Amino Acids (2012) 42:337–345
DOI 10.1007/s00726-010-0811-0

ORIGINAL ARTICLE

Study on the decrease of renal D-amino acid oxidase activity in the rat after renal ischemia by chiral ligand exchange capillary electrophoresis

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Received: 1 September 2010 / Accepted: 8 November 2010 / Published online: 26 November 2010

Abstract D-Amino acid oxidase (DAAO) in mammal kidney regulates the renal reactive oxygen species (ROS) levels directly and plays a leading role in the development of ROS-mediated renal pathologic damages based on its crucial role in the oxidative deamination of D-amino acids and the consequent generation of H_2O_2. Quantitative measurement of DAAO activity in the process of renal ischemia, which could help to understand the molecular mechanisms of this gripping acute renal disease, was conducted through the determination of chiral substrate by capillary electrophoresis (CE) in our study. In this study, a chiral ligand exchange CE method was explored with Zn(II)-L-alaninamide complex as the chiral selector to investigate DAAO activity by determining the decreased concentration of the chiral substrate of DAAO-mediated enzymatic reaction. Then, the change of DAAO activity following 60-min acute renal ischemia in rats was observed with the proposed method. The study showed that the operation of renal ischemia resulted in a 45.49 ± 8.30% ($n = 8$) decrease in the DAAO-induced consumption of substrate, indicating a sharp decrease in renal DAAO activity following this acute renal injury. This phenomenon, with the possible reason of metabolic acidosis, could pave a new way for the study of oxidative stress in the development of renal ischemia injury.

Keywords D-Amino acid oxidase (DAAO) · Renal ischemia · Chiral ligand-exchange capillary electrophoresis · D,L-Methionine

Abbreviations

DAAO	D-Amino acid oxidase
D-AAs	D-Amino acids
ROS	Reactive oxygen species
CE	Capillary electrophoresis
ARF	Acute renal failure
L-AAs	L-Amino acids
L-Aln	L-Alaninamide
Dns-D,L-Met	Dansylated D,L-methionine
I–R injury	Ischemia–reperfusion injury

Introduction

As a frequent acute problem encountered during surgical operations, renal ischemia has attracted great attention over the last several decades because it can cause dysfunction, injury, and death of the cells in the kidney and thus result in a huge cost of public health burden (Tilney and Guttmann 1997; Aronson and Blumenthal 1998; Gok et al. 2003; Eisenberg et al. 2010). Recent progress in the study of renal ischemia has demonstrated that studying the molecular mechanisms of local ischemic stroke, such as energy decline, ion channel dysfunction, signal chain variation, and oxidative stress, is of great importance in protective

Electronic supplementary material The online version of this article (doi:10.1007/s00726-010-0811-0) contains supplementary material, which is available to authorized users.

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therapeutics for the ischemic injury because these molecular mechanisms lead to cell swelling, intracellular disruption, and eventual cell death (Conger et al. 1995; Chatterjee 2007; Fujii et al. 2003). On a molecular level, reactive oxygen species (ROS) are recognized to be the most attractive chemical species related to the renal function impairment resulted from renal ischemia as well as the subsequent development of ischemic acute renal failure (ARF), and also are measured as an indicator of ischemic injury of the transplanted kidneys (Hasegawa et al. 2010; Plotnikov et al. 2007; Guz et al. 2007; Kim et al. 2009, 2010; Rodrigoand Rivera 2002). Thus, understanding the change of ROS during renal ischemia process can provide abundant information, which is crucial to study the molecular mechanisms of related pathological conditions.

D-Amino-acid oxidase (EC 1.4.3.3, DAAO), a marker enzyme associated with the generation of ROS in peroxisomes, is a flavoenzyme catalyzing the oxidative deamination of most neural D-amino acids (D-AAs) and producing the corresponding α-keto acids, ammonia and H_2O_2 (Tishkov and Khoronenkova 2005; Angermüller et al. 2009). Increasing evidence has demonstrated that DAAO located in the peroxisomes of the epithelial cells of the proximal tubules in the kidney plays a leading role in some chronic renal pathologic damages, such as D-serine-induced nephrotoxicity and nephrotoxicity of D-propargylglycine (Veenhuis and Wendelaar bonga 1977; Konno et al. 2000; Maekawa et al. 2005; Young et al. 1994; Imai et al. 1998). Reliable study has indicated that the chronic renal diseases linked to this local enzyme are ascribed to the intracellular DAAO-mediated generation of H_2O_2, which in turn can produce even more aggressive ROS to damage the straight proximal tubule (Krug et al. 2007; Silbernagl et al. 1999; Cortés-Rojo et al. 2007; Williams et al. 2005). Therefore, the renal ROS level exhibits strong dependence on this local H_2O_2-generating enzyme, which present a pressing need to explore the behavior of DAAO in different renal pathological states. Till now, rare study has been conducted to study DAAO and its effect on ROS in the pathogenesis of acute renal ischemia, which has drawn considerable attention because of its high morbidity and mortality. Thus, development of effective methods to study the state of DAAO in different physiological and pathological conditions could give insight into its effect on the change of ROS.

Common protocols used for the determination of DAAO activity are based on the measurement of products obtained from the DAAO-mediated oxidation of D-AAs, such as the detection of hydrogen peroxide coupled with indicator reactions using *o*-dianisidine or tyramine, the measurement of ammonia coupled with some reactions with changing NAD^+ and NADH amounts, and a highly sensitive HPLC method measuring α-keto acids after derivatization with hydrazone (Watanabe et al. 1978; Biondi et al. 1991; Holme and Goldberg 1982; Mora et al. 2009; Tanaka et al. 2007; Konno 1998). However, these procedures all have a severe limitation: the same products of the assay are present in the tissues derived from abundant L-amino acids (L-AAs) or other substances via various pathways. As a consequence, an accurate and reliable method used for the determination of DAAO activity is highly desired for physiological and pathological investigations.

Different from former study of DAAO activity through the detection of products, we have been interested in the development of new analytical protocols for exact measurement of physiologically important DAAO from the determination of substrates, D-AAs (Qi et al. 2009). Considering the interferential influence from large amounts of L-AAs in complex biological fluid, study of the DAAO activity by using the determination of reactants essentially makes the chiral separation a key issue in the development of such methodology. Efforts have revealed that the use of capillary electrophoresis (CE) could offer a solution to the need of chiral separation procedure based on its merit of high chiral separation efficiency, small sample loading and economical equipment compared with HPLC and GC (Preinerstorfer et al. 2009; Ward and Baker 2008; Vespalec and Boček 2000). As one kind of chiral separation mode used in CE, chiral ligand-exchange mode is relied on the formation of diastereomeric ternary mixed metal complexes between the chiral selector ligand and the analytes to get excellent chiral separation and is valuable because of its specificity and simplicity (Gassman et al. 1985; Gozel et al. 1987; Davankov 2003). By taking advantage of the excellent chiral separation ability of Zn(II)-L-alaninamide (Zn(II)-L-Aln) complex and the efficient application in the quantitative analysis of D- and L-labeled methionine, we have recently developed a facile chiral ligand-exchange capillary electrophoretic method to study the distribution of DAAO in different organs and measure the change of renal DAAO activity following renal ischemia, which could offer indirect information about the change of renal ROS level during this acute pathological process.

Materials and methods

Chemicals

All D- and L-AA standards, dansyl chloride, L-Aln, horseradish peroxidase (HRP) and DAAO (from porcine kidney) were the products of Sigma Chemical Co. (St. Louis, MO, USA). Acetone-D6 was obtained from Cambridge Isotope Laboratories (MA, USA). Lithium carbonate, zinc sulfate, boric acid, ammonium acetate, *o*-phenylenediamine, Tris(hydroxymethyl)aminomethane (Tris) and other chemicals

were all of analytical reagent grade from Beijing Chemical Factory (Beijing, China).

Preparation of sample solution and dansylation of AAs

All aqueous solutions were prepared with triply distilled water produced by a distillation apparatus model SZ-93 (Yarong Biochemical Instrument Co., Shanghai, China) and stored at 4°C. Standard stock solutions of 2.0 mg/mL D- and L-AAs were prepared in 40 mM lithium carbonate buffer (adjusted to pH 9.5 with 0.1 M HCl) and diluted to desired concentrations in degassed solutions with 40 mM lithium carbonate by 10–10^4 fold to get work solutions.

Derivative solution was freshly prepared by dissolving 3.0 mg dansyl chloride in 2.0 mL acetone. Dansylation of AAs was according to previous studies (Qi et al. 2009). Briefly, an aliquot of 100 μL AAs, 100 μL 40 mM lithium carbonate buffer and 100 μL labeling solution of dansyl chloride were all mixed in a 0.50-mL vial, which was placed in the dark at room temperature for 30 min to make the derivation process complete adequately. After addition of 5 μL 2% ethylamine to terminate the reaction, the reacted solution was either directly injected for CE separation or kept at 4°C for future analysis.

Animal experiment and preparation of organ homogenates

Male rats (Sprague-Dawley, 6 weeks old, 200–250 g) were obtained from Health Science Center, Peking University (Beijing, China). Animals were housed on a 12:12 h light–dark schedule at room temperature and were allowed ad libitum access to food and water. Experimental protocols and animal care methods in the experiment were approved by the Experimental Animal Care Committee at Institute of Chemistry Chinese Academy of Sciences.

Rats were anesthetized with an intraperitoneal injection of 3% sodium pentobarbital (initial dose of 30 mg/kg ip with additional doses of 5 mg/kg ip as needed to maintain anesthesia). Then, the organs (kidney, liver, lung and heart) were quickly removed and washed with buffer. Each organ was cut up and homogenized on ice with 3× volumes of 50 mM Tris–HCl (pH 8.2) using a glass homogenizer. These homogenates were centrifuged at 10,000*g* for 10 min, and the obtained supernatants were either used for the DAAO assay or stored frozen.

To induce renal ischemia, the left kidney of anesthetized rat was exposed through a small flank incision. Then, the left renal artery and vein were occluded with a nontraumatic clamp for 60 min. Throughout the surgery, the body temperature of animals was maintained at 37°C with a heating pad. Immediately after surgery, kidneys exposed and not exposed to ischemia were collected for homogenization (processing method the same as other organs) and DAAO activity assay.

Excised kidneys used for light microscopic observation were treated according to standard procedures. Isolated kidneys were firstly preserved in phosphate-buffered 10% formalin, then chopped into small pieces, embedded in paraffin wax, cut at 3 μm and stained with hematoxylin and eosin.

DAAO assay using D,L-methionine (D,L-Met) as the substrate

Enzymatic reactions were performed in 0.5-mL polypropylene tubes at 37°C in 50 mM Tris–HCl (pH 8.2) with a final assay volume of 100 μL. Reaction for standard DAAO was initiated by adding 33 μL DAAO solution of different concentration (0, 0.5, 1 and 2 U/mL) into 67 μL D,L-Met solution (1.5 mM) and stopped by heating in boiling water for 10 min after 15 min incubation. Then, the solutions were centrifuged at 10,000*g* for 10 min. A 20 μL of the supernatants was transferred into 0.5-mL polypropylene tubes and dried in a vacuum drying equipment model WMZK-08A (Yashilin laboratory equipment Co., Beijing, China) at 50°C for 60 min. The obtained solid samples were dissolved in 20 μL of 40 mM lithium carbonate buffer (adjusted to pH 9.5 with 0.1 M HCl) and experienced the same dansylation steps as standard AAs. The dansylated samples were siphoned for 10.0 s at 15.0 cm height and analyzed in the CE system at 254 nm.

The determination conditions for DAAO in organ homogenates were as follows: 10 μL of 100 mM D,L-Met solution in Tris–HCl buffer, 75 μL of the tissue samples and 15 μL of 50 mM Tris–HCl buffer (pH 8.2) were mixed in a 200 μL tube. After being incubated at 37°C for 0 min or 60 min, the mixed solution experienced the same heating, centrifugation, dehydration and derivation steps as the processing method of standard DAAO reaction.

CE system used for the analysis of labeled D,L-AAs

The CE system consisted of a 1229 HPCE analyzer (Beijing Institute of New Technology and Application, Beijing, P. R. China), a UV detector and an uncoated fused-silica capillary (Yongnian Optical Fiber Factory, Hebei, China) of 50 μm id × 65 cm (50 cm effective). Before sample introduction, the bare fused-silica capillary was sequentially washed with 0.1 M HNO_3, water, 0.1 M NaOH, water and running electrolyte for 2 min, respectively. A sample was siphoned for 10.0 s at 15.0 cm height and separated at −18 kV. CE running buffer for this study, unless stated otherwise, were composed of 5 mM ammonium acetate, 100 mM boric acid, 7 mM $ZnSO_4 \cdot 7H_2O$ and 14 mM L-Aln, adjusted to pH 8.2 with Tris. Before use, all

the running buffers were filtered through a membrane filter with 0.45 μm pores and degassed by sonication for 2 min. UV detection was set at the anodic end of capillary, and wavelength at 254 nm for Dns-AAs. Peaks were identified by spiking relative standard AAs in sample solutions. The peaks with increased height were considered to be the targets. All separation processes were conducted at room temperature.

All the detected Dns-D,L-Met concentrations were reported as mean (SE). Differences between groups were analyzed by paired *t* test. We used SPSS (version 14.0, SPSS) statistical software and considered a P value < 0.05 as statistically significant.

Result

Optimizing the CE separation conditions

Zn(II)-L-Aln complex was selected as the chiral selector because it still remains to be well explored. Considering that the principle of chiral ligand-exchange is based on the interchange between analyte and ligand in the coordination sphere of a central ion, the chiral separation conditions of Dns-AAs were optimized by changing the pH of buffer solution and the concentration of Zn (II) complex (Kurganov 2001; Schmid et al. 2001).

The pH value of running electrolyte is a crucial factor in the chiral ligand-exchange separation model based on its significant influence on the complexation between the central ion and chiral ligand, the dissociation of analytes and also the dissociation of silanol groups on the inner surface of capillary. As shown in Fig. 1a, the effect of pH on the enantioresolution and migration time of Dns-D,L-Met was investigated. When buffer pH was increased from 7.9 to 8.2, the formed Zn(II)-L-Aln complex showed a step-up stability and increased enantioselectivity, which resulted in a rising chiral resolution. But further increase of pH led to an obvious increase in the migration time and a minor decrease in the enantioselectivity. The decreased enantioselectivity may be explained by the conjecture that higher pH lead to high stability of the Zn(II)-ligand complex, which makes it difficult for the analyte enantiomers to replace the ligand in the Zn(II)-ligand complex. Considering the fact that pH value should be selected by compromise between resolution and running speed, running buffer at pH 8.2 was adopted.

To determine the optimum selector concentration, the effect of Zn(II)-L-Aln complex concentration on the enantioresolution and migration time of Dns-D,L-Met was studied. The concentration range of Zn(II) was investigated from 4 to 8 mM at increments of 1 mM while the molar ratio of zinc to the chelating acid amide was always kept at

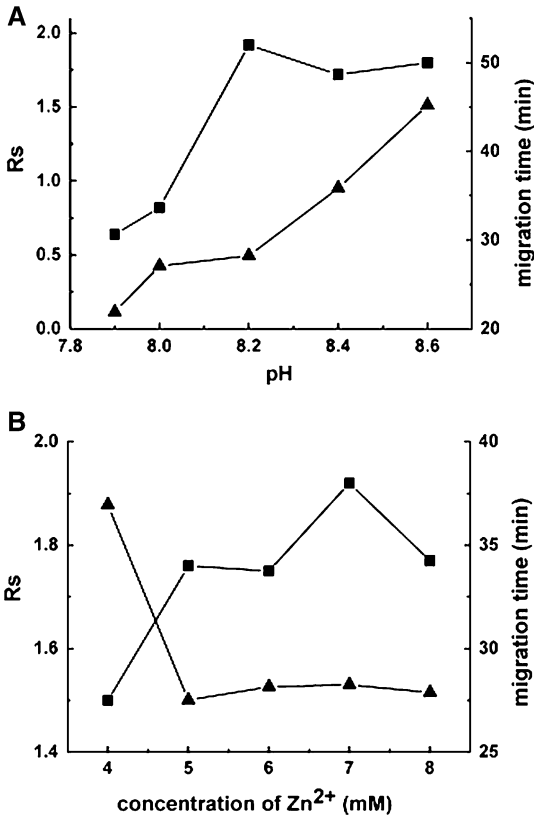

Fig. 1 Dependence of (*filled triangle*) migration time and (*filled square*) enantioresolution (Rs) of Dns-D,L-Met on the running buffer pH (**a**) and concentration of Zn(II)-L-Aln complex (**b**)

1:2. The results reported in Fig. 1b show that the highest Rs of Dns-D,L-Met was obtained when the concentration of Zn(II) complex was 7 mM. The migration time of Dns-D,L-Met decreased sharply first and then kept in a plateau phase with the increasing concentration of Zn(II) complex in the running electrolyte. Based on comprehensive consideration of CE requirements, such as high resolution, low electrolyte concentration, low electric current and stable baseline, we selected 7 mM Zn(II), 14 mM L-Aln as the optimum selector concentration in this work.

Under the optimal condition, dansylated D,L-Met and several other pairs of AA enantiomers were effectively separated. Some mixed labeled D,L-AAs were also successfully separated (Fig. 2).

Chiral ligand-exchange CE method validation

To validate and further reveal the quantitative feature of this new method, quantitation of Dns-D,L-Met was conducted. To obtain the linear calibration curve, standard solutions containing Dns-D-Met and Dns-L-Met from 10 to 1,750 μM were sampled and the resulting peak areas were analyzed. Typical regression equations for peak areas versus concentrations

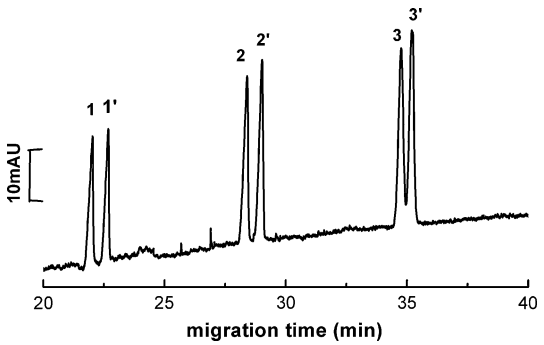

Fig. 2 Electropherogram measured from some mixed pairs of Dns-D,L-AAs using a running buffer of 100 mM boric acid, 5 mM ammonium acetate, 7 mM Zn(II) and 14 mM L-Aln, adjusted to pH 8.2 with solid Tris. Peak identity: *1* D-Ile, *1′* L-Ile, *2* D-Met, *2′* L-Met, *3* D-Asn, *3′* L-Asn

(μM) were as follows: $y = 565.5x - 1.51 \times 10^4$ ($r^2 = 0.998$) for Dns-D-Met, $y = 586.2x - 1.75 \times 10^4$ ($r^2 = 0.997$) for Dns-L-Met. The concentration detection limits for Dns-D,L-Met were both 2.5 μM.

The repeatability of this developed method was determined by five injections of artificially prepared Dns-D,L-Met standard solutions (250 μM respectively). The run-to-run RSDs of migration time and peak area were less than 1.6 and 1.0%, respectively. The recovery of the method was determined by spiking 500 μM of standard Dns-D,L-Met into the dansylated D,L-Met sample obtained after being incubated with liver homogenates. The found content averaged over five measurements were 102.7 ± 6.6% for Dns-D-Met and 96.5 ± 1.4% for Dns-L-Met.

Standard DAAO assay

In the assay about DAAO, D-Met has been chosen as the detected substrate because it belongs to essential AAs and presents itself as a nice substrate of DAAO (Vanoni et al. 1997; Sacchi et al. 2002). Using the chiral ligand-exchange CE method, the effect of DAAO on the catalytic reaction was obviously observed. It has been found that the higher the DAAO concentration was, the less the D-Met remained when D,L-Met samples were incubated with DAAO for 15 min (Fig. 3). It should be noted that although the peak area of Dns-D-Met decreased sharply with the increase of DAAO concentration, the peak area of Dns-L-Met maintained a constant level. This result strongly indicated that the present DAAO assay procedure could be used as a suitable platform for sensitive and accurate determination of DAAO activity.

Determination of DAAO activity in different rat organs

By using the present assay procedure, DAAO activity was determined in four different organs of rat. The

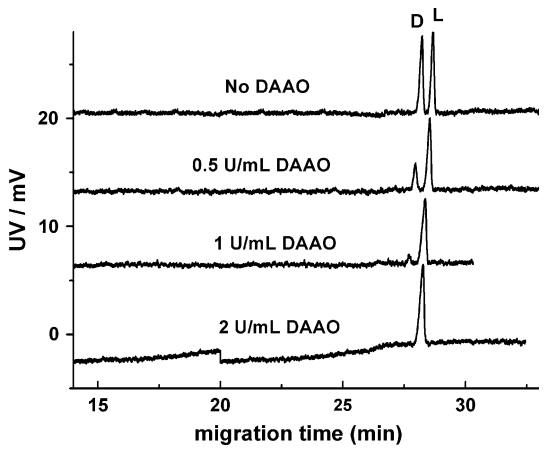

Fig. 3 Electropherogram measured from D,L-Met incubated with standard DAAO at different concentration. Other conditions are the same as in Fig. 2

electropherogram obtained from labeled D,L-Met after incubation with a kidney homogenate sample for different time and the statistical results for four organs are shown in Fig. 4.

Considering the interfering factors in the quantitative analysis of DAAO in biological fluid here including the complex biofluid composition and other simultaneous

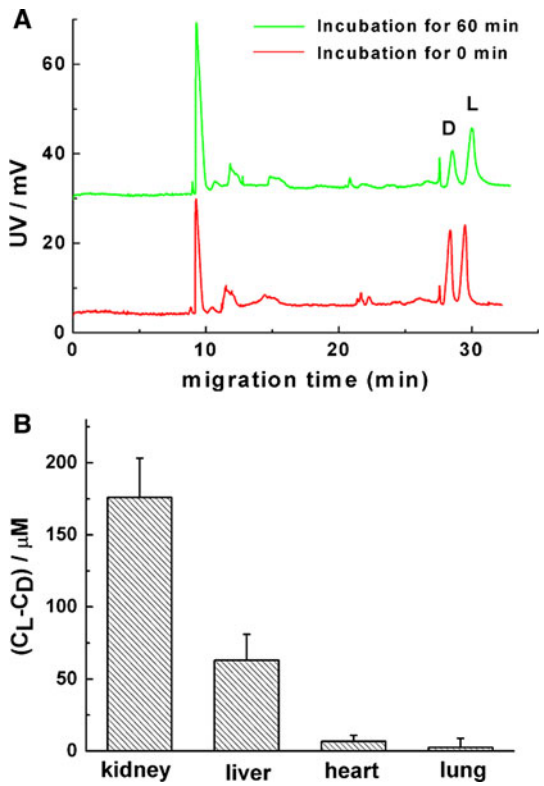

Fig. 4 Electropherogram measured from D,L-Met incubated with a normal kidney homogenates (**a**) and the activity of DAAO determined in the homogenates of the heart, liver, lung and kidney (**b**).Values are mean ± SEM ($n = 3$ rats per tissue)

enzymatic reaction related to D-Met and L-Met, such as the transamination of Met by aminotransferase and formation of *S*-adenosyl-methionine by methionine adenosyltransferase from Met and ATP, L-Met was added into the enzymatic reaction solution to eliminate quantitative disturbance and obtain the precise change of D-Met ascribed to DAAO (Mitchell and Benevenga 1978; Martinov et al. 2010; Zeisel et al. 1989). It should be noted that because of the presence of high concentration of L-AAs in biofluid, D,L-Met (not D-Met) need to be used in our enzymatic reaction to mimic real biological condition (Zinellu et al. 2007; Xu et al. 2005). Therefore, the detected concentration of L-Met minus that of D-Met ($C_L - C_D$) was used to indicate DAAO activity without any interference.

After incubating D,L-Met (5 mM respectively) with homogenates of kidney and liver for 60 min, DAAO-induced consumption of D-Met was determined to be 176.04 ± 17.15 μM and 62.94 ± 18.08 μM ($n = 3$), respectively, (Fig. 4b). In contrast, incubation of D,L-Met with homogenates of heart and lung did not result in obvious gap between the concentration of D-Met and L-Met. Significant difference in the DAAO-induced consumption of D-Met among four organs is shown in Fig. 4b. Paired *t* test showed that the DAAO-induced consumption of D-Met concentration in rat kidney homogenate was significantly higher than those in the rat liver homogenate, followed by those in the heart and lung homogenates ($P < 0.05$). This result displayed that highly expressed DAAO activity appeared in the kidney as well as the liver and minimal DAAO activity was present in the heart and lung. The results we found here are in accord with those reported in the literature and could offer a straightforward basis for the research on the physiological and pathological process associated with DAAO in the kidney (Hamase et al. 2006; Xin et al. 2005; D'Aniello et al. 1993).

Measurements of DAAO activity in rat renal ischemia model

Histopathological results (Fig. 5) showed evident lesions in the rat ischemic kidney, which was characterized by slightly wrinkled glomerular and mild shedding of renal tubular epithelial cells, compared to the normal kidney with no tissue damage.

Figure 6 shows the electropherogram obtained from D,L-Met incubated with an ischemic kidney homogenate sample and the change of local DAAO activity in the kidney following renal ischemia. As shown in Fig. 6a, incubation of D,L-Met (5 mM respectively) with homogenate of an ischemic kidney for 60 min resulted in a slight difference between the concentration of L-Met and D-Met. The DAAO-induced consumption of D-Met resulted from the incubation of D,L-Met with the homogenate samples of normal contralateral kidney was determined to be 145.99 ± 29.71 μM ($n = 8$), which is accordant with the previous figure obtained from normal animal. After the surgery of renal ischemia for 60 min, the gap between detected concentration of L-Met and D-Met was decreased to 78.64 ± 15.21 μM ($n = 8$), which is statistically different from the value of the kidney under normal condition ($t = 7.071$, $P < 0.001$). The operation of renal ischemia resulted in a 45.49 ± 8.30% ($n = 8$) decrease in the DAAO-induced consumption of D-Met, indicating that the renal DAAO activity was easily influenced by renal ischemia. This novel phenomenon was also confirmed by a traditional method for the determination of DAAO activity, which was based on the measurements of hydrogen peroxide and coupled with an indicator reaction using HRP and *o*-phenylenediamine (see the Online resource, Fig. 7).

The observed change in the level of local DAAO activity may be explained by the markedly reduced pH value in kidney upon ischemia, which leads to the blockage of renal blood flow, deficient supply of oxygen, increased demand of metabolism consumption, increased anaerobic metabolism and dysfunction of bicarbonate reabsorption (Prathapasinghe et al. 2008; Phifer 1983; Garcia et al. 2007). The research work from Raska illustrated that the decreased ammonia formation following renal ischemia was an key interference with the ability of the kidney to regulate the acid–base balance, resulting in metabolic acidosis (Raska 1943). According to Prathapasinghe and his

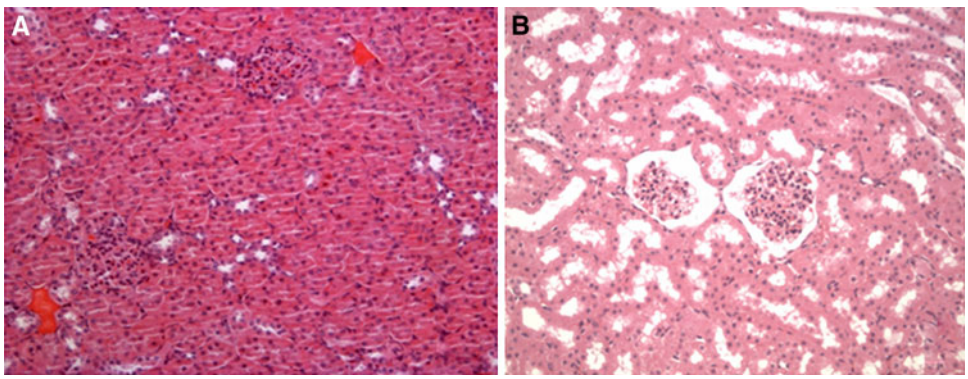

Fig. 5 Light microscopy of the cortex zone of the kidney without (**a**) and with (**b**) renal ischemia (hematoxylin and eosin staining, magnification, ×200)

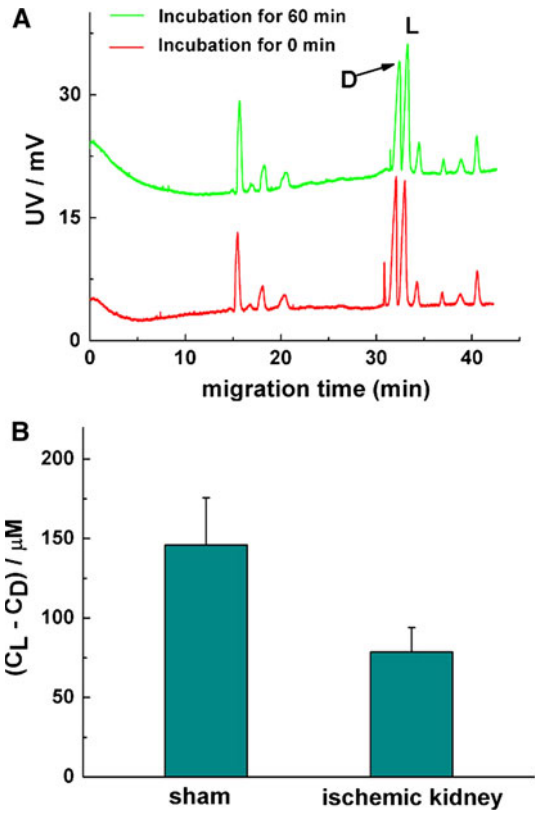

Fig. 6 Electropherogram measured from D,L-Met incubated with an ischemic kidney homogenates (**a**) and the effect of renal ischemia on the renal local DAAO activity (**b**). Values are mean ± SEM ($n = 8$)

co-workers' report, the kidney tissue pH is at 7.1 in the normal condition and decreases to 6.7 after ischemia for 60 min. Meanwhile, the optimal activity of DAAO in vitro is recorded at pH 8.2 (Prathapasinghe et al. 2008). There is a profound connection between the native FAD group of DAAO and pH in the redox-sensitive regulation of enzyme activity (Pernot et al. 2008; Denu and Fitzpatrick 1992; Kurtz et al. 2000; Pollegioni et al. 2000). In a word, the metabolic acidosis during ischemia may be the critical factor responsible for the reduction of DAAO activity in the kidney.

The reduced level of DAAO activity following renal ischemia was demonstrated in detail in our study and regarded as a pivotal supplement to intensive research about the metabolism of an ischemic kidney. Because the DAAO-mediated oxidation of D-AAs is accompanied by the generation of cytotoxic H_2O_2, which is considered to be an vital precursor of ROS, the change of renal DAAO activity during renal ischemia influence the local production of ROS, such as $HO_2^{\cdot}$ or $O_2^{\cdot-}$ (Silbernagl et al. 1999; Cortés-Rojo et al. 2007; Williams et al. 2005). In the previous studies, the establishment of renal ischemia–reperfusion resulted in the development of oxidative stress, e.g., via the generation of superoxide anions $O_2^{\cdot-}$ and the hydroxyl radical (·OH) (Chatterjee 2007). But this study substantially suggests that the first ischemia step of the renal transplantation operation has no contribution to the generation of ROS in the development of renal ischemia–reperfusion (I–R) injury and ARF. Excessive ROS generation present in the following reperfusion step is deduced. The result demonstrated here is believed to pave a new way for the study of oxidative stress in the development of renal I–R injury and ischemic ARF.

Conclusion

We took the advantage of chiral ligand exchange CE to develop a novel method for the detection of D-Met and L-Met after D, L-Met had been incubated with rat tissue homogenate samples, which is very useful for the determination of DAAO activity. The renal DAAO activity decreased sharply following 60 min renal ischemia with the possible reason of metabolic acidosis. Considering the DAAO-mediated oxidation of D-AAs accompanied by the generation of cytotoxic H_2O_2, which is considered to be a vital precursor of ROS, the change of renal DAAO activity during renal ischemia influence the local production of ROS, such as $HO_2^{\cdot}$ or $O_2^{\cdot-}$. The result demonstrated in detail here is believed to give new insight into the study of oxidative stress in the development of renal ischemia injury and related molecular mechanism of ischemic stroke-mediated major organ and tissue dysfunction.

Acknowledgments The present research was financially supported by National Natural Science Foundation of China (no. 20875091 and no. 20935005), Ministry of Science and Technology of China (no. 2007CB714504), and Chinese Academy of Sciences. We also thank Dr. Lei Liu in Department of Urology, Peking University Third Hospital for his helpful animal surgery.

References

Angermüller S, Islinger M, Völkl A (2009) Peroxisomes and reactive oxygen species, a lasting challenge. Cell Biol 131:459–463

Aronson S, Blumenthal R (1998) Perioperative renal dysfunction and cardiovascular anesthesia: concerns and controversies. J Cardiothorac Vasc An 12:567–586

Biondi PA, Guidotti L, Negri A, Secchi C (1991) High-performance liquid chromatographic determination of d-amino acid oxidase activity. J Chromatogr B 566:377–382

Chatterjee PK (2007) Novel pharmacological approaches to the treatment of renal ischemia-reperfusion injury: a comprehensive review. N-S Arch Pharmacol 376:1–43

Conger J, Robinette J, Villar A, Raij L, Shultz P (1995) Increased nitric oxide synthase activity despite lack of response to endothelium-dependent vasodilators in post-ischemic acute renal failure in rats. J Clin Invest 96:631–638

Cortés-Rojo C, Clemente-Guerrero M, Saavedra-Molina A (2007) Effects of D-amino acids on lipoperoxidation in rat liver and kidney mitochondria. Amino Acids 32:31–37

D'Aniello A, D'Onofrio G, Pischetola M, D'Aniello G, Vetere A, Petrucelli L, Fisher GH (1993) Biological role of D-amino acid oxidase and D-aspartate oxidase. Effects of D-amino acids. J Biol Chem 268:26941–26949

Davankov VA (2003) Enantioselective ligand exchange in modern separation techniques. J Chromatogr A 1000:891–915

Denu JM, Fitzpatrick PF (1992) pH and kinetic isotope effects on the reductive half-reaction of D-amino acid oxidase. Biochemistry 31:8207–8215

Eisenberg MS, Brandina R, Gill IS (2010) Current status of laparoscopic partial nephrectomy. Curr Opin Urol 20:365–370

Fujii T, Kurata H, Takaoka M, Muraoka T, Fujisawa Y, Shokoji T, Nishiyama A, Abe Y, Matsumura Y (2003) The role of renal sympathetic nervous system in the pathogenesis of ischemic acute renal failure. Eur J Pharmacol 481:241–248

Garcia MA, Meca R, Leite D, Boim MA (2007) Effect of renal ischemia/reperfusion on gene expression of a pH-sensitive K^+ channel. Nephron Physiol 106:1–7

Gassman E, Kuo JE, Zare RN (1985) Electrokinetic separation of chiral compounds. Science 230:813–814

Gok MA, Pelsers M, Glatz JF, Shenton BK, Peaston R, Cornell C, Talbot D (2003) Use of two biomarkers of renal ischemia to assess machine-perfused non-heart-beating donor kidneys. Clin Chem 1:172–175

Gozel P, Gassmann E, Michelsen H, Zare RN (1987) Electrokinetic resolution of amino acid enantiomers with copper(II)-aspartame support electrolyte. Anal Chem 59:44–49

Guz G, Oz E, Lortlar N, Ulusu NN, Nurlu N, Demirogullari B, Omeroglu S, Sert S, Karasu C (2007) The effect of taurine on renal ischemia/reperfusion injury. Amino Acids 32:405–411

Hamase K, Nagayasu R, Morikawa A, Konno R, Zaitsu K (2006) Sensitive high-performance liquid chromatographic assay for D-amino-acid oxidase activity in mammalian tissues using a fluorescent non-natural substrate, 5-fluoro-D-tryptophan. J Chromatogr A 1106:159–164

Hasegawa K et al (2010) Kidney-specific overexpression of Sirt1 protects against acute kidney injury by retaining peroxisome function. J Biol Chem 285:13045–13056

Holme DJ, Goldberg DM (1982) Development of a fluorometric assay for amino-acid oxidase activity and its application to the study of human tissues. Biochem Med 28:51–61

Imai K, Fukushima T, Santa T, Homma H, Huang Y, Shirao M, Miura K (1998) Whole body autoradiographic study on the distribution of ^{14}C-D-serine administered intravenously to rats. Amino Acids 15:351–361

Kim J, Seok YM, Jung KJ, Park KM (2009) Reactive oxygen species/oxidative stress contributes to progression of kidney fibrosis following transient ischemic injury in mice. Am J Physiol-Renal 297:461–470

Kim J, Jung KJ, Park KM (2010) Reactive oxygen species differently regulate renal tubular epithelial and interstitial cell proliferation after ischemia and reperfusion injury. Am J Physiol-Renal 298:1118–1129

Konno R (1998) Methods for the detection of D-amino-acid oxidase. Biol Proced Online 1:27–31

Konno R, Ikeda M, Yamaguchi R, Ueda Y, Niwa A (2000) Nephrotoxicity of D-propargylglycine in mice. Arch Toxicol 4:473–479

Krug AW, Völker K, Dantzler WH, Silbernagl S (2007) Why is D-serine nephrotoxic and -aminoisobutyric acid protective? Am J Physiol-Renal 293:382–390

Kurganov A (2001) Chiral chromatographic separations based on ligand exchange. J Chromatogr A 906:51–71

Kurtz KA, Rishavy MA, Cleland WW, Fitzpatrick PF (2000) Nitrogen isotope effects as probes of the mechanism of D-amino acid oxidase. J Am Chem Soc 122:12896–12897

Maekawa M, Okamura T, Kasai N, Hori Y, Summer KH, Konno R (2005) D-amino acid oxidase is involved in D-serine-induced nephrotoxicity. Chem Res Toxicol 18:1678–1682

Martinov MV, Vitvitsky VM, Banerjee R, Ataullakhanov FI (2010) The logic of the hepatic methionine metabolic cycle. BBA Proteins Proteom 1804:89–96

Mitchell AD, Benevenga NJ (1978) The role of transamination in methionine oxidation in the rat. J Nutr 108:67–78

Mora MF, Giacomelli CE, Garcia CD (2009) Interaction of D-amino acid oxidase with carbon nanotubes: implications in the design of biosensors. Anal Chem 81:1016–1022

Pernot P, Mothet JP, Schuvailo O, Soldatkin A, Pollegioni L, Pilone M et al (2008) Characterization of a yeast D-amino acid oxidase microbiosensor for D-serine detection in the central nervous system. Anal Chem 80:1589–1597

Phifer TJ (1983) Metabolic control of cellular pH after renal ischemia. Circ Shock 10:274–274

Plotnikov EY, Kazachenko AV, Vyssokikh MY, Vasileva AK, Tcvirkun DV, Isaev NK, Kirpatovsky VI, Zorov DB (2007) The role of mitochondria in oxidative and nitrosative stress during ischemia/reperfusion in the rat kidney. Kidney Int 72:1493–1502

Pollegioni L, Porrini D, Molla G, Pilone MS (2000) Redox potentials and their pH dependence of D-amino acid oxidase of *Rhodotorula gracilis* and *Trigonopsis variabilis*. Eur J Biochem 267:6624–6632

Prathapasinghe GA, Siow YL, Xu ZB OK (2008) Inhibition of cystathionine-β-synthase activity during renal ischemia-reperfusion: role of pH and nitric oxide. Am J Physiol-Renal 295:912–922

Preinerstorfer B, Lämmerhofer M, Lindner W (2009) Advances in enantioselective separations using electromigration capillary techniques. Electrophoresis 30:100–132

Qi L, Qiao J, Yang GL, Chen Y (2009) Chiral ligand-exchange CE assays for separation of amino acid enantiomers and determination of enzyme kinetic constant. Electrophoresis 30:2266–2272

Raska SB (1943) The metabolism of the ischemic kidney I. The respiration and the oxidase activity of the ischemic kidney. J Exp Med 78:75–89

Rodrigo R, Rivera G (2002) Renal damage mediated by oxidative stress: a hypothesis of protective effects of red wine. Free Radical Bio Med 33:409–422

Sacchi S, Lorenzi S, Molla G, Pilone MS, Rossetti C, Pollegioni L (2002) Engineering the substrate specificity of D-amino acid oxidase. J Biol Chem 277:27510–27516

Schmid MG, Grobuschek N, Lecnik O, Gübitz G (2001) Chiral ligand-exchange capillary electrophoresis. J Biochem Biophys Methods 48:143–154

Silbernagl S, Völker K, Dantzler WH (1999) D-Serine is reabsorbed in rat renal pars recta. Am J Physiol-Renal 276:857–863

Tanaka H, Yamamoto A, Ishida T, Horiike K (2007) Simultaneous measurement of D-serine dehydratase and D-amino acid oxidase activities by the detection of 2-oxo-acid formation with reverse-phase high-performance liquid chromatography. Anal Biochem 362:83–88

Tilney NL, Guttmann RD (1997) Effects of initial ischemia/reperfusion injury on the transplanted kidney. Transplantation 64:945–947

Tishkov VI, Khoronenkova SV (2005) D-Amino acid oxidase: structure, catalytic mechanism, and practical application. Biochemistry (Moscow) 70:40–54

Vanoni MA, Cosma A, Mazzeo D, Mattevi A, Todone F, Curti B (1997) Limited proteolysis and X-ray crystallography reveal the origin of substrate specificity and of the rate-limiting product release during oxidation of D-amino acids catalyzed by mammalian D-amino acid oxidase. Biochemistry 36:5624–5632

Veenhuis M, Wendelaar Bonga SD (1977) The cytochemical demonstration of catalase and D-amino acid oxidase in the microbodies of teleost kidney cells. Histochem J 9:171–181

Vespalec R, Boček P (2000) Chiral separations in capillary electrophoresis. Chem Rev 100:3715–3754

Ward TJ, Baker BA (2008) Chiral separations. Anal Chem 80:4363–4372

Watanabe T, Motomura Y, Suga T (1978) A new colorimetric determination of D-amino acid oxidase and urate oxidase activity. Anal Biochem 86:310–315

Williams RE, Major H, Lock EA, Lenz EM, Wilson ID (2005) D-Serine-induced nephrotoxicity: a HPLC–TOF/MS-based metabonomics approach. Toxicology 207:179–190

Xin YF, Zhou XJ, Cheng X, Wang YX (2005) Renal D-amino acid oxidase mediates chiral inversion of NG-Nitro-D-arginine. J Pharmaol Exp Ther 312:1090–1096

Xu HH, Zhang W, Zhu W, Wang D, Ye J, Yamamoto K, Jin LT (2005) Simultaneous determination of total homocysteine, cysteine and methionine in hypothyroid patients' plasma by liquid chromatography using platinum/poly(methyl violet) modified electrode. Anal Chim Acta 545:182–188

Young GA, Kendall S, Brownjohn AM (1994) D-Amino acids in chronic renal failure and the effects of dialysis and urinary losses. Amino Acids 6:283–293

Zeisel SH, Zola T, Dacosta KA, Pomfret EA (1989) Effect of choline deficiency on *S*-adenosylmethionine and methionine concentrations in rat liver. Biochem J 259:725–729

Zinellu A et al (2007) Plasma methionine determination by capillary electrophoresis-UV assay: application on patients affected by retinal venous occlusive disease. Anal Biochem 363:91–96