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Sequence of the gene encoding cat (*Felis domesticus*) serum albumin *

(PCR; DNA amplification; allergen; homology)

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SUMMARY

The complete nucleotide (nt) sequence of the gene encoding cat serum albumin has been determined. The nt and deduced amino-acid sequences were compared to those of other known mammalian serum albumins (SA).

Serum albumin (SA) is the major protein component in plasma. The protein is produced in the liver. It functions as a multipurpose transport molecule and is responsible for maintaining normal osmolarity in the blood stream. SA is also found in tissues and body secretions. The proportion of this extravascular fraction constitutes about 60% of the total albumin pool. In a recent study (Spitzauer et al., 1994) it has been shown that 35% of patients allergic to dogs display IgE reactivity to dog SA. In our laboratory we found a variable pattern of allergic cross-reactions between SA of various domestic animals (data not shown). The sequences of a number of albumins are known, some of them with a particular relevance to allergies: bovine SA (BSA; Holowachuk, 1991), horse SA (ESA; Ho et al., 1993), porcine SA (PSA; Weinstock and Baldwin, 1988), rat SA (RSA; Sargent et al., 1981) and sheep SA (SSA; Brown, 1989). In order to compare the amino acid (aa) sequences of different SA and to identify cross-reactive epitopes we decided to analyse the gene coding for cat SA.

The sequence of the cloned cDNA is presented in Fig. 1. The overall nt sequence homology of the cat SA-encoding gene (SA) to human (HSA, Lawn et al., 1981), bovine, horse, porcine and sheep SA is 84–85%. The rat SA sequence has a lower homology (77%) to the cat gene. The deduced aa sequence shows a high homology to different other SA: HSA (82%), PSA (80%), BSA, ESA, SSA (78%) and RSA (75%). The cat preproalbumin is composed of 608 aa with a signal sequence of 18 aa, a propeptide sequence of 6 aa and 584 aa of mature SA. The same number of residues is found in RSA. HSA contains one more aa, SSA, BSA, ESA and PSA contain one less. All 35 Cys residues are conserved as compared to the other SA. The seven SA analysed show a total of 53% identical aa. Besides the highly conserved regions, stretches of aa which are heterogenous between albumins of different species do exist. However in these non-conserved regions some aa sequences are common to two or three albumins, e.g., aa 216–220 (²¹⁶SSAKE²²⁰) of cat SA are shared by ESA. It is conceivable that human allergic cross-reactions exist against these aa stretches common to the SA of different animal species.

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* On request, the authors will supply detailed experimental evidence for the conclusions reached in this Brief Note.

Abbreviations: aa, amino acid(s); BSA, bovine serum albumin; cDNA, DNA complementary to RNA; ESA, horse SA; HSA, human SA; dNTP, deoxyribonucleoside triphosphate(s); nt, nucleotide(s); PCR, polymerase chain reaction; PSA, porcine SA; RSA, rat SA; RT, reverse transcription(tase); SA, serum albumin(s); SA, gene encoding SA; SSA, sheep SA.

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9TCGACCTTTGGCAAGTAAAGTAACTTTATTTCCCTCCCTCCCTCTTACCTCTGCTTATTCGAGGGGCTGACTCGTGGAGAACACATCAGAGTGAAGTTGCTCATCGGTTCAATGATTGGGAGAGAACATTTTCAGAGGC 150
      N K N V T F I S L L L L F S S A Y S R G V T R R E A H Q S E I A H R F N D L G E R E H F R G 145
CTGGTACTGGTTGCTTTTCTCAATATCTCCAGCAGTGTCCATTTGAAAGATCATGTAAATTTAGTGAATGAACTAACTGAGTTGCGAAAGGATGTGTGGCTGACCCAGTCAGAGCCAACTGTGAAAGTCACTTCACGAACTCTTAGGA 300
      L V L V A F S Q Y L Q Q C F F B D H V R L V M H V T S F A K G C V A D Q S A A N C E K S L E H L L G 295
GATAAAGTGTGTACAGTTGCTCTCTCGCGACAAGTATGTTGAGATGCTGATGCTGTGAGAAAAAGAACCTGAGAGAAACGAATGCTTCTGACACAAAGATGACAAATCCCGGCTTGGTCAAGTGGTACCCAGAGGCTGAT 450
      D R L C T V A S L R D K Y G E M A D C C E K K E P E R N B C F L Q H K D D N P G F G Q L V T P B A D 245
GCCATGTGCACTGCTTCCAGAAAATGAACAGAGGTTCTGGGCAAACTACTATGAAATTCGAGAGAGACCTCTACTTTTATGCCCGAGAACTCTTTACTATGCCGAGAAATCAAGAGGTTTTTACAGAACTGCGAAGCT 600
      A M C T A F H E N B Q R F L G K Y L Y B I A R R H P Y F Y A P B L L Y Y A E B Y K G V F T E C C E A 195
CCAGATAAAGTGGCTGCTGACACCAAGGTTGATGCTTTGAGAGAAAAATGCTGCTTCCGCAAAAGAGAGACTCAAGTGTGACAGCTCCAGAAATTCGGAGAAAGAGCTTTCAAGCATGCTCACTAGTCTGCTGAGCCAG 750
      A D K A A C L T P K V D A L R B K V L A S S A X E R L K C A S L Q K P G R R A P F X A M S V A R L S Q 245
AAATTTCCCAAGGCTGAATTTGAGAGAGTTCCAAAGTTAGTACAGATCTTCCCAAAATCCACAGGAATGCTGCCATGTGACCTGCTTGAATGTGACAGATGACAGGGCGGAGCTTCCCAAGTATATATGAAAAATCAAGATTCAATC 900
      X F P K A B F A B I S K L V T D L A K I H K R C C H G D L L E C A D D R A D L A X Y I C B N Q D S I 295
TCCACTAGCTGAAGGAATGCTGTGAAGCTGTGTGGAAGATCCCATGCTGAGCGAGGTGAGAAAGAGATGAGTTGCTGCTGACCTGCCCGCTTAGCTGTGATTTTGTGAGAGTAAAGAGTTTTCAGAGCATATCAGGAG 1050
      T C L K K R C C G K V S L R E K S H C I S E V B R L D F A D L P L A V D P V B R V E C K N Y Q B 345
GCAAGAGATGTGTCTGCGACGCTTTTGTATGAATACCAAGAGGACCTGAAATGCTGTCTATGCTACTGAGTTGCCAAGGAATATGAAGCCACCTAGAGAAAGTGTGCTGCACTGATGATCTCTCTGCTGCTATGCC 1200
      A K D V F L G T P L Y E Y S R R H F E Y S V S L L R L A K A B D Y L S A V L N R L C V L H E K T P F V S A 395
CATGTGTTGATGAATTTAAAGCTCTTGTGAGAGAGCTCACAATTTAGTCAAAACAAAGCTGTGAATTTTGAAGAACTGGAGAAATGAGCTTCCAAATGCGCTTGTAGTGTGATACCAAGAAAGTACCTCAAGTGTCACTCCA 1350
      H V F D E F K P L V E E P H N L V X T H C B L F R K L G E Y G F Q N A L L V R Y T T K K V P Q V S T P 445
ACTCTGTGAGGCTCAAGAGGCTAGGAAAAATGGCGAGCAAGTGTGTACGCACTCTGACAGAGAGAGCTGCTGCTGCTGAGAGCTATCTGTGCTGCTGCTGAGAGCTGTGTGTGTGATGATGAGAGAGAGCTGTGAGCGAG 1500
      T L V E V S R S L G K V G S K C T H B E A B R L S C A B D Y L S A V L N R L C V L H E K T P F V S A 495
AGAGTTACCAAAATGCTGACAGAGTCTTGGTGAACAGAGACCTGCTTTTCTGCTGCTGCAAGTCAATGAACATATGTTCCCAAGAAATTTAGTGTGAAACATTACCTTCCATGCAATTTATGCACTTCTGAGGCTGAGAAA 1650
      R V T K C C T B S L V N R R P C F S A L Q V D B T Y V P K E F S A E T F T P B A D L C T L P B A E K 545
CAATCAAGAAACAACTGCACTGTTGAGCTGTTGAAACACAAAGCCCAAGCAACAGAGGAAACAACTGAAACTGTCAATGGGAGTTTGGAGCTTTGTAGACAAAGTGTGCGAGCTGAAGACAGAGGAGGCTGCTTGTGAGGAG 1800
      Q I K K Q S A L V R L L K H K P K A T E R Q L K T V M G D P G S F V D E C C A A R D K E A C P A B R 595
GTCCTCAAGCTGTGCTGCTGAGCTCAAGCTGCTTAGCTTAAACACAAATCACAACATCTCAAGCTACCTGAGAGTGCAGC
      G P K L V A A A Q A A L A *

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Fig. 1. The nt sequence of the cDNA encoding cat SA and the deduced aa sequence of the coding region. By comparison with BSA, ESA, HSA, PSA and RSA, nt 16–69 are identified as coding for the signal peptide, nt 70–87 coding for the propeptide and nt 88–1839 coding for the mature SA. The nt sequences corresponding to the PCR primers (albuFor and albuRev, see below) are indicated with underlined letters. The GenBank/EMBL accession No. is X84842. **Methods:** Total cellular RNA was extracted from cat liver by acid guanidinium thiocyanate-phenol-chloroform extraction (Chomczynski et al., 1987). Reverse transcription was performed using 5 µg of total cellular RNA/500 ng of reverse primer albuRev/0.4 mM of each dNTP/40 units (u) RNasin (Promega, Madison, WI, USA)/200 u M-MLV RT (BRL) and RT buffer (BRL) in a total volume of 50 µl. Prior to RT, RNA was suspended in 5 µl of water and denatured for 5 min at 65°C. RNA was reverse transcribed for 2 h at 37°C. The reaction was terminated by heating at 65°C for 5 min. PCR was performed with 5 µl of the cDNA in a 100 µl reaction volume with 200 ng primer albuFor/200 ng albuRev/200 µM of each dNTP/1.5 mM MgCl₂/50 mM KCl/10 mM Tris-HCl (pH 9 at 25°C)/0.1% Triton X-100/2.5 u *Taq* DNA polymerase (Promega). Samples were heat denatured for 5 min at 95°C and subjected to 25 cycles of 1 min at 95°C, 1 min at 62°C, 3 min at 72°C. The final extension step was 10 min at 72°C. The amplified DNA was cloned into the *Sall* site of pBluescript (Stratagene) and sequenced by the dideoxy chain-termination method. The nt sequences of two clones obtained from independent reverse transcription and PCR were determined for both DNA strands. The nt sequences of the PCR primers were derived from highly homologous regions found at the 5' and 3' ends of different SA nt sequences. A *Sall* site was incorporated at the 5' end of each primer (underlined): albuFor, 5'-GACAGAGTTCGACTTTTGGCACAAATGAAGTGGGT; albuRev, 5'-TGATGGTTCGACTCTCAGGGTAGGCTGAGATG.

REFERENCES

- Brown, W.M., Dziegielewska, K.M., Foreman, R.C. and Saunders, N.R.: Nucleotide and deduced amino acid sequence of sheep albumin. *Nucleic Acids Res.* 17 (1989) 10495.
- Chomczynski, P. and Sacchi, N.: Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* 162 (1987) 156–159.
- Ho, J.X., Holowachuk, E.W., Norton, E.J., Twigg, P.D. and Carter, D.C.: X-ray and primary structure of horse serum albumin (*Equus caballus*) at 0.27 nm resolution. *Eur. J. Biochem.* 215 (1993) 205–212.
- Holowachuk, E.W. and Stoltzenberg, J.K.: Bovine serum albumin: cDNA sequence and expression. EMBL accession Nos. M73993 and M73215, 1991.
- Lawn, R.M., Adelman, J., Bock, S.C., Franke, A.E., Houck, C.M., Najarian, R.C., Seeburg, P.H. and Wion, K.L.: The sequence of human serum albumin cDNA and its expression in *E. coli*. *Nucleic Acids Res.* 9 (1981) 6103–6114.
- Sargent, T.D., Yang, M. and Bonner, J.: Nucleotide sequence of cloned rat serum albumin messenger RNA. *Proc. Natl. Acad. Sci. USA* 78 (1981) 243–246.
- Spitzauer, S., Schweiger, C., Sperr, W.R., Pandjaitan, B., Valent, P., Mühl, S., Ebner, C., Schreiner, O., Kraft, D., Rumpold, H. and Valenta, R.: Molecular characterization of dog albumin as a cross-reactive allergen. *J. Allerg. Clin. Immunol.* 93 (1994) 614–627.
- Weinstock, J. and Baldwin, G.S.: Nucleotide sequence of porcine liver albumin. *Nucleic Acids Res.* 16 (1988) 9045.