

Sequencing of cDNA Encoding Serum Albumin and Its Extrahepatic Synthesis in the Mongolian Gerbil, *Meriones unguiculatus*

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Abstract

We have sequenced serum albumin cDNA from liver of the Mongolian gerbil, *Meriones unguiculatus*. The deduced amino acid sequence showed 82.6% and 73.6% identity with the corresponding proteins from rats and humans, respectively. Identical cDNA was detected in pancreas by reverse transcription followed by polymerase chain reaction (RT-PCR). Further amplification of cDNA by nested PCR revealed the presence of the same cDNA in the brain and kidney. These results indicate that serum albumin is expressed in some extrahepatic tissues. In rats, an albumin-related 70-kDa protein (P70) has been proposed to be associated with cobalt-induced epilepsy (Onozuka et al. (1995) *Neurochem. Res.*, **20**, 901–905). We intensively searched for a P70-like protein in the brain of an epilepsy-prone gerbil strain, MGS/Idr, by the RT-PCR and nested PCR using several pairs of primers based on the albumin cDNA sequence. However, we found only mRNA for albumin itself.

Key words: cDNA sequence of serum albumin; epilepsy-prone strain; extrahepatic expression; Mongolian gerbil

In the experimental epilepsy induced by cobalt, animals such as mice and rats develop epileptic seizures several days after application of cobalt powder to a motor area of the cerebral cortex.^{1,2} Onozuka et al. showed that a 70-kDa protein (P70) increased activity in the cobalt-induced epileptogenic cortex of the rat prior to onset of the epileptogenic electrocorticogram pattern.³ Purification and partial characterization of P70 revealed that its amino acid composition was very similar to that of rat serum albumin and that its N-terminal heptadecapeptide was identical with that of albumin. In another experimental epilepsy, bicuculline-induced short-lasting epileptic seizures led to an increase of endogenous serum albumin content in the cerebral cortex.⁴ Furthermore, it has become increasingly clear that serum albumin is capable of affecting several types of ion channels of various cells, such as cultured neurons, *Xenopus* oocytes, and cardiac myocytes.^{5–7} P70, however, differed from serum albumin in several points: (i) unlike albumin, P70 reacted positively with periodic acid-Schiff reagent, suggesting that P70 is a glycoprotein; (ii) two-dimensional

electrophoresis distinguished P70 from serum albumin; and (iii) P70, but not albumin, elicited seizure discharges on the electrocorticogram and behavioral seizures when applied to the cerebral cortex of normal rats. These results, together with the finding that the amino acid composition of P70 was slightly different from that of albumin, suggest that P70 is a new protein whose chemical characteristics are very similar to, but not identical with, those of serum albumin, and that P70 may play an important role in epileptogenesis.³

The Mongolian gerbil (*Meriones unguiculatus*) exhibits epileptic motor seizures in response to a variety of stimuli and serves as an experimental animal to understand the mechanisms underlying epilepsy. By selective breeding with sister-brother mating, Seto-Ohshima et al. established a seizure-prone strain, MGS/Idr, in which virtually all individuals exhibit behavioral seizures during development.⁸ We therefore hypothesized that P70-like protein (P70-LP) is constitutively synthesized in the brain of MGS/Idr strain, while in the seizure-resistant strain it is only induced under some circumstances such as cobalt application. To substantiate this hypothesis, we used the following strategy: (i) isolation and sequencing of cDNA encoding serum albumin from liver since the sequence of gerbil albumin is not known, (ii) preparation of appropriate primers for RT-PCR amplification

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1  GACCTTTCTCTCAACCCCACTAGCCACTGGCACAATGAAGTGGTAACCTTCTCTCTCTCTTTGTCTCTGGCTCTGCTTTCTCCAGSGGTGTGTTTCGCCGAGATGCAGCACACA
1  M K W V T F L L L F V S G S A F S R G V F R R D A A H K

121 AGAGTGAGATCGCTCATCGTATAAAGATTGGGAGAAAAATATTTCAAGGCGCTTGCTGTCACACCTTTTCCAGTATCTCCAGAAATGCTCTATGAAGAATATGTAATTAGTGA
30  S E I A H R Y K D L G E K Y F K G L V L Y T F S Q Y L Q K C S Y E E H V K L V R

241 GAGAAGTAAGTACTTTGCAAGCAACTGTGCTAAAGATGAGTCTGCTGAAAACTGTGACAAATCACTTCACACTCTTTTGGAGACAAGTTATGCTCTCTACCAAATTTTGGTGAATAAT
70  E V T D F A S N C A K D E S A E N C D K S L H T L F G D K L C S L P N F G E K Y

361 ATGCTGAAATGGCGACTGCTGTGCAAAACAAGAGCCGGAAGAAATGAATGTTTCTGCAACACAAAGATGACAACCCCACTGCCCCATTCAAGAGGGGGAGCGCTGATGCCATGT
110  A E M A D C C A K Q E P E R N E C F L Q H K D D N P Q L P P F K R A E P D A M C

481 GTACTGCTTCCAGGAAAATGCTGAAGCATTTATGGGACATTACTGCAATGAAGTGCAGAGACATCCTTATTCTACGGCCCGAGCTCTTTACTTGGCTGATAAATACACTGCAG
150  T A F Q E N A E A F M G H Y L H E V A R R H P Y F Y G P E L L Y L A D K Y T A V

601 TCCTGACTGAGTGTGTCAGCTGATGACAAAGAGCGCTGCCTGACACCAAGCTTGATGCGCTGAAGGAGAAAGCATTGGTTTCAGCTGTCCGACAAAGACTGAAGTGTCAAGTATGA
190  L T E C C A A D D K G A C L T P K L D A L K E K A L V S A V R Q R L K C S S M K

721 AGAAATTCGGTGAGAGAGCTTTCAAGGCGAGTGCATGCAATGAGCCAGACATCCCCAATGCTGACTTGCAGAAATACCAAACTGCCAACAAGACCTTACCAAAGTCAACCGAG
230  K F G E R A F K A W A V A R M S Q T F P N A D F A E I T K L A T D L T K Y T Q E

841 AGTGCTGCCATGGTGACCTGCTTGAATGTGAGATGACAGGGCGAGCTTGCCAAAGTACATGTGTGAAAACAGGGCTCTATCTCCAGCAAGCTGCAGCGCTTGTGTGATAAAGAAATGT
270  C C H G D L L E C A D D R A E L A K Y M C E N Q A S I S S K L Q A C C D K E M L

961 TGCAGAAATCCAGTGCCTTGTGAGGTGAACATGACGACATGCCTGCGATCTGCTGATTAACCTGCTGATTTGTGAGGATAAGGATGTGCAAGAATATGCTGAGGCCAAAG
310  Q K S Q C L A E V E H D D M P A D L P A L T A D F V E D K D V C K N Y A E A K D

1081 ATGTCTTCTGGGACGCTTTTGTATGAATTTCAAGAAGGCAACCTGAGTACTCGTTTCTGTTGCTGAGACTTGAAGAAATACGAAGCCACACTGGAAAAATGCTGTGCTGAAG
350  V F L G T F L Y E Y S R R H P E Y S V S L L L R L A K K Y E A T L E K C C A E A

1201 CCGACCCATCATGCTGCTATGCGCATGTGTTGATGAATTTAAGCCTTGTGAGGAGCGCTCAGAACTTAGTCAAAAGCAACTGTGAACCTTTATGAGAAGCTTGGAGAGTATGGGTTC
390  D P H A C Y G H V F D E F K P L V E E P Q N L V K S N C E L Y E K L G E Y G F Q

1321 AAAATGCCGTCTAGTTCGCTACACCAAGAAAGCACCTCAGGTGTCGACTCCGACTCTGCTGAGGCTGCCAAGGCTAGGAAGAGTGGGACCCATTGCTGTGCACTTCCGGAAAAAGA
430  N A V L V R Y T K K A P Q V S T P T L V E A A R S L G R Y G T H C C A L P E K K

1441 AGAGACTGCCCTGTGTTGAGGACTATCTGTCTGCGATCCTGAACCGTGTGCTTGTCTCCAGSAGAAGACCCAGTGAGTGAACAGGTACCAAGTGTCTTCTGGATCCCTGCTGGAGC
470  R L P C V E D Y L S A I L N R V C L L H E K T P V S E Q V T K C C S G S L V E R

1561 GCGGCCATGCTTCTGCTCTGCGGTTGATGAACATACGTCCTCCCAAGGAGTTTAAAGCTGAGACCTTCACTTCCATGCCAATATCTGCACGCTTCCGGAGAAGGAGAAGCAGATGG
510  R P C F S A L P V D E T Y V P K E F K A E T F T F H A N I C T L P E K E K Q M E

1681 AGAAGCAACGGCACTTGTGAGCTGCTGAAACACAAGCCCGAGCTACAGAAGAGCAGCTGAAGAAAGTGTGCGTGACTTTGCGAGAATCTGGAGAAGTGTGCAAGCAGGAAGACA
550  K Q T A L A E L V K H K P Q A T E E Q L K K V M G D F A E F L E K C C K Q E D K

1801 AGGAAGCATGCTTCTCCACAGAGGTCCTCAAACTTGTGCTGAAAGCCAAAGGCTTGGCCTAAACACATCACAACCAAGCATCTCAGTCTACCTTGAAAAACAAGAGACATGAAG
590  E A C F S T E G P K L V A E S Q K A L A *

1921 ACTCAGGACTCATCTTTCTGTTGGTGAATAATACACCCCAAGGAACACAAATTTCTTCAACATTTGACTTCTTCTCTGTACTTCAATTAATAAGAATGAAAAGATCT

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Figure 1. Nucleotide sequence of cDNA and its deduced amino acid sequence of MGS/Idr gerbil serum albumin. Total RNA was extracted from the Mongolian gerbil liver by ISOGEN (Nippongene, Tokyo) as described previously.⁹ The complete cDNA sequence was determined by the three-step strategy, i.e., (i) RT-PCR, (ii) 3'-rapid amplification of cDNA ends (RACE) and (iii) 5'-RACE essentially as described previously⁹ except for the following. (i) RT-PCR: primers were designed based on the regions showing high homology among the known albumins; P1 (sense), 5'-ATGAAGTGGGTAAACCTTT-3' and P2 (antisense), 5'-CAGGGTAGCCTGAGATG-3'. (ii) 3'-RACE: the liver mRNA was amplified by PCR using P3 (sense, specific for gerbil albumin), 5'-TGTTGAGGACTATCTGTCTG-3' and oligo(dT)₂₀-M4 (antisense), 5'-GTTTTCCAGTACAGACTT...TT-3' (Takara); the resulting first round RACE product was further amplified by P4 (sense), 5'-AATTCCTGGAGAAGTGCTGC-3', and M4 (antisense). (iii) 5'-RACE: the liver mRNA was reverse-transcribed using P5 (antisense, specific for gerbil albumin, 5'-CCTGGAAGGCAGTACACATG-3') and then tailed by oligo(dA)_n; the product was subsequently amplified by PCR using oligo(dT)₂₀-M4 and P6 (antisense), 5'-GCATTCTTGGAGATACTGGG-3'. The nucleotide sequencing was carried out by the dideoxynucleotide method of Sanger et al.¹⁰ The upper and lower lines represent nucleotide and predicted amino acid sequences, respectively. Numbering starts with the start codon for both sequences. The underline indicates the polyadenylation signal.

of putative P70-LP cDNA from the brain of epilepsy-prone strain, and (iii) sequence determination followed by preparation of recombinant P70-LP for testing its epileptogenic activity.

As shown in Fig. 1, the total RNA extracted from a gerbil liver was found to contain cDNA encoding serum albumin. The sequence spans a stretch of 2035 nucleotides with one open reading frame (ORF) coding for 609 amino

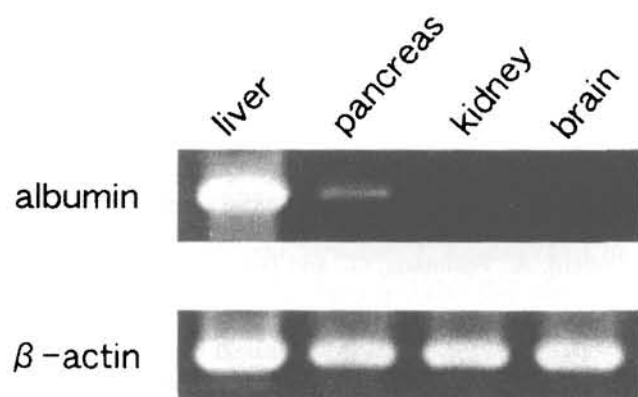


Figure 2. RT-PCR amplification of serum albumin mRNA. Total RNA was extracted from liver, pancreas, kidney, and brain, and a portion (1 μ g) was subjected to RT-PCR amplification (30 cycles) as described in the legend to Fig. 1. As a control, RT-PCR amplification of β -actin mRNA was carried out by the same method.

acid residues and is 82.6% and 73.6% identical with those of rat and human albumins, respectively. By comparison with several mammalian homologues, gerbil albumin was concluded to consist of an 18-residue signal peptide, a 6-residue propeptide and a 585-residue mature protein. The comparison also revealed that gerbil albumin contains an extra alanine between the propeptide and mature protein. Based on this nucleotide sequence, several pairs of primers for PCR and nested PCR, including the one corresponding to the N-terminal sequence, were synthesized and used for RT-PCR amplification to obtain the putative P70-LP mRNA in MGS/Idr brain. All attempts failed, but the cDNA identical with that of serum albumin was repeatedly amplified. Virtually no difference was found between the epilepsy-prone and -resistant strains. These results suggest that P70-LP is not constitutively synthesized in the brain of epilepsy-prone gerbils.

The presence of albumin transcript in the adult gerbil brain led us to examine whether or not albumin transcript is detectable in other organs, since albumin is thought to be synthesized predominantly in the liver in adult mammals. As shown in Fig. 2, the band identical to albumin cDNA in the liver was detected in the pancreas, whereas it was barely detectable in the brain or kidney. However, when a portion (5 μ l) of the PCR amplification mixture (100 μ l) was further subjected to the nested PCR, the brain and kidney yielded the band whose sequence was identical with albumin cDNA in the liver (data not shown). In some tissues, the expression of albumin genes is known to be developmentally regulated. Dot hybridization analysis on newborn and 25-day-old rats demonstrated the presence of albumin transcript in the liver, pancreas, lung, and kidney but not in the brain.¹¹ The *in situ* hybridization technique which

can detect small amount of transcripts concentrated in a limited area, showed albumin mRNA in the brain of the fetal, but not 3-month-old, rats.¹² In this study, we detected albumin transcripts in the brain of 4-month-old gerbil. This apparent difference between rat and gerbil brains seems to be due to the sensitivity of the methods used, although the species difference cannot be ruled out.

In man, mouse and rat, the albumin superfamily consists of four genes, i.e., serum albumin, α -fetoprotein, α -albumin, and vitamin D-binding protein.¹³ The latter three proteins all differ to a great extent from albumin in the N-terminal heptapeptide sequence, indicating that none of them correspond to P70. These four genes are tandemly linked, and the chromosome walking experiment suggests the absence of other genes belonging to this family. It is known¹⁴ that serum albumin exists in a number of conformationally different states and that it can bind a number of small molecules and cations, especially transition metal cations. Mild oxidative stress elicited by trace metals affects the binding properties of human serum albumin *via* purely conformational changes. It is also known that circulating albumin contains a fraction, termed procoagulant albumin, which is capable of inducing or altering hemostatic properties of vascular endothelial cells.¹⁵ This procoagulant albumin is shown to be an albumin fraction complexed with phospholipid.¹⁵ These results suggest that pinpoint denaturation or localized modification of the albumin molecule as well as interaction with small ligands leads to alterations in the physicochemical properties of a small fraction of the albumin molecule, while the majority of the molecule remains conformationally unaltered. It is interesting to note that rat albumin contains a potential glycosylation site,¹⁶ Asn-X-Ser/Thr where X is any amino acid. Since P70 was reported to be a glycoprotein, it may be a glycosylated albumin synthesized *in situ* under neuropathological conditions, such as that seen with cobalt application. The gerbil albumin sequence obtained in this study does not have the potential glycosylation site and similar modification is unlikely. Thus, the present results suggest that P70 is a conformational isoform of albumin which is induced by cobalt powder, although other possibilities cannot be excluded.

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