Cloning and characterisation of the cDNA and gene for sheep liver arginase

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ABSTRACT

Arginase (arginine amidinohydrolase, EC 3.5.3.1) is a ubiquitous enzyme, notably found in the liver of ureotelic animals. It plays a critical role in the hepatic metabolism of most higher organisms as a cardinal component of the urea cycle (Jenkinson *et al.*, 1996). Arginase has also been identified in numerous organisms and tissues where there is no functioning urea cycle. In animals, many extrahepatic tissues have been shown to contain a second form of arginase, closely related to the hepatic enzyme but encoded by a distinct gene or genes and involved in a host of physiological roles. Recent interest in arginase has been stimulated by it's demonstrated involvement with the metabolism of nitric oxide. Subcloning the sheep hepatic cDNA sequence would allow a ruminant arginase to be compared with other known arginases. Probing a sheep genomic library for the arginase gene could ultimately lead to the characterisation of regulatory elements of the gene.

Partial purification of sheep liver arginase was carried out to develop a DNA probe to screen a sheep liver cDNA library for the cDNA sequence but the protein was N-terminally blocked. An attempt was made to electroelute arginase from an SDS-PAGE gel with a view to cleaving the purified protein and sequencing some of the resulting peptides. But arginase could not be purified sufficiently for successful electoelution.

Total RNA was isolated from both sheep and rat liver. A product of the expected size was produced by RT-PCR on the rat RNA template, but could not be subcloned into a vector. PCR performed on a sheep cDNA library generated a PCR product which was subcloned and sequenced. The sequence had no similarity with known arginase sequences, and showed that the reverse primer sequence was present at both ends of the PCR product.

A region of the human arginase cDNA sequence was PCR amplified from the expression plasmid pTAA12. The PCR product was radiolabelled, and used as a probe to screen a sheep liver cDNA library. No positive clones were identified. Northern blot analysis of RNA isolated from sheep liver was carried out. The blot was probed with a fragment of the human arginase cDNA sequence. Nonspecific binding was observed.

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LIST OF ABBREVIATIONS

A_{260}	Absorbance at 260 nm
A_{280}	Absorbance at 280 nm
AMP	Ampicillin
APS	Ammonium persulphate
AR	Analtical reagent
ATP	Adenosine triphosphate
bp	Base pair(s)
BRL	Bethesda research laboratories
BSA	Bovine serum albumin
CAPS	3-(Cyclohexylamino)-1-propanesulfonic acid
C/EBP	CCAAT/enhancer binding protein
cAMP	Cyclic adenosine monophosphate
cDNA	Complementary DNA
CIP	Calf intestinal phosphatase
CM	Carboxymethyl
CSPD®	Disodium 3-(4-methoxyspiro{1,2-dioxetane-3,2'-(-5'-chloro) tricyclo [3.3.1.1^{3,7}] decan}-4-yl) phenyl phosphate
dATP	Deoxyadenosine triphosphate
dCTP	Deoxypyrocytidine triphosphate
dNTP	Deoxynucleotide triphosphate
DEPC	Diethylpyrocarbonate
Dept	Department
DIG	Digoxigenin
DNA	Deoxyribonucleic acid
DNase	Deoxyribonuclease
DTT	Dithiothreitol
EDTA	Ethylene diamine tetra-acetate
EEO	Electroendosmosis
h	Hours(s)
HIS	Histidine
HNF-4	Hepatocyte nuclear factor-4
IEF	Isoelectric focussing
IPTG	Isopropyl β-D-thiogalactopyranoside
kb	Kilobase
kDa	Kilodalton
LB	Luria-bertani
min	Minutes(s)
Mn	Manganese
MOPS	3-(N-Morpholino) propanesulfonic acid
mRNA	Messenger RNA
M_r	Relative molecular weight
NA	Not applicable
NADH	Nicotinamide adenine dinucleotide
PCR	Polymerase chain reaction

PEG	Polyethylene glycol
Pfu	Plaque forming units
pKS	pBluescript® KS II
PMSF	Phenylmethylsulfonyl fluoride
PNK	Phosphonucleotide kinase
Q	Ubiquinone
RNA	Ribonucleic acid
RNase	Ribonuclease
rpm	Revs per minute
rRNA	Ribosomal RNA
RT	Reverse transcriptase
RT-PCR	Reverse transcriptase-polymerase chain reaction
s	Second(s)
S	Subunit
SD	Standard deviation
SDS	Sodium dodecyl suphate
SDS-PAGE	Sodium dodecyl suphate-polyacrylamide gel electrophoresis
Taq	*Thermus aquaticus*
TAE	Tris acetate EDTA
TEMED	N,N,N′,N′-tetramethylethylenediamine
Tris	Tris-(hydroxymethyl) aminomethane
TsAP	Temperature sensitive alkaline phosphotase
T4 PNK	T4 phosphonucleotide kinase
U	Unit
UV	Ultraviolet
(v/v)	Volume: volume ratio
(w/v)	Weight: volume ratio
X-gal	5-bromo-4-chlor-3-indoyl β-D-galactopyranoside