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Microassay of 5′-Nucleotidase and Adenosine Deaminase Activity in Microdissected Nephron Segments

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The present study describes a new method for microassay of the activity of 5′-nucleotidase (5′-ND) and adenosine deaminase (ADA) in the microdissected nephron segments. The nephron segments including glomeruli, proximal convoluted and straight tubules (PCT and PST), cortical and medullary thick ascending limbs, and cortical and medullary collecting ducts were microdissected. 5′-ND and ADA in the nondenatured lysate of 20-mm microdissected tubules and 20 glomeruli were separated by agarose gel electrophoresis and by isoelectric focusing, respectively. The gels were incubated with specific substrates and staining dyes to exhibit the dephosphorylation by 5′-ND or deamination by ADA. The enzyme activity was estimated by measuring the intensity of the reaction bands on the gels. The 5′-ND activity was detected in all microdissected tubular segments and glomeruli. Among these nephron segments, PCT and PST exhibited the greatest enzyme activity, averaging 1142 and 939 mU/mg tissue protein, respectively. The activity of ADA was also detected in all tubular segments and glomeruli. However, the greatest activity of this enzyme was found in the glomeruli (649.8 mU/mg protein). Using reverse transcriptase–polymerase chain reaction technique, we verified the presence of mRNA of 5′-ND and ADA in all microdissected tubular segments and glomeruli. Based on these results, we conclude that 5′-ND and ADA are present in all nephron segments studied, but the activity of these enzymes is nonuniformly expressed along the nephron. This microassay is a highly specific, sensitive, and reliable method for the segmental analysis of adenosine metabolism in the kidney.

Key Words: **adenosine; 5′-nucleotidase; adenosine deaminase; glomerulus; tubule; kidney; isoelectric focusing; gel electrophoresis.**

Adenosine is widely recognized as a regulator of renal function. It participates in the control of renal hemodynamics, hormone and neurotransmitters release, and tubular reabsorption under physiological conditions (1–3). Recent studies indicated that adenosine plays an important role in protecting renal tubules and glomeruli from pathological injury (4–6). An abnormality of adenosine metabolism in the kidney could cause or exaggerate renal diseases and kidney-related systemic diseases such as acute renal failure, hypertension, glomerular sclerosis, and diabetic nephropathy (6–10). Although the physiological and pathological significance of renal adenosine has been extensively studied, the mechanism of adenosine metabolism in the kidney is poorly understood. There are very few tools that can be used to quantify the adenosine metabolism along the renal nephrons. Previous studies indicated that proximal tubules and thick ascending limb produced adenosine in response to hypoxia or other stimuli (10, 11), but these studies required a bulk isolation of tubular segments and failed to reveal the profile of the adenosine metabolism along the nephron and the contribution of the individual enzymes such as 5′-nucleotidase (5′-ND)[2] and adenosine deaminase (ADA) to the adenosine metabolism in these nephron segments. Using immunohistochemical methods, an ecto-5′-ND and ADA have been localized along the nephron. It has been shown that 5′-ND is present on the proximal tubules and glomeruli, and that ADA is highly expressed in the glomeruli (12–15). However, these immunohistochemical studies only detected protein amount of the enzymes, but not the enzyme activ-

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[2] Abbreviations used: 5′-ND, 5′-nucleotidase; ADA, adenosine deaminase; RT–PCR, reverse transcriptase–polymerase chain reaction; PCT, proximal convoluted tubule; PST, proximal straight tubule; CTAL, cortical thick ascending limb; MTAL, medullary thick ascending limb; CCD, cortical collecting duct; MCD, medullary collecting duct; Glm, glomeruli; 5′-AMP, adenosine 5′-monophosphate; EHNA, erythro-9-(2-hydroxyl-3-nonyl) adenine; DU, densitometric units.

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ity. Although histochemical techniques were used to study the distribution of the 5′-ND activity along the rat nephron in tissue slices (16), those assays only provided a qualitative analysis of the 5′-ND activity along the nephron, and the low sensitivity and specificity largely plagued the usefulness of the histochemical assay. In the present study, we have developed a new microassay method for the measurement of the 5′-ND and ADA activity in the microdissected nephron segments based on isoelectric focusing and native gel electrophoresis techniques. Since isoelectric focusing or gel electrophoresis was used to separate 5′-ND and ADA from other possible enzymes that may react with their substrates, this microassay exhibited a great sensitivity and specificity. To verify the distribution of 5′-ND and ADA, we also detected the mRNA expression of both enzymes in these microdissected nephron segments using a RT–PCR technique.

MATERIALS AND METHODS

Microdissection of nephron segments. Microdissection was performed as described previously (17, 18). Briefly, Sprague–Dawley rats weighing between 250 and 300 g were anesthetized with Inactin (80 mg/kg body wt., ip), and the aorta below left renal artery was isolated and cannulated. After ligating the aorta at a site between the origin of the left and right renal arteries, the left kidney was perfused with 20 ml ice-cold dissection solution containing 135 mM NaCl, 3 mM KCl, 1.5 mM $CaCl_2$, 1 mM $MgSO_4$, 2 mM KH_2PO_4, 5.5 mM glucose, 5 mM L-alanine, and 5 mM Hepes (pH 7.4). Then, 10 ml of the digestion solution was prepared by adding 1 mg/ml collagenase (243 U/mg, Worthington), 1 mg/ml trypsin inhibitor, and 0.5 mg/ml proteinase inhibitor, aprotinin, in the dissection solution. Following perfusion, the kidney was removed and cut into 1- to 2-mm-thick sections containing the entire corticomedullary axis. The sections were incubated at 37°C for 30 min in the same digestion solution with gentle shaking. During incubation, the samples were bubbled with 100% O_2. The sections were then rinsed twice with collagenase-free dissection solution and transferred into petri dishes filled with ice-cold dissection solution containing 0.1 mg/ml trypsin inhibitor and 20 μg/ml aprotinin. A petri dish was mounted on the microscope stage and maintained at 4°C during dissection. Microdissection was performed under a Leica MZ8 stereomicroscope with dark-field illumination. The following segments were dissected, and the length of these tubules was measured with a calibrated eyepiece micrometer: proximal convoluted tubule (PCT), proximal straight tubule (PST), cortical thick ascending limb (CTAL), medullary thick ascending limb (MTAL), cortical collecting duct (CCD), and medullary collecting duct (MCD). The glomeruli (Glm) were counted under microscope. The time period for dissection was limited to 1 h. In general, 20 glomeruli and 20-mm tubule segments were pooled to use as one sample.

Assay of the 5′-ND activity. Microdissected nephron segments were lysed in 8 μl of 0.15 M sodium phosphate buffer (pH 6.8), containing 0.75% Zwittergent 3-14 (Calbiochem, La Jolla, CA) and sonicated for 15 s three times at 45 W using a microsonicator. The lysates of the nephron segments or 1 mU purified 5′-ND protein (Sigma) were incubated in the presence of 0.75% Zwittergent 3-14 overnight at 4°C to release 5′-ND from bound membrane lipid. Then, 5′-ND was electrophoretically separated in a 0.5-mm thin layer of agarose gel (EEO = 0) prepared with 25 mM barbital buffer (pH 8.2) using a horizontal electrophoresis apparatus (Multi Temp III, Pharmacia Biotech) as described by Tucker-Pian *et al.* (19). Electrophoresis was carried out in 50 mM barbital buffer (pH 8.2) at 20 V/cm across the gel for 2.5 h at 4°C. After electrophoresis, the gel was incubated in the reaction solution containing Tris–maleate buffer (pH 7.0, 50 mM), adenosine 5′-monophosphate (5′-AMP, 1 mM), lead nitrate (2 mM), and manganous nitrate (50 mM) (16) at 37°C for 3 h. Control gels were incubated in the reaction solution omitting the substrate, 5′-AMP. To verify the specificity of the reaction, a selective inhibitor of 5′-ND, α,β-methyleneadenosine 5′-diphosphate (5 μM) was added into the reaction solution in additional gel incubations. Following incubation, the gel was rinsed with distilled water, and the bands of the enzyme reaction were made visible with a 2% sodium sulfate solution. After being washed and cleaned, the gel was dried. The intensity of the enzyme reaction bands on the gel was measured using a densitometer. The activity of 5′-ND was estimated by comparing the densitometric units (intensity) of unknown samples with that of purified 5′-ND (1 mU) on the same gel. The protein concentration of the lysates of microdissected nephron segments was measured using a Bio-Rad protein assay kit according to the microassay procedures described by manufacturer.

Assay of the ADA activity. Microdissected nephron segments were lysed in 8 μl Tris–HCl buffer (10 mM Tris–Cl, 1 mM EDTA, 1 mM mercaptoethanol, pH 7.4) for 5 min and then frozen and thawed twice by placing the sample tubes in liquid nitrogen. The samples were sonicated for 15 s twice at 45 W before being loaded on the gel. ADA was separated by isoelectric focusing. Isoelectric focusing was carried out in 0.5-mm gels containing 4.85% acrylamide, 0.15% bisacrylamide, 2% (v/v) preblended ampholine, pH 3.5–9.5 (Pharmacia Biotech), 300 mM sucrose, and 2 μM riboflavin. The electrode solutions were composed of 150 mM acetic acid for the anode and 150 mM ethanolamine for the

cathode. The samples were electrically focused at 150 V/cm at 4°C for 3 h. Immediately after isoelectric focusing, the gel was overlaid with 1% agar–Noble gel mixture containing 1.5 mM adenosine, 0.2 mM tetrazolium salt MTT, 0.3 mM phenazine methosulfate, 0.3 U xanthine oxidase, and 3 U nucleoside phosphorylase in 0.1 M sodium phosphate buffer (pH 7.5) (20, 21). After incubation for 2 h at 37°C, a blue band representing the ADA activity was exhibited. Control gels were incubated with an overlay gel omitting the substrate, adenosine. A selective inhibitor of ADA, erythro-9-(2-hydroxyl-3-nonyl) adenine (EHNA) (1 μM) was added into the overlay gel mixture in additional gel incubations. The activity of ADA was estimated by measuring the intensity of gel bands and comparing with purified ADA (1 mU) on the same gel.

RNA extraction and RT–PCR of 5′-ND and ADA. To extract total RNA, the microdissected segments were transferred into individual tubes containing 450 μl TRIzol reagent (Gibco BRL, Life Technologies) and then incubated at room temperature for 5 min. Total RNA was extracted, precipitated, and washed according to the protocol described by the manufacturer. The resultant RNA was resuspended in 8 μl of RNase-free water.

A first-strand cDNA synthesis kit (Pharmacia Biotech) was used to synthesize cDNA by reverse transcriptase (RT) from mRNA. As described by the instructions of the manufacturer, 8 μl of total RNA was heated at 65°C for 10 min, rapidly chilled on ice, and then mixed with 7 μl of the reagents supplied with the kit. The reaction mixture contained 0.2 μg random hexadeoxynucleotides, 45 mM Tris (pH 8.3), 68 mM KCl, 15 mM DTT, 9 mM $MgCl_2$, 0.08 mg/ml BSA, 1.8 mM dNTPs, and 100 U M-MuLV reverse transcriptase. The reaction mixture was incubated at 37°C for 60 min and then heated to 65°C for 10 min to inactivate the reverse transcriptase activity and to denature cDNA hybrids.

PCR reactions were performed in a total volume of 50 μl using a PCR Supermix kit (Gibco BRL) containing: 22 mM Tris–HCl (pH 8.4), 55 mM KCl, 1.65 mM $MgCl_2$, 200 μM dNTPs, 5 μl RT reaction mixture, 22 U recombinant *Taq* DNA polymerase, and 400 pmol of the specific primer pairs for ADA, 5′-ND, or β-actin. The reactions were cycled 30 times from 94°C for 1 min to 58°C for 1 min, and then 72°C for 1.5 min. Samples were incubated at 72°C for an additional 5 min after the last cycle was completed. ADA, 5′-ND, and β-actin primers span fragments of 149, 219, and 350 bp from their respective cDNAs (22–24). Negative control PCRs with a substitution of dissection solution or total RNA without RT reaction were performed in parallel.

The structure of the primers was as follows: ADA, sense 5′-CCG TGG TGG CTA TGG ACT, antisense 5′-ACG CAC AAC CTC AGG AGA; 5′-ND, sense 5′-CAG TTC ACC CGC TCA A, antisense 5′-CGC CGC CCA CAC ACA G; and β-actin, sense 5′-AAC CGC GAG AAG ATG ACC CAG ATC ATG TTT; antisense 5′-AGC AGC CGT GGC CAT CTC TTG CTC GAA GTC. All primers were synthesized by OPERON. Base sequences of the primer pair for β-actin were based on previous work by Briggs *et al.*, who reported that this primer pair had high efficiency for PCR product in microdissected glomeruli or tubular segments (22).

PCR products were separated by a 1.5% agarose gel electrophoresis (200 V for 1 h) in 1× TBE buffer, stained with ethidium bromide (0.5 μg/ml), and visualized under UV light, and a photograph was made. The identity of the PCR products of 5′-ND and ADA was determined by restriction digestion using two endonuclease specific to each cDNA sequence as described previously (25). The endonucleases for ADA were *Bsm*I and *Hae*III, and those for 5′-ND were *Sac*I and *Hae*III. These endonucleases were chosen based on restriction site mapping using GCG computer program. The restriction digestions were performed at 37°C for 1 h in the buffer provided by the manufacturer. The digestion products were separated on a 2% agarose gel in 1× TBE buffer, stained with ethidium bromide (0.5 μg/ml), and visualized under UV light, and a photograph made.

Statistics. Data are presented as means ± SE. The significance of differences within and between groups was evaluated using an analysis of variance and a Student's *t* test. $P < 0.05$ was considered statistically significant.

RESULTS

Construction of standard curve for the 5′-ND and ADA activity. Purified 5′-ND produced a specific activity band on nondenaturing agarose gel after electrophoresis. Figure 1A presents concentration-dependent changes in the intensity of 5′-ND reaction bands on the gel. The intensity of the reaction bands increased as the 5′-ND concentrations increased. Correlation analysis showed a linear relationship between the densitometric units of the reaction bands and 5′-ND activity with a correlation coefficient of 0.984. A regression equation for calculation of the 5′-ND activity from the densitometric units of the gel bands was described as 5′-ND (mU) = 0.007 DU − 0.79, where DU is the densitometric units of the reaction bands on the gel. The minimum detectable 5′-ND activity was 0.5 mU. When the 5′-ND substrate, 5′-AMP, was omitted from the reaction solution or the 5′-ND inhibitor, α,β-methyleneadenosine 5′-diphosphate, was added, no reaction band was detected on the gel, even at the highest loaded concentration of 5′-ND (8 mU) (data not shown).

As shown in Fig. 2, purified ADA also produced con-

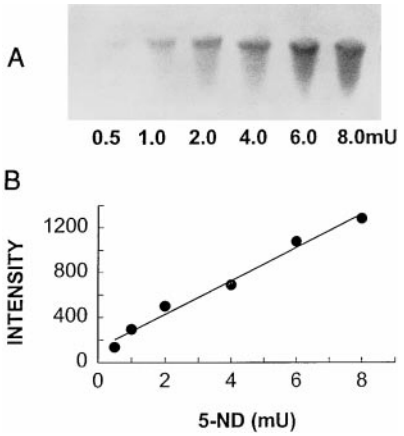

FIG. 1. (A) A photograph depicting the activity of purified 5′-nucleotidase (5′-ND) separated by nondenaturing agarose gel electrophoresis. (B) A plot shows the correlation relationship between the concentrations of purified 5′-nucleotidase and the densitometric units of gel band specific to 5′-nucleotidase.

centration-dependent changes in the intensity of the reaction bands on the isoelectric focusing gel when stained with the agar overlay mixture (Fig. 2A). Correlation analysis showed a linear relationship between the densitometric units of the reaction bands and ADA activity units, and the correlation coefficient was 0.991. A regression equation for calculation of the ADA activity from the densitometric units of the gel bands was described as ADA (mU) = 0.001 DU + 0.149, where DU is the densitometric units of reaction bands on the gel. The minimum detectable ADA activity was 0.2 mU. When adenosine was omitted from the agar overlay mixture or ADA was cooked at 95°C for 10 min, no reaction band was detected. In the presence of the ADA

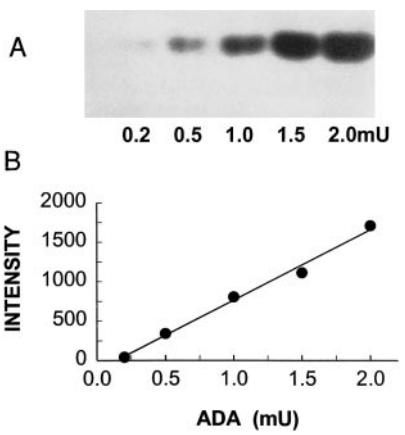

FIG. 2. (A) A photograph depicting the activity of purified adenosine deaminase (ADA) separated by isoelectric focusing. (B) A plot shows the correlation relationship between the concentrations of purified adenosine deaminase and the densitometric units of gel bands specific to adenosine deaminase.

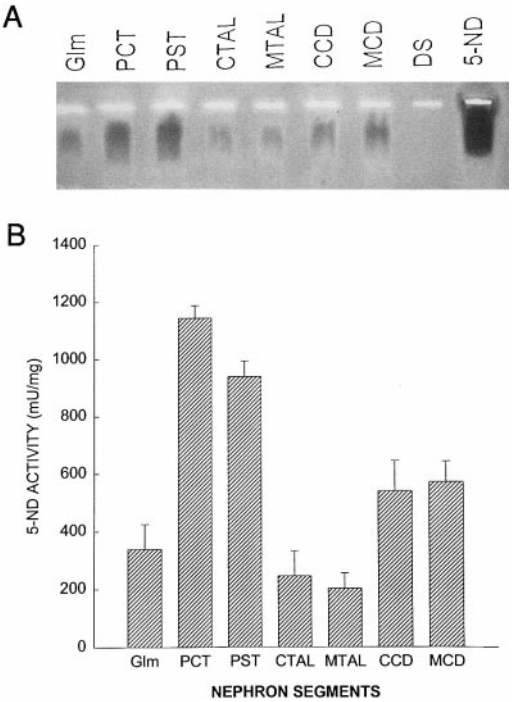

FIG. 3. (A) A photograph presenting the 5′-nucleotidase activity from microdissected nephron segments of rats. (B) A summary of the 5′-nucleotidase activity in microdissected nephron segments. Values are means ± SE of 7 independent experiments. Glm, glomerulus; PCT, proximal convoluted tubule; PST, proximal straight tubule; CTAL, cortical thick ascending limb of loop of Henle; MTAL, medullary thick ascending limb of loop of Henle; CCD, cortical collecting duct; MCD, medullary collecting duct; DS, dissection solution.

inhibitor, EHNA, no reaction band was detected on the gel as well.

5′-ND activity in microdissected nephron segments. Since 5′-ND is tightly lipid-bound enzyme in the tissue lysate, a slight detergent was required to separate 5′-ND from the lipid and to increase its mobility on the gel, but not to alter its activity. We have tested *n*-butanol, Triton 100, and Zwittergent 3-14, which all were reported to increase the 5′-ND mobility on the gel. We found that Zwittergent 3-14 was the best detergent for the separation of 5′-ND from lipid and the preservation of its activity.

A typical photograph depicting the 5′-ND activity in microdissected nephron segments is presented in Fig. 3A. A well-separated reaction band specific to substrate 5′AMP was identified in the lysate from all nephron segments. No band was detected in the dissection solution. The greatest 5′-ND activity was found in proximal tubules. As shown in Fig. 3B, the 5′-ND activities of PCT and PST were 1142 and 939 mU/mg protein, which were significantly higher than that detected in other nephron segments. The 5′-ND activity in these nephron segments was completely lost by preheating the lysate at 98°C for 10 min and the addition

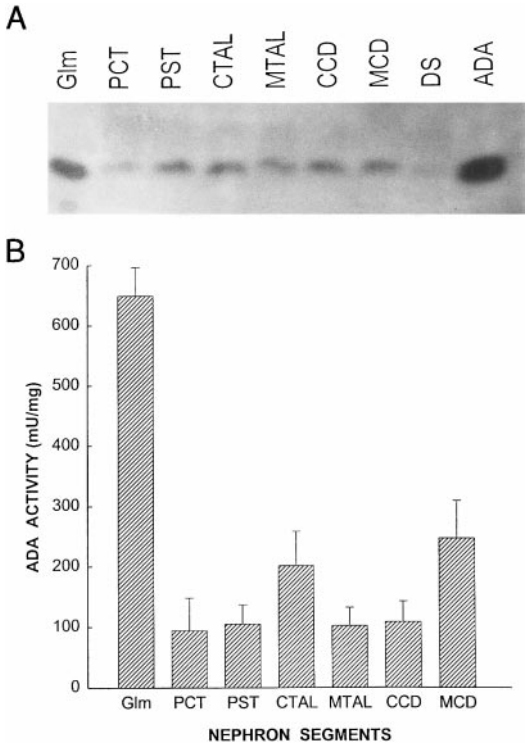

FIG. 4. (A) A photograph presenting the adenosine deaminase activity from microdissected nephron segments of rats. (B) A summary of the adenosine deaminase activity in microdissected nephron segments. Values are means ± SE of 6 independent experiments. Abbreviations are same as those in Fig. 3.

of 5′-ND inhibitor, α,β-methyleneadenosine 5′-diphosphate, in the reaction mixture or omission of 5′-AMP (data not shown).

ADA activity in microdissected nephron segments. A typical photograph depicting the ADA activity in microdissected nephron segments is presented in Fig. 4A. The lysates of all nephron segments exhibited a well-separated reaction band with pI 4.6. Occasionally, a very light band was detected in the dissection solution contained tissue, which may be associated with ADA release from tissue interstitium. To rule out the influence of this contamination in data analysis, the densitometric values of reaction bands of all nephron segments were corrected by subtracting background value in dissection solution. Figure 4B depicts the distribution of the ADA activity along the nephron. The greatest activity was detected in the glomeruli (649.8 mU/mg protein). Among the tubular segments, CTAL and MCD expressed greater ADA activity. Preheating the lysate, addition of ADA inhibitor, EHNA in the reaction overlay mixture, and omission of substrate completely inactivated the ADA activity in the nephron segments.

Distribution of 5′-ND mRNA and ADA mRNA along the nephron. Figure 5 shows a typical photograph of ethidium bromide-stained PCR products of 5′-ND, ADA, and β-actin. The signal of each lane on the gel represents the PCR products only from 0.7-mm dissected tubules or 1 glomerulus. Both 5′-ND and ADA mRNA were expressed in all microdissected nephron segments. The sizes of the PCR products of 5′-ND and ADA were 219 and 149 bp, respectively, which were identical with the expected cDNA sizes. The PCR product of β-actin was also consistently detected with a predicted size of 350 bp. Addition of RNA extracted from all dissected nephron segments without reverse transcription to the PCR mixture did not produce any signal, suggesting that PCR products were derived from cDNA synthesized by reverse transcription. An example of negative control without RT using PCT RNA was shown in Fig. 5 (RT (−)). Using β-actin, a housekeeping gene, as a reference, it seems that the expression of 5′-ND mRNA was greater in PST, PCT, and Glm, but ADA mRNA expression was the same in all microdissected nephron segments.

Figure 6 presents a typical photograph of full-length or restriction-digested PCR products of 5′-ND and ADA. Digestion of the PCR product of 5′-ND with *Hae*III produced two pieces of cDNA with sizes of 115 and 104 bp. Digestion with *Sac*I also produced two pieces of cDNA with sizes of 119 and 100 bp. The PCR product of ADA was cut into two pieces of cDNA with sizes of 92 and 57 bp by *Hae*III, and 94 and 55 bp by *Bsm*I, respectively. The sizes of these digestion products and restriction cutting sites all were predicted from reported cDNA sequence of 5′-ND and ADA.

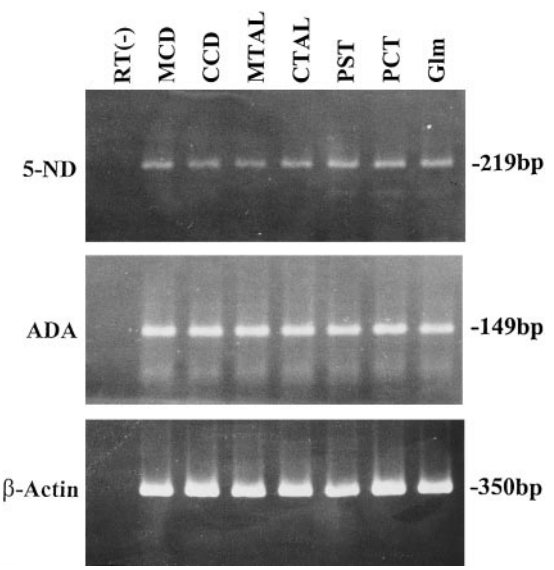

FIG. 5. RT–PCR products of 5′-nucleotidase, adenosine deaminase, and β-actin in microdissected nephron segments stained by ethidium bromide. The sizes of PCR products of 5′-nucleotidase, adenosine deaminase, and β-actin are 219, 149, and 350 bp, respectively. Abbreviations are same as those in Fig. 3.

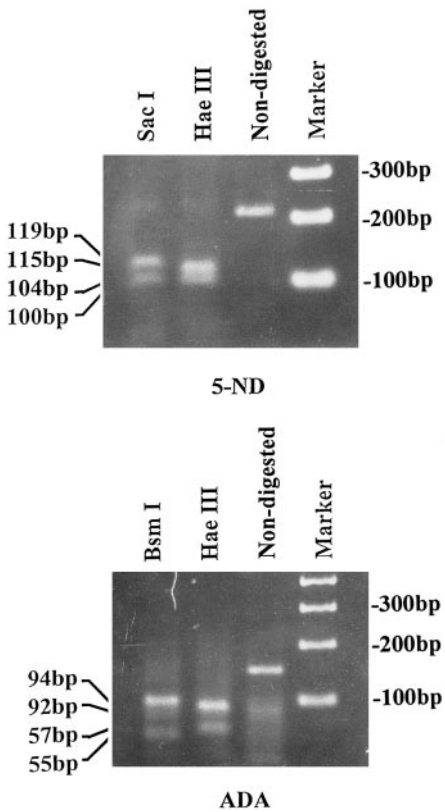

FIG. 6. Restriction digestion of PCR products of 5′-nucleotidase and adenosine deaminase. Top panel shows cDNA of 5′-nucleotidase with or without restriction digestion by *Sac*I and *Hae*III, respectively. Bottom panel shows cDNA of adenosine deaminase with or without restriction digestion by *Bsm*I and *Hae*III, respectively. Marker represents a 100-bp ladder DNA marker. Labels of molecular sizes on the left of photo are predicted sizes by computer mapping analyses.

DISCUSSION

The present study measured 5′-ND and ADA activity in the microdissected cortical and medullary nephron segments using isoelectric focusing and gel electrophoresis in combination with specific staining of the enzyme reaction products. We demonstrated that both 5′-ND and ADA are present in all microdissected cortical and medullary tubule segments and glomeruli. This provides the first evidence of a quantitative colocalization of 5′-ND and ADA activity in the cortical and medullary nephron segments. We found that the distribution profiles of both enzymes were different. The greatest activities of 5′-ND and ADA were detected in the proximal tubules and glomeruli, respectively. Importantly, we found that medullary tubules such as medullary thick ascending limb and medullary collecting ducts expressed the activity of 5′-ND and ADA. Medullary collecting ducts appeared to be rich for both 5′-ND and ADA. These results indicate that the medullary tubules are capable of producing and metabolizing adenosine. However, previous studies using a qualitative histochemical assay reported that both 5′-ND and ADA are not detectable in the renal medullary tubules in tissue slices (12, 13, 16). The reason for this discrepancy is unknown, but it is possible that electrophoresis-based quantitative microassay used in the present study is more sensitive than previous histochemical estimations.

In previous studies, different gel electrophoresis and isoelectric focusing were used to identify the isozymes of 5′-ND and ADA from tissue and blood cells (19, 21, 26). In the microdissected nephron segments, we did not identify any isoforms for 5′-ND and ADA. However, unspecific reactions due to other related enzymes such as alkaline phosphatase were differentiated and ruled out by electrophoresis or isoelectric focusing. In the kidney homogenate, some reaction bands produced by the action of alkaline phosphatase were detected even only in the presence of substrate for 5′-ND (data not shown), but these bands were separated far from that of 5′-ND on the gel. This unspecific reaction for 5′-ND and ADA was also reported and discussed in previous studies (19, 27). It may interfere with the accuracy of the activity assay of 5′-ND and ADA if they are not well separated. This nonspecificity of the substrates challenged the use of histochemical and biochemical assays, by which neither 5′-ND nor ADA was separated from other enzymes. To further determine the specificity of the microassay used in the present study, we examined whether the 5′-ND and ADA activities were inhibited by denaturing the enzyme proteins in the lysate, omission of reaction substrates, and incubation of the gel with specific inhibitors. When the lysate proteins were preheated at 98°C, the activities of both 5′-ND and ADA were not detectable. When the substrates were removed from the incubation mixtures or specific inhibitors of 5′-ND and ADA were added, the enzyme reaction bands also completely disappeared. Taken together, these results indicate that the microassay developed in the present study is a specific method for measurement of the 5′-ND and ADA activity on the single nephron level. We found that the minimally detectable activity was 0.5 mU for 5′-ND and 0.2 mU for ADA. As shown in Figs. 3 and 4, the lowest activity detected in microdissected nephron segments was 0.8 mU for 5′-ND (230 mU/mg protein in MTAL) and 0.24 mU for ADA (94 mU/mg protein in PCT). Therefore, the sensitivity of this microassay for both 5′-ND and ADA is adequate to segmental analysis on the single nephron level.

Using RT–PCR, we demonstrated that 5′-ND and ADA mRNA was expressed in all nephron segments studied. This verified the data obtained by the microassay of the enzyme activity. It seems that the distribution profile of 5′-ND mRNA was similar to that of 5′-ND activity. However, we did not find a distribution

profile of ADA mRNA similar to that of the ADA activity. Since RT–PCR used in the present study could not quantitatively measure mRNA, the mechanism for the dissociation of ADA mRNA expression with its activity along the nephron remains to be further determined.

In summary, we developed a novel method for measurement of the 5′-ND and ADA activities in microdissected nephron segments. This method was highly sensitive, specific, and reliable. 5′-ND and ADA are present in all microdissected cortical and medullary tubule segments and glomeruli, but the activities of 5′-ND and ADA distribute heterogeneously along the nephron. The proximal tubules exhibited the greatest 5′-ND activity, while the glomeruli expressed the greatest ADA activity.

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