Molecular cloning of cDNA for nonhepatic mitochondrial arginase (arginase II) and comparison of its induction with nitric oxide synthase in a murine macrophage-like cell line

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Abstract Arginase exists in two isoforms. Liver-type arginase (arginase I) is expressed almost exclusively in the liver and catalyzes the last step of urea synthesis, whereas the nonhepatic type (arginase II) is expressed in extrahepatic tissues. Arginase II has been proposed to play a role in down-regulation of nitric oxide synthesis. A cDNA for human arginase II was isolated. A polypeptide of 354 amino acid residues including the putative NH_2-terminal presequence for mitochondrial import was predicted. It was 59% identical with arginase I. The arginase II precursor synthesized in vitro was imported into isolated mitochondria and proteolytically processed. mRNA for human arginase II was present in the kidney and other tissues, but was not detected in the liver. Arginase II mRNA was coinduced with nitric oxide synthase mRNA in murine macrophage-like RAW 264.7 cells by lipopolysaccharide. This induction was enhanced by dexamethasone and dibutyryl cAMP, and was prevented by interferon-γ. Possible roles of arginase II in NO synthesis are discussed.

Key words: Arginase II; Gene induction; Mitochondria; Nitric oxide synthase; Macrophage; Lipopolysaccharide

1. Introduction

Arginase catalyzes hydrolysis of arginine to urea and ornithine. Liver-type arginase (arginase I) is expressed almost exclusively in the cytosol of the liver of ureotelic animals [1,2] and catalyzes the last step of urea synthesis. The enzyme is markedly induced in the perinatal period in coordination with other urea cycle enzymes [3,4]. Arginase I and the other urea cycle enzymes are regulated by dietary protein [5,6] and hormones such as glucagon and glucocorticoids [7–9]. cDNA clones were isolated from the rat [10,11] and human [12,13] liver, and the structures of the rat [14] and human [15] genes were determined. Promoter [16–18] and enhancer [17] regions were analyzed (reviewed in [19]).

An isoform of arginase (arginase II) that differs from arginase I in catalytic, molecular and immunological properties is present in nonhepatic tissues such as kidney, small intestine and lactating mammary gland [20–23]. Arginase II appears to be located in the mitochondria [23]. Arginase II activity in the kidney is markedly elevated in patients with arginase I deficiency [24]. Recently, arginase activity as well as nitric oxide synthase (NOS) activity was found to be induced in murine macrophage-like RAW 264.7 cells by bacterial lipopolysaccharide (LPS) and the induced arginase was shown to be arginase II by immunochemical analysis [25]. These results suggest that arginase II plays a role in down-regulation of NO synthesis by decreasing the arginine availability for the NOS reaction. Three different forms of nonhepatic arginase cDNAs from *Xenopus laevis* were cloned [26].

We report here the cloning of a full-length cDNA for human arginase II and a partial cDNA for the rat enzyme. The human enzyme contains 354 amino acid residues including the putative NH_2-terminal mitochondria-targeting presequence. Mitochondrial import of the arginase II precursor synthesized in vitro with concomitant proteolytic processing was shown. Induction of mRNAs for arginase II and inducible form of NOS (iNOS or NOS2) by LPS and other compounds in RAW 264.7 cells was also reported.

2. Materials and methods

2.1. Cloning of human and rat arginase II cDNAs

A partial cDNA for a candidate human arginase II corresponding to nucleotides 18–541 of the human arginase isolog reported in the TIGR database, was isolated by reverse transcription-polymerase chain reaction (RT-PCR) using HepG2 mRNA. A full-length cDNA was isolated by screening a HepG2 cDNA-λZAP library (Stratagene, La Jolla, CA, USA) using the PCR product as a probe. The obtained cDNA was linker attached and inserted into *Bam*HI site of the plasmid pGEM-3Zf(+) (Promega, Madison, WI, USA), yielding pGEM-hAII. To obtain a mammalian expression plasmid, the plasmid pCAGGS [27] was cut with *Eco*RI, and arginase II cDNA was inserted after linker attachment, yielding pCAGGS-hAII. A partial rat arginase II cDNA clone was isolated by PCR using mRNA from the small intestine of a male Wistar rat. PCR was carried out using the human arginase II primers corresponding to nucleotides 294–541. The obtained product was inserted into *Hin*cII site of pGEM-3Zf(+), yielding pGEM-rAII-1.

2.2. In vitro translation and mitochondrial import

mRNA for human arginase II was synthesized by in vitro transcription of plasmid pGEM-hAII using SP6 RNA polymerase. The procedures of in vitro translation and the import into isolated rat liver mitochondria were described previously [28].

2.3. Cell culture and DNA transfection

Monkey kidney COS-7 cells and mouse macrophage-like RAW 264.7 cells were grown in 10-cm dishes in Dulbecco's modified Eagle's medium and Eagle's minimal essential medium, respectively, each supplemented with 10% fetal calf serum. Transfection to COS-7 cells was carried out using TransIT-LT1 Polyamine (PanVera Corp., Madison, WI, USA) according to the protocol supplied by the manufacturer with 15 μg of the expression plasmid. After transfection, cells were cultured for 36 h prior to harvesting.

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Abbreviations: LPS, lipopolysaccharide; NO, nitric oxide; NOS, nitric oxide synthase; iNOS or NOS2, inducible isoform of NOS; OTC, ornithine transcarbamylase; PCR, polymerase chain reaction

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2.4. RNA blot analysis

RNA extraction, agarose gel electrophoresis and membrane transfer were performed as described previously [29]. Hybridization was performed using as probes random-primed ^{32}P-labeled human arginase II cDNA or human β-actin cDNA, or digoxigenin-labeled antisense RNAs for rat arginase II or rat iNOS. The antisense RNAs were synthesized using as templates pGEM-rAII-1 or pcDNAII-riNOS [29] cut by an appropriate restriction enzyme. The radioactive bands were visualized by autoradiography on X-ray films. Chemiluminescence signals derived from hybridized probes were detected on X-ray films using a DIG luminescence detection kit (Boehringer Mannheim, Germany).

2.5. Measurement of arginase activity

Cultured cells and rat liver were homogenized in 20 mM HEPES-KOH (pH 7.5) containing 1% Triton X-100, 20% glycerol and 1 mM dithiothreitol. After centrifugation, the supernatants were used for measurement of arginase activity [5].

3. Results and discussion

3.1. Cloning of human arginase II cDNA

A partial cDNA sequence (THC124562-1) that is a candidate for arginase II was reported in the TIGR human cDNA database. This cDNA of 664 bp contained the protein coding sequence corresponding the NH_2-terminal two-thirds of the suggested entire sequence of arginase II. A full length cDNA was isolated by combination of PCR cloning and screening of a HepG2 cDNA library. The cDNA contained the 5′-untranslated sequence of 20 bp, protein coding sequence of 1062 bp (354 amino acid residues), 3′-untranslated sequence of 272 bp and a poly(A) tract (the sequence is deposited in the DDBJ/EMBL/GenBank nucleotide sequence databases with the accession number D86724).

Fig. 1 shows a sequence comparison of the putative human arginase II with human arginase I and *X. laevis* nonhepatic arginase. The predicted molecular weight of the putative arginase II is 38 577. This polypeptide was 59% identical with arginase I and had additional NH_2-terminal sequence of about 18 residues. This extrapeptide portion contained three arginine residues and no acidic residue, and appeared to be the presequence for mitochondrial targeting and import (reviewed in [30]). The putative human arginase II precursor was 73% identical with nonhepatic form (probably also the precursor form, xArg1 in [26]) of *X. laevis* arginase.

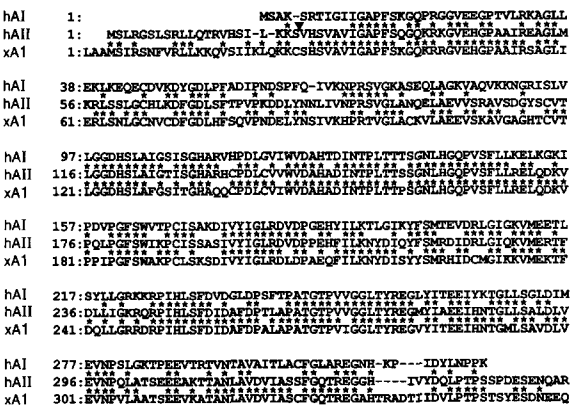

Fig. 1. Predicted amino acid sequence of putative human arginase II precursor (hAII) and comparison with those of human arginase I (hAI) and of *X. laevis* nonhepatic arginase (xAl, xArgl in [26]). Gaps were introduced to increase the homology and identical amino acids are shown by asterisks. The triangle indicates the putative cleavage site.

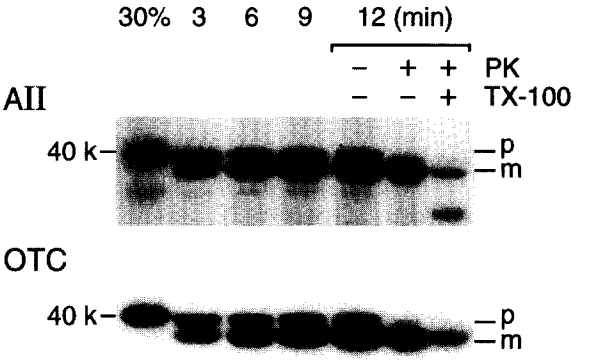

Fig. 2. In vitro import of human arginase II precursor (AII) and human OTC precursor (OTC) into isolated mitochondria. The ^{35}S-labeled precursor proteins synthesized in rabbit reticulocyte lysate were incubated with rat liver mitochondria (100 μg of protein) at 25°C for the indicated times. The mitochondria were reisolated and subjected to SDS-PAGE and fluorography. Where indicated, the isolated mitochondria were treated with 20 μg/ml of proteinase K (PK) with or without 0.1% Triton X-100 (TX-100) for 15 min on ice prior to SDS-PAGE. 30%, 30% of input precursors. p, precursor form; m, mature form. 40 k, position of the OTC precursor.

3.2. Expression of arginase II in COS-7 cells

cDNA for the putative arginase II precursor was subcloned into a potent mammalian expression vector pCAGGS and was transfected into COS-7 cells. In RNA blot analysis, expression of a hybridized RNA was observed in the transfected cells. Arginase activity was not detectable in control cells, whereas a high enzyme activity (2.1 μmol/min/mg protein) was detected in the transfected cells. This value was comparable to that of arginase I in rat liver that was measured simultaneously.

3.3. Import of the arginase II precursor into isolated mitochondria

The putative arginase II precursor and as a positive control, ornithine transcarbamylase (OTC) precursor [28] were synthesized in rabbit reticulocyte lysate and subjected to mitochondrial import assay. The arginase II precursor synthesized in vitro migrated in SDS-PAGE as a polypeptide of about 40 kDa (Fig. 2). This value was close to that predicted from the amino acid sequence. When the putative precursor was incubated with isolated rat liver mitochondria, it was imported into the organelle with concomitant processing to the mature form of about 38 kDa in a time-dependent manner. Import across the mitochondrial membranes was verified by the proteinase K digestion experiments. About 55% of the added precursor was imported into the mitochondria. The import and processing pattern resembled that for the OTC precursor. These results show that arginase II is initially synthesized as a larger precursor of about 40 kDa with the NH_2-terminal presequence, imported into the mitochondria and processed to the mature form of about 38 kDa. We speculate that cleavage occurs between Ser-22 and Val-23 (Fig. 1) because a basic residue dominates at position −2 or positions −2 and −3 among many precursor proteins [30].

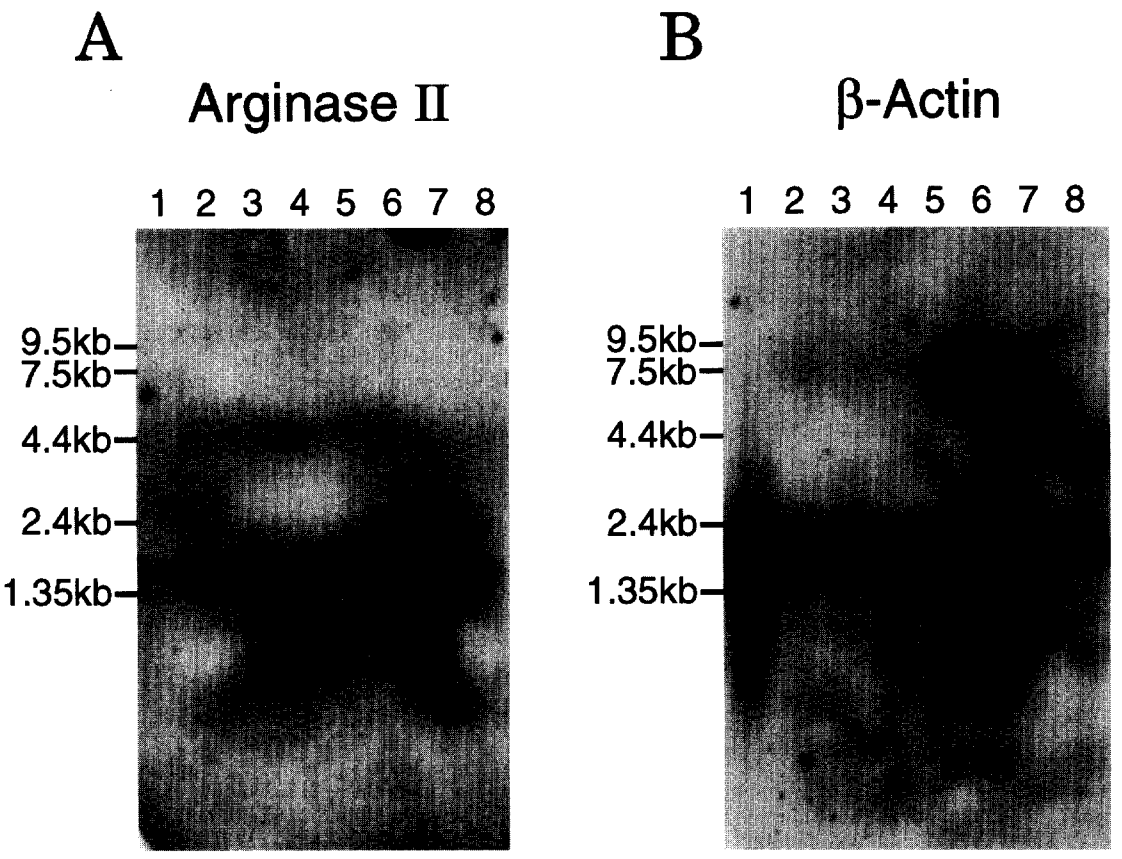

Fig. 3. RNA blot analysis for arginase II in human tissues. The filter with human tissue poly $(A)^+$ RNAs (Clontech, Palo Alto, CA, USA) was hybridized using as probes ^{32}P-labeled DNA for human arginase II (A) or human β-actin (B). Lane 1, heart; lane 2, brain; lane 3, placenta; lane 4, lung; lane 5, liver; lane 6, skeletal muscle; lane 7, kidney; lane 8, pancreas. Molecular markers (Clontech) are shown on the left.

3.4. Expression of arginase II mRNA in human tissues

RNAs from human tissues were subjected to RNA blot analysis. Arginase II mRNA of about 1.8 kb was detected in various tissues (Fig. 3). The mRNA was expressed most strongly in the kidney, much less strongly in the brain, skeletal muscle, placenta and lung in this order, but apparently not in the liver, heart and pancreas. The high mRNA in the kidney is in accord with a high arginase II activity in rat kidney [22].

3.5. Coinduction of mRNAs for arginase II and iNOS in LPS-activated RAW 264.7 cells and effects of dexamethasone, cAMP and interferon-γ

Wang et al. [25] reported that activities of arginase and NOS are coinduced RAW 264.7 cells by LPS. The induced arginase was shown to be arginase II by immunoprecipitation experiments. We examined whether the increased arginase activity is due to induction of its mRNA. A partial rat cDNA (the sequence is deposited in the DDBJ/EMBL/GenBank nucleotide sequence databases with the accession number D86928) obtained by PCR was used as a probe. When RAW 264.7 cells were treated with 100 μg/ml LPS for 24 h, iNOS mRNA was markedly induced (Fig. 4). This induction of iNOS mRNA by LPS appeared to be somewhat enhanced by dexamethasone and interferon-γ, but was little affected by dibutyryl cAMP. Arginase II mRNA was also induced by LPS. This induction was enhanced by dexamethasone and more strongly by dibutyryl cAMP, but was completely prevented by interferon-γ. The effects of LPS and interferon-γ on the mRNAs agree with the report [25] on effects of these compounds on iNOS and arginase II activities. When 10 μg/ml LPS was used, induction of iNOS mRNA was similar to that with 100 μg/ml LPS, whereas induction of arginase II mRNA was much less evident (data not shown). Induction of arginase I mRNA was not observed under these conditions (data not shown).

It was proposed that arginase II plays a role in down-regulation of NO synthesis by decreasing the availability of arginine for the NOS reaction [25]. Both iNOS and arginase II were induced by LPS, but the effects of interferon-γ and cAMP differed between the two enzymes. Interferon-γ may stimulate NO synthesis by suppressing arginase II induction as well as by inducing iNOS. On the other hand, dexamethasone and cAMP may suppress NO synthesis by inducing arginase II. This may partly explain the therapeutic effects of dexamethasone and catecholamines in the shock treatment. Common and discrete steps in signal transduction and gene cascades appear to be involved in regulation of these two genes. The cDNAs for arginase II will provide an important tool for the studies on regulation of the enzyme gene and its role in NO synthesis.

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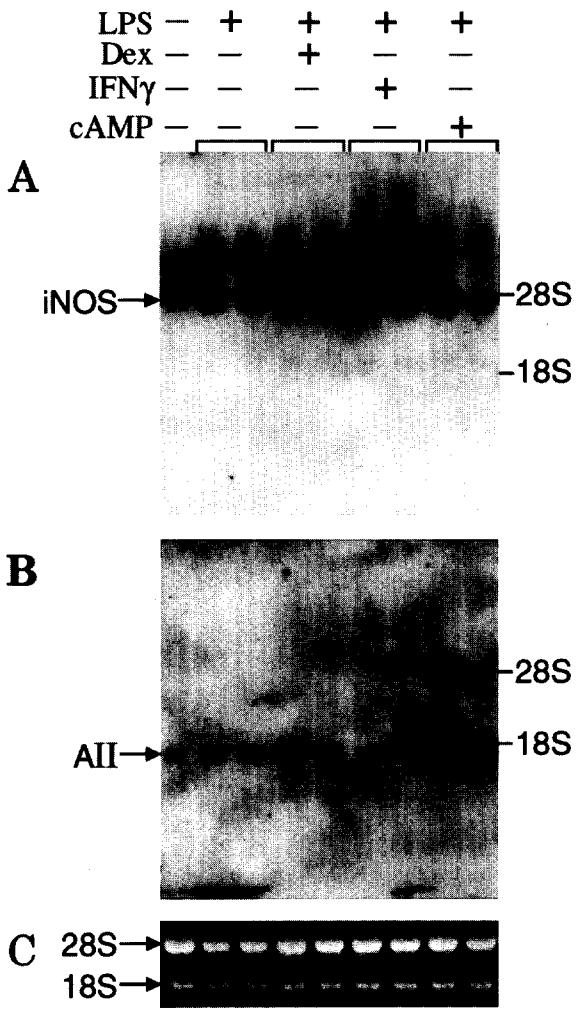

Fig. 4. Effects of LPS, dexamethasone, interferon-γ and cAMP on iNOS (A) and arginase II (AII) (B) mRNAs in RAW 264.7 cells. The cells were treated with various combination of *Escherichia coli* LPS (100 μg/ml) (serotype 0127:68, Sigma), dexamethasone (Dex, 1 μM), interferon-γ (IFNγ, 100 units/ml), and dibutyryl cAMP (cAMP, 1 mM) for 24 h. Total RNAs (5 μg) were subjected to blot analysis. The positions of 28 S and 18 S rRNAs are shown on the left. C shows ethidium bromide staining of 28S and 18S rRNAs.

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