sodium 1-octanesulfonate. After the separation of compounds, derivatization of sulfur-containing compounds was achieved by mixing at a flow rate of 0.6 mL min$^{-1}$ at 40°C with hexaiodoplatinate reagent (100 mM phosphate buffer (pH 2.2) containing 100 $\mu$M hexachloroplatinate and 100 $\mu$M potassium iodide) after the analytical column. The derivatization reaction was performed in a PEEK tube coil (0.25-mm i.d. × 10 m). The brownish colour of the hexaiodoplatinate reagent in the acidic solution was eliminated by the exchange reaction of sulfur-containing compounds and iodine ions co-ordinated with platinum. The decrease in absorbance at 500 nm was measured using a spectrophotometric detector and an integrator. The retention times for NAC, cysteine, and dithiothreitol were 7.7, 11.3, and 14.5 min, respectively. The peak areas were used for quantification. The calibration curve for cysteine was linear from 20 pg to 10 ng.

### Antibody preparation

A New Zealand White rabbit was initially immunized subcutaneously at 20 sites with 0.1 mL of an antigen emulsion consisting of porcine acylase I (1 mg mL$^{-1}$ phosphate-buffered saline (PBS)) and complete Freund's adjuvant (1 mL). The animal was boosted three weeks later with 1 mg acylase I in the same manner. Three weeks after the second immunization, 1 mg acylase I without adjuvant was injected subcutaneously at multiple sites. Serum was obtained eight days after the final injection and was immobilized by incubation for 30 min at 56°C. The IgG was isolated from the serum using HiTrap rProtein A Columns (1 mL, Amersham Pharmacia Biotech) according to the method specified by the manufacturer.

### SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting

SDS-PAGE was performed on 10% polyacrylamide gels (0.1 × 9.0 × 7.5 cm) in the presence of 0.1% SDS as described by Laemmli (1970). Proteins were separated by SDS-PAGE and electrophoretically transferred to a polyvinylidene difluoride membrane with Semi-Dry Electroblotter (Sartorius AG, Goettingen, Germany). After washing three times with Tris-buffered saline at room temperature for 8 min with gentle shaking, the membrane was incubated with the blocking solution in Tris-buffered saline for 30 min and then with 14 $\mu$g mL$^{-1}$ rabbit anti-acylase I IgG overnight at room temperature. After washing with Tris-buffered saline, the transblots were incubated with peroxidase-coupled goat anti-rabbit IgG (1:5000 dilution) in Tris-buffered saline containing 0.05% Tween 20 and 0.01% thimerosal for 3 h at room temperature. The membrane was washed three

**Table 1** Tissue distribution of NAC-deacetylating activity in various animal species.

| Tissue | Enzyme activity (nmol $min^{-1}$ $mg^{-1}$) | | | |
|---|---|---|---|---|
| | Rabbit (n = 3) | Rat (n = 6) | Monkey (n = 3) | Dog (n = 3) |
| Kidney | 674.9±71.6** | 491.0±21.0** | 69.3±1.5** | 41.2±4.5** |
| Liver | 143.4±6.7 | 52.1±7.6 | 7.3±0.4 | 0.2±0.3 |
| Heart | N.D. | 10.6±4.1 | 2.9±0.0 | 0.1±0.0 |
| Small intestine | N.D. | 14.5±1.3 | 1.6±0.0 | 0.3±0.0 |
| Lung | N.D. | 9.4±2.0 | 0.7±0.0 | 0.9±0.1 |
| Brain | N.D. | 11.3±3.2 | 1.1±0.1 | 0.1±0.0 |
| Pancreas | N.D. | 11.6±2.1 | 0.7±0.2 | 7.6±0.8 |

After cytosolic protein (0.05–1.0 mg $mL^{-1}$) from each organ was incubated with 4 mM NAC at 37°C for 10 min, cysteine formation was determined using post-column-derivatization HPLC. Experiments were performed in triplicate. Data represent means±s.d. **$P < 0.01$ compared with the activities of other tissues. N.D., not determined.

times with Tris-buffered saline, and acylase I was detected using the Peroxidase Stain Kit for immunoblotting.

### Immunohistochemistry

FITC, an amine-reactive fluorescein derivative, is one of the most widely used fluorescent labelling reagents. The FITC techniques described here represent the basic procedure recommended for the immunohistochemical determination of specific protein present in the tissues with high resolution and high sensitivity. Paraffin-embedded tissue sections were mounted on slides and heated for 1 h at 60°C. The paraffin was removed with xylene, and the slides were hydrated with an ethanol gradient (100–70%) and washed with water and PBS. The slides were blocked with normal goat serum in a humid chamber for 10 min at room temperature and then incubated with primary antibody. Another tissue section on each slide was used as the negative control and was treated similarly without primary antibody. The slides were then incubated with biotinylated goat anti-rabbit IgG antibody for 10 min at room temperature in the humid chamber, incubated with FITC-labelled streptavidine (1:50 dilution) prepared in PBS for 10 min at room temperature, and then washed three times with PBS for 3 min.

The specimens obtained were examined using a microscope (BX50, Olympus Optical Co., Ltd, Tokyo, Japan) equipped with an incident-light fluorescence system (BX-FLA, Olympus Optical). A cube (U-MNIBA, Olympus Optical) with an excitation filter (BP 470-490) and an absorption filter (BA 515-550) was used for FITC examination.

### Statistical analysis

All experimental results regarding enzyme activities are given as means±s.d. and the statistical analysis in Table 1 was performed using the unpaired Student's *t*-test (two-tailed) with a significance level of $P < 0.05$.

## Results and Discussion

### Tissue distribution of NAC-deacetylating activity in different species

The deacetylation of NAC was determined in the kidney and liver of rabbits, rats, monkeys, and dogs using the post-column-derivatization HPLC method in which the reaction product, cysteine, was specifically measured. The activities in the pancreas, brain, small intestinal mucosa, and heart were measured also in all species except for the rabbit. The results are shown in Table 1. Deacetylase activity was highest in the kidney in all species studied. Renal enzyme activity in the different species was ranked as follows: rabbit > rat ≫ monkey > dog. Hepatic enzyme activity values were 21%, 11%, and 10% of renal enzyme activity values in the rabbit, rat, and monkey, respectively. With respect to total enzyme activity per organ, NAC deacetylation in the kidney and liver may be comparable because the weight of the liver is more than five-times greater than that of the kidneys in the rat and the rabbit. In other tissues, NAC-deacetylating activity was very low.

Hepatic activity was barely detectable in the dog (< 1% of renal activity). Results in the dog were markedly different from those in other species, with substantial activity (18% of renal activity) observed in the pancreas,

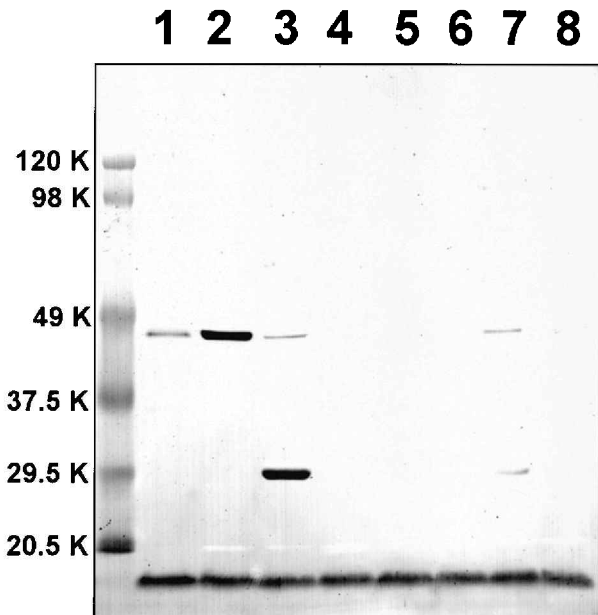


**Figure 1** Western blot analysis of anti-acylase I IgG immunoreactive proteins in cytosol from various tissues of the monkey. IgG purified from the serum of a rabbit immunized with porcine acylase I was used for the analysis. Cytosolic proteins (10 $\mu$g) were analysed by SDS-PAGE, after which immunostaining was performed as described in Materials and Methods. Lane 1: 40 ng of porcine kidney acylase I. Lanes 2–8: 10 $\mu$g of monkey cytosol protein from the kidney (lane 2), liver (lane 3), pancreas (lane 4), brain (lane 5), lung (lane 6), small intestinal mucosa (lane 7), and heart (lane 8). Protein molecular markers are shown in the left lane.

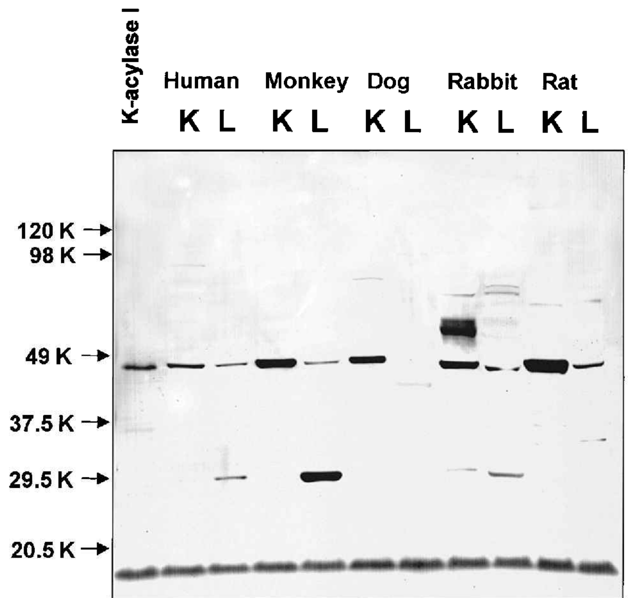


**Figure 2** Western blot analysis of anti-acylase I IgG immunoreactive proteins in the cytosol from the kidney and liver of the man, monkey, dog, rabbit, and rat. K-acylase I: 40 ng porcine kidney acylase I; K: 10 $\mu$g kidney cytosol protein; L: 10 $\mu$g liver cytosol protein. The arrows indicate molecular weights.

but not in the liver. In a study by Chasseaud (1974), it was reported that of the various animals species investigated, which included the guinea-pig, rabbit, rat, pigeon, ferret, and mouse, the dog had the lowest hepatic NAC-deacetylating activity. Others have reported also that the liver cytosol of the dog is much less active than that of the rat, mouse, or man in terms of the hydrolysis of NAC (Sjödin et al 1989). The results of this study are consistent with these previous observations.

### Immunoblotting

After cytosolic protein was subjected to SDS-PAGE, immunoblotting studies were carried out using rabbit IgG purified from specific antisera against porcine acylase I. Figure 1 shows immunoblots of anti-acylase I antibody reactive proteins in the cytosol from different organs in the monkey. The acylase I purified from porcine kidney showed a band at 46 kDa. A single band at 46 kDa, corresponding to acylase I, was detected in the kidney. The liver cytosol showed a weakly immunostained band at 46 kDa and a strongly stained band at 29 kDa. Both of these bands were weakly detected in the small intestinal mucosa as well. No protein bands reacting with the anti-acylase I antibody were seen in the pancreas, brain, lung, or heart.

In Figure 2 immunoblotting analyses of cytosolic fractions from the kidney and liver of the rat, rabbit, dog, monkey, and man are shown. An immunoreactive protein band at 46 kDa, corresponding to acylase I, was detected in the kidney of all species. Another unknown band at approximately 55 kDa was observed only in the rabbit kidney. In the liver cytosol, bands at 46 kDa and/or 29 kDa were detected in the man, monkey, and rabbit. A 29-kDa band was not seen in rat liver cytosol. Notably, no appreciable bands appeared in the dog liver cytosol, indicating that the dog liver lacked acylase I. These findings were consistent with the extremely low activity of NAC deacetylase observed in dog liver cytosol.

The aminoacylases were well characterized decades ago. Acylase I preferentially catalyses the hydrolysis of neutral and aliphatic *N*-acyl $\alpha$-amino acids, including NAC. cDNAs encoding the complete amino acid sequence of porcine kidney acylase I have been identified, and the enzyme is known to be a homodimer consisting of subunits with an $M_r$ of 46216 (Mitta et al 1992). A human liver acylase I consisting of two subunits with an

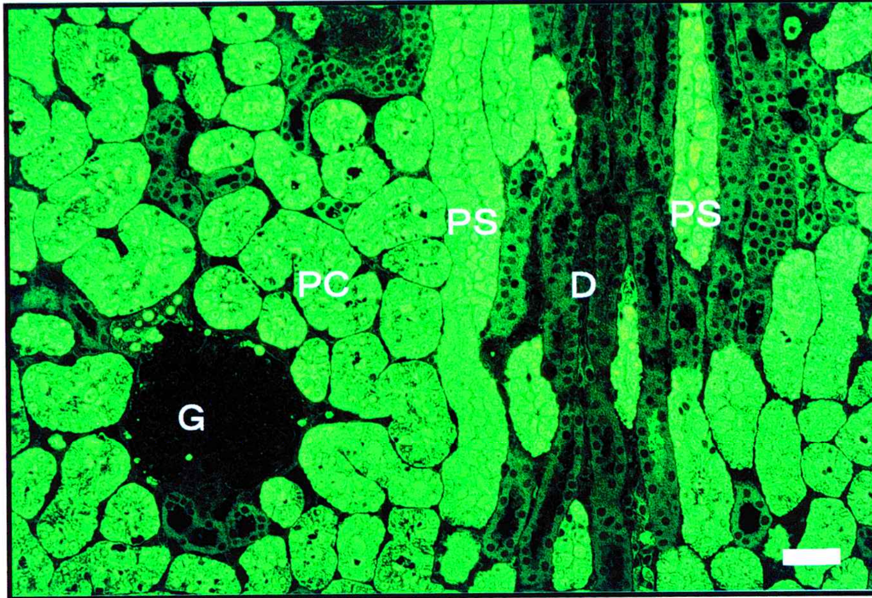


**Figure 3** Immunohistochemistry of monkey kidney tissue stained with anti-acylase I antibody. Original magnification: 180×; bar = 50 $\mu$m. Distinct staining was seen in the proximal convoluted (PC) and straight (PS) tubules. Particularly in the proximal straight tubules, staining was most intense in the nephron. Less intense staining was seen in the distal tubules (D). No significant staining was observed in other structures, including the glomeruli (G), Bowman's capsules, and interstitial cells, as compared with the sections stained without primary antibody.

$M_r$ of 45885 (Cook et al 1993) has been reported also. Since the homology of acylase I in the two species was calculated to be approximately 90%, we decided to use an antibody against porcine acylase I, which is commercially available, to detect acylase I-like immunoreactive proteins. The results of this study indicated that acylase I was present in the kidney of the rat, rabbit, dog, monkey, and man, as well as in the liver of all of the species except for the dog. The tissue distribution of acylase I determined by immunoblotting analysis was found to be consistent with the differences in NAC deacetylase activity observed in various organs.

With regard to aspartoacylase (acylase II), the cloning of its cDNA from the human kidney has been reported and its $M_r$ has been calculated to be 35735 (Kaul et al 1993), although the cDNA of acylase III has not yet been cloned. Since acylase I has no homology with acylase II, the antibody used in this study is not likely to cross-react with acylase II. The unknown immunoreactive protein band at 29 kDa in the liver cytosol (Figures 1 and 2) remains to be investigated.

## Immunohistochemistry

Using the purified IgG from specific antisera against porcine acylase I characterized in Figures 1 and 2, the localization of acylase I in the kidney was determined in the monkey and man using an immunohistochemical method.

Figure 3 shows the immunohistochemical localization of anti-acylase I antibody immunoreactive proteins in the monkey kidney. Distinct antibody staining was observed in the proximal convoluted and straight tubules of the kidney. Staining was most intense in the nephrons, particularly in the proximal straight tubules. Less distinct staining was seen in the distal tubules and the loop of Henle. No significant staining was detected in the other structures, including the glomeruli, Bowman's capsules, and interstitial cells. In the human kidney (Figure 4) a positive reaction with anti-acylase I antibody was observed in the proximal convoluted and straight tubules, as was the case in the monkey kidney. The staining of the distal tubules was less intense than that of the proximal tubules. No difference in the staining of the cytoplasm of endothelial cells was seen between the apical and basal portions in both the monkey and human kidney. No fluorescent immunostaining was observed in either monkey or human kidney tissue sections incubated without primary antibody (data not shown).

This study has demonstrated for the first time that acylase I is localized specifically to the renal proximal straight and convoluted tubules in primates. Previous immunocytochemical studies showed that acylase I was

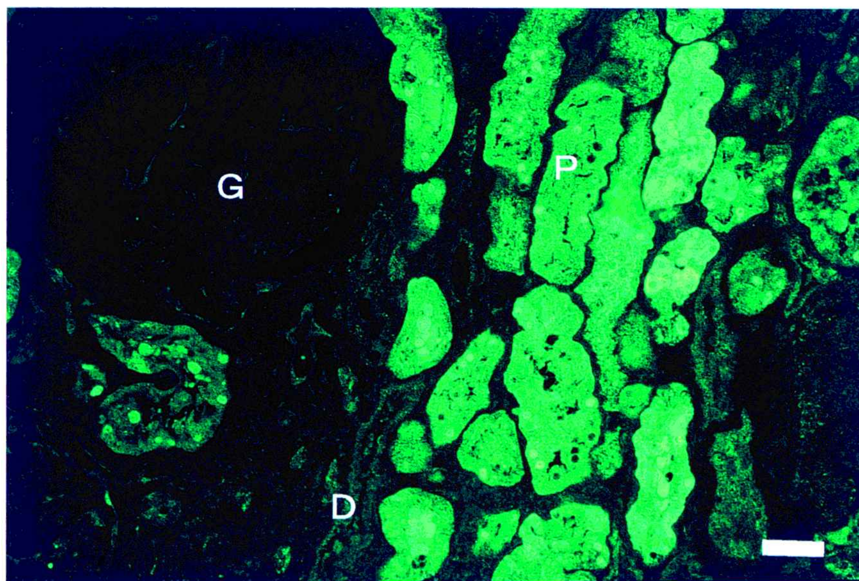


**Figure 4** Immunohistochemistry of human kidney tissue stained with anti-acylase I antibody. Original magnification: 180×; bar = 50 $\mu$m. A positive reaction with anti-acylase I IgG was observed in the proximal tubules (P), and a less positive reaction was seen in the distal tubules (D). No significant staining was observed in other tissues, including the glomeruli (G), Bowman's capsules, and interstitial cells, as compared with the sections stained without primary antibody.

localized to the distal tubule in the swine kidney (Löffler et al 1982) and that the enzyme was uniformly distributed in the glomeruli and the proximal and distal convoluted tubules in the rat kidney (Uttamsingh et al 2000). Following the intravenous infusion of NAC, NAC undergoes renal elimination and subsequent reabsorption in the renal tubules. Renal acylase I present in the proximal tubules may play an important role in the systemic supply of cysteine from the salvaged NAC.

NAC has been used clinically for approximately 40 years (Gregory & Kelly 1998), primarily as a mucolytic agent in pulmonary diseases (Webb 1962) and later for the treatment of acetaminophen hepatotoxicity (Prescott et al 1977; Lauterburg et al 1983). NAC is currently thought to be clinically useful in conditions characterized by a decrease in glutathione or by oxidative stress such as HIV infection (Roederer et al 1993), cancer (Meyskens 2000), and heart disease (Horowitz 1991). It has been thought that NAC acts as a source of SH groups, thus stimulating glutathione synthesis and glutathione transferase activity (Lauterburg et al 1983), and it may also act directly as a scavenger of reactive oxidant radicals (Aruoma et al 1989). However, the disposition of NAC in man has not been elucidated. The results of this study suggested that the kidney and liver were the organs responsible for the biotransformation of NAC to cysteine in mammals other than the dog. No acylase I activity or anti-acylase I antibody immunoreactive proteins were observed in the dog liver cytosol. In fact, slow elimination of radioactivity from plasma has been reported in the dog following the oral administration of [$^{35}$S]NAC (Bonanomi & Gazzaniga 1980). It is noteworthy that the dog is not a suitable experimental animal for assessing the nutritional value of parenteral NAC. Further investigations are required to elucidate the systemic distribution and pharmacokinetics of NAC administered by intravenous infusion.

## References

Anders, M. W., Dekant, W. (1994) Aminoacylases. *Adv. Pharmacol.* **27**: 431–448

Aruoma, O. I., Halliwell, B., Hoey, B. M., Butler, J. (1989) The antioxidant action of *N*-acetylcysteine: its reaction with hydrogen peroxide, hydroxyl radical, superoxide, and hypochlorous acid. *Free Radic. Biol. Med.* **6**: 593–597

Bonanomi, L., Gazzaniga, A. (1980) Toxicological, pharmacokinetic and metabolic studies on acetylcysteine. *Eur. J. Respir. Dis.* (*Suppl.*) **111**: 45–51

Bruns, F. H., Schulze, C. (1962) Acylase I. Reiidarstellung, physikalisch-chemische eigenschaften und identität mit ''Hippurikase''. *Biochem. Z.* **336**: 162–181

Chasseaud, L. F. (1974) Reaction with electrophiles after enzyme-catalysed deacetylation of *N*-acetylcysteine. *Biochem. Pharmacol.* **23**: 1133–1134

Cook, R. M., Burke, B. J., Buchhagen, D. L., Minna, J. D., Miller, Y. E. (1993) Human aminoacylase-1. Cloning, sequence, and expression analysis of a chromosome 3p21 gene inactivated in small cell lung cancer. *J. Biol. Chem.* **268**: 17010–17017

Cooper, A. J. (1983) Biochemistry of sulfur-containing amino acids. *Annu. Rev. Biochem.* **52**: 187–222

Endo, Y. (1978) *N*-Acyl-L-aromatic amino acid deacylase in animal tissues. *Biochim. Biophys. Acta* **523**: 207–214

Gaull, G., Sturman, J. A., Raiha, N. C. (1972) Development of mammalian sulfur metabolism: absence of cystathionase in human fetal tissues. *Pediatr. Res.* **6**: 538–547

Gaull, G., Sturman, J. A., Schaffner, F. (1974) Homocystinuria due to cystathionine synthase deficiency: enzymatic and ultrastructural studies. *J. Pediatr.* **84**: 381–390

Goldstein, F. B. (1976) Amidohydrolases of brain: enzymatic hydrolysis of *N*-acetyl-L-aspartate and other *N*-acyl-L-amino acids. *J. Neurochem.* **26**: 45–49

Gregory, S., Kelly, N. D. (1998) Clinical applications of *N*-acetylcysteine. *Alt. Med. Rev.* **3**: 114–127

Harada, D., Naito, S., Kawauchi, Y., Ishikawa, K., Koshitani, O., Hiraoka, I., Otagiri, M. (2001) Determination of reduced, protein-unbound, and total concentrations of N-acetyl-L-cysteine and L-cysteine in rat plasma by postcolumn ligand substitution high-performance liquid chromatography. *Anal. Biochem.* **290**: 251–259

Horowitz, J. D. (1991) Thiol-containing agents in the management of unstable angina pectoris and acute myocardial infarction. *Am. J. Med.* **91**: 113S–117S

Kaul, R., Gao, G. P., Balamurugan, K., Matalon, R. (1993) Cloning of the human aspartoacylase cDNA and a common missense mutation in Canavan disease. *Nat. Genet.* **5**: 118–123

Kawano, S., Yasui, Y., Hayashi, M., Shono, T., Chone, Y., Hirai (Endo), S., Aoki, M. (1997) Post-column derivatization using hexaiodoplatinate for determination of sulfur-containing amino acids by high performance liquid chromatography. *Shimadzu Hyouron* **54**: 3–8

Laemmli, U. K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**: 680–685

Lauterburg, B. H., Corcoran, G. B., Mitchell, J. R. (1983) Mechanism of action of *N*-acetylcysteine in the protection against the hepatotoxicity of acetaminophen in rats in vivo. *J. Clin. Invest.* **71**: 980–991

Löffler, H. G., Schneider, F., Aumüller, G., Unsicker, K. (1982) Immunzytochemische untersuchungen zur lokalisation der aminoacylase I (EC 3.5.1.14) in der schweineniere. *Acta Histochem.* (*Suppl.*) **25**: 57–60

Magnusson, I., Ekman, L., Wangdahl, M., Wahren, J. (1989) *N*-Acetyl-L-tyrosine and *N*-acetyl-L-cysteine as tyrosine and cysteine precursors during intravenous infusion in humans. *Metabolism* **38**: 957–961

Meyskens, F. L. (2000) Criteria for implementation of large and multiagent clinical chemoprevention trials. *J. Cell. Biochem. Suppl.* **34**: 115–120

Mitta, M., Ohnogi, H., Yamamoto, A., Kato, I., Sakiyama, F., Tsunasawa, S. (1992) The primary structure of porcine aminoacylase 1 deduced from cDNA sequence. *J. Biochem. Tokyo* **112**: 737–742

Neuhäuser, M., Grötz, K. A., Wandira, J. A., Bässler, K. H., Langer, K. (1986) Utilization of methionine and *N*-acetyl-L-cysteine during long-term parenteral nutrition in the growing rat. *Metabolism* **35**: 869–873

Prescott, L. F., Park, J., Ballantyne, A., Adriaenssens, P., Proudfoot, A. T. (1977) Treatment of paracetamol (acetaminophen) poisoning with *N*-acetylcysteine. *Lancet* **2**: 432–434

Roederer, M., Staal, F. J., Ela, S. W., Herzenberg, L. A., Herzenberg, L. A. (1993) *N*-Acetylcysteine: potential for AIDS therapy. *Pharmacology* **46**: 121–129

Sheffner, A. L., Medler, E. M., Bailey, K. R., Gallo, D. G., Mueller, A. J., Sarett, H. P. (1966) Metabolic studies with acetylcysteine. *Biochem. Pharmacol.* **15**: 1523–1535

Sjödin, K., Nilsson, E., Hallberg, A., Tunek, A. (1989) Metabolism of *N*-acetyl-L-cysteine. Some structural requirements for the deacetylation and consequences for the oral bioavailability. *Biochem. Pharmacol.* **38**: 3981–3985

Sturman, J. A., Gaull, G., Raiha, N. C. (1970) Absence of cystathionase in human fetal liver: is cystine essential? *Science* **169**: 74–76

Suzuki, S., Tateishi, M. (1981) Purification and characterization of a rat liver enzyme catalyzing *N*-deacetylation of mercapturic acid conjugates. *Drug Metab. Dispos.* **9**: 573–577

Uttamsingh, V., Keller, D. A., Anders, M. W. (1998) Acylase I-catalyzed deacetylation of *N*-acetyl-L-cysteine and S-alkyl-*N*-acetyl-L-cysteines. *Chem. Res. Toxicol.* **11**: 800–809

Uttamsingh, V., Baggs, R. B., Krenistsky, D. M., Anders, M. W. (2000) Immunohistochemical localization of the acylases that catalyze the deacetylation of *N*-acetyl-L-cysteine and haloalkene-derived mercaputurates. *Drug Metab. Dispos.* **28**: 625–632

Webb, W. R. (1962) Clinical evaluation of a new mucolytic agent acetylcysteine. *J. Thorac. Cardiovasc. Surg.* **44**: 330–343