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Comparison of PSP Amino Acid Sequences between Domestic Animals

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Summary

cDNA of PSP in pig and chick was sequenced and its amino acid sequences were determined. Among mammalians, the identity between PSP is over 80%, whereas the amino acid sequence of chick PSP significantly differs from that of mammalians PSP and is about only 70% identity by comparison with mammalians.

Key words: perchloric acid-soluble protein (PSP), sequence, comparison

Introduction

In recent papers, we reported the isolation and characterization of a perchloric acid-soluble protein (PSP) of rat liver [8], rat kidney [1], rat brain [8], chick liver [7], pig liver [4] and rat lung [5]. PSP is a homodimer consisting of two identical subunits with a molecular mass of 14kDa and its N-terminal is acetylated. The cDNA of rat liver PSP contained a 411bp, encoding a 137 amino acid protein with a molecular mass of 14149 Da. PSP is mainly expressed in differentiated tissues such as liver and kidney, but its expression in undifferentiated tissues such as monocyte [10] and tumor [1] is low. Because Morishita et al. [6] reported that rat liver PSP had mRNA-specific RNase activity, PSP may inhibit protein synthesis to suppress cell proliferation and maintain differentiation, suggesting that PSP is a regulating factor of cell differentiation and proliferation.

PSP-like proteins were also isolated from various other living sources such as humans [10], mice [9], cattle [3], goats [2], *E.coli* [12] and so on. The amino acid sequences of these PSP-like proteins are highly similar to each other. Translational inhibitor protein p14.5, the human homologue of rat liver PSP, shows 87.5% sequence identity with the latter and even YjgF protein, the *E.coli* homologue of rat liver PSP, shows about 40%. The high degree of evolutionary conservation of these proteins may reflect an involvement in basic cellular regulation.

In the present study, we describe cloning and sequencing of cDNA encoding PSP-like proteins from pig and chick, and comparison of these proteins with various other living sources or each other.

Materials and Methods

Total RNA of pig and chick was extracted from fresh livers using QuickPrep™ Total RNA

Table 1. Sequences of primers.

Use	Name	Sequence(5'-3')	Comments
5'end amplification for pig	5'-PSP	AgAgggAAggMTTAgC	Used with PLPSP3'-2
	PLPSP3'-2	CTTTTgggYAAAgCAgCAAC	Used with 5'-PSP
	PLPSP5'-2	CAAgCTCTTACAAACATggg (for pig)	Used with M13M4
	CLPSP5'-2	ggTgCCTACAgYCARC(for chick)	Used with M13M4
	M13M4	gTTTTCCCAgTCACgAC	Used with PLPSP5'-2 and CLPSP5'-2
5'-RACE for chick	Chick-RT	(phosphate)AACCTgAAAggATAC	Used for reverse transcript
	Chich5R-F1	CTgCAggCTgTgACTATAgC	Used for 1st PCR with chick5R-R1
	Chick5R-F2	TCAAATCAAACTgCCCATC	Used for 2nd PCR with chick5R-R1
	Chick5R-R1	TCTATACCTATCTgTCCTgC	Used with chick5R-F1 and chick5R-F2

Extraction Kit (Amersham Biosciences) according to the manufacturer's instructions. These cDNAs were synthesized using TaKaRa RNA PCR Kit (AMV) Ver.2.1(TaKaRa) modified according to the manufacturer's instructions. Primers for PCR were synthesized on the basis of amino acid sequences of chick [7] and pig [4], and of nucleotide sequences of rat [8] and human [10] (table 1). PCR conditions consisted of an initial denaturation at 94 ℃ for 5 min, 30 cycles of amplification consisted of denaturation at 94 ℃ for 30 sec, annealing at 50 ℃ (5'-PSP/PLPSP3'-2) or 54 ℃ (PLPSP5'-2 and CLPSP5'-2/M13M4) for 30 sec, extension at 72 ℃ for 1 min and final extension at 72 ℃ for 5 min. For 5'-RACE of chick PSP, primers were designed based on the sequence of the 3'-RACE product from chick PSP (table 1), the mRNA of chick PSP was purified using Oligotex™-dT30 <Super> mRNA purification Kit from total RNA (TaKaRa), and 5'-Full RACE Core Kit (TaKaRa) was used for 5'-RACE. The cDNA synthesis of chick PSP for 5'-RACE was performed using 5'-phosphate chick-RT primer at 42 ℃ for 1 hr. First PCR conditions (primer set : chick5R-F1/chick5R-R1) consisted of initial denaturation at 94 ℃ for 5 min, 30 cycles of amplification consisted of denaturation at 94 ℃ for 15 sec, annealing at 58 ℃ for 15 sec, extension at 72 ℃ for 30 sec and final extension at 72 ℃ for 5 min. Nested PCR conditions (primer set : chick5R-F2/chick5R-R1) consisted of the same conditions as the first PCR except for the annealing temperature of 54 ℃. PCR products within the partial PSP-like gene were cloned into pCR 2.1 vector included in the TA Cloning Kit (Invitrogen), according to the instructions of the producer. Bacterial colonies containing recombinant plasmid DNA were lysed. Plasmid DNAs were purified and concentrated using GFX™ Micro Plasmid Prep Kit (Amersham Biosciences) for the sequence analysis, which was conducted with the use of PE Applied Biosystems ABI PRISM 310 or 3100.

Results and Discussion

Amino acid sequences in PSP of pig and chick were deduced from cDNA cloning (table 2). The identity of PSP between mammalians is over 80%. While PSP of goats and cattle particularly have high similarity (97.8%) with each other, PSP of chick has about 70% identity with other species (Table 3). In addition, the chick PSP comprises 138 amino acid residues containing 2 cysteine residues, although PSP of other species comprises 136 amino acid residues containing 1 cysteine residue. The deduced proteins of pig and chick have calculated molecular weights of 14199 and 14688 Da, respectively (Table 2).

Table 2. PSP amino acid sequences of various animals.

Rat	MSSIIRKVIS	TSKAPAAIGA	YSQAVLVDRT	IYVSGQIGMD	40
Pig	＊＊A L V＊＊＊＊＊	＊V＊＊＊＊＊＊＊P	＊＊＊＊＊＊＊＊＊＊	＊＊I＊＊＊＊＊＊	40
Chick	＊A＊V V＊＊I＊＊	＊A＊＊＊＊P L＊＊	＊＊＊＊＊＊＊＊＊＊	M＊I A＊＊＊＊I E	40
Cattle	＊＊＊L V＊＊I＊＊	＊A＊＊＊＊＊＊＊P	＊＊＊＊＊＊＊＊＊＊	＊＊I＊＊＊L＊＊＊	40
Human	＊＊＊L I＊R＊＊＊	＊A＊＊＊G＊＊＊P	＊＊＊＊＊＊＊＊＊＊	＊＊I＊＊＊＊＊＊	40
Mouse	＊＊＊＊＊＊＊＊＊＊	＊T＊＊＊A＊＊＊P	＊＊＊＊＊Q＊＊＊＊	＊＊I＊＊＊V＊L＊	40
Goat	＊＊＊L V＊R I＊＊	＊A＊＊＊A＊＊＊P	＊＊＊＊＊＊＊＊＊＊	＊＊I＊＊＊L＊＊＊	40

Rat	PSSGQLVPGG	VAEEAKQALK	NLGEILKAAG	CDFTNVVKTT	80
Pig	＊A＊＊＊＊＊＊＊＊	＊V＊＊＊＊＊＊＊T	＊M＊＊＊＊＊＊＊＊	＊＊＊＊＊＊＊＊＊＊	80
Chick	＊＊N＊＊＊＊S＊＊	I K＊＊T＊＊＊F K	＊＊＊＊＊＊＊＊＊＊	＊＊Y S＊＊＊＊＊＊	80
Cattle	＊A＊＊＊＊＊＊＊＊	＊＊＊＊＊＊＊＊＊T	＊I＊＊＊＊＊＊＊＊	＊＊＊＊＊＊＊＊A＊	80
Human	＊＊＊＊＊＊＊S＊＊	＊＊＊＊＊＊＊＊＊＊	＊M＊＊＊＊＊＊＊＊	＊＊＊＊＊＊＊＊＊＊	80
Mouse	＊＊＊＊＊＊＊＊＊＊	＊V＊＊＊＊＊＊＊＊	＊＊＊＊＊＊＊＊＊＊	＊＊＊N＊＊＊＊＊＊	80
Goat	＊A＊＊＊＊＊＊＊＊	＊V＊＊＊＊＊＊＊T	＊I＊＊＊＊＊＊＊＊	＊＊＊＊＊＊＊＊A＊	80

Rat	VLLADINDFG	TVNEIYKTYF	QGNLPARAAY	QVAALPKGSR	120
Pig	＊＊＊＊＊＊＊＊＊S	＊＊＊D＊＊＊Q＊＊	＊＊＊F＊＊＊＊＊＊	＊＊＊＊＊＊＊＊G＊	120
Chick	＊F＊＊＊＊K＊＊N	DM＊＊＊＊GQF＊	K S＊C＊S＊V S F	＊＊＊＊＊＊＊＊A＊	120
Cattle	＊＊＊＊＊＊＊＊＊S	＊＊＊D V＊＊Q＊＊	＊S S F＊＊＊＊＊＊	＊＊＊＊＊＊＊＊G＊	120
Human	＊＊＊＊＊＊＊＊＊N	＊＊＊＊＊＊＊Q＊＊	K S＊F＊＊＊＊＊	＊＊＊＊＊＊＊＊＊＊	120
Mouse	＊＊＊＊＊M＊＊＊＊	＊＊＊＊＊＊＊＊＊＊	＊＊S＊＊＊＊＊＊	＊＊＊＊＊＊R＊＊＊	120
Goat	＊＊＊＊＊＊＊＊＊S	A＊＊D V＊＊Q＊＊	＊S S F＊＊＊＊＊＊	＊＊＊＊＊＊＊＊G＊	120

Rat	IEIEAIAVQG	PF--TTAGL	137
Pig	V＊＊＊＊＊＊I＊＊	＊L - - V＊＊S＊	137
Chick	V＊＊＊＊＊＊I＊＊	＊I QNV P＊S＊	139
Cattle	V＊＊＊＊＊＊＊＊＊	＊L - - ＊＊＊ - -	135
Human	＊＊＊＊＊V＊I＊＊	＊L - - ＊＊＊S＊	137
Mouse	V＊＊＊＊＊＊＊＊＊	＊＊ - - I K＊ - -	135
Goat	V＊＊＊＊＊＊V＊＊	＊L - - ＊＊＊S＊	137

＊ Identical amino acid residues.

Table 3. Percentages of PSP amino acid sequences identity among the animals.

	Pig	Chick	Rat	Human	Mouse	Goat	Cattle
Pig		70.6%	86.0%	87.5%	82.8%	90.4%	91.0%
Chick			69.1%	72.8%	66.4%	68.4%	67.9%
Rat				87.5%	89.6%	82.4%	82.8%
Human					81.3%	86.0%	84.3%
Mouse						81.3%	81.3%
Goat							97.8%
Cattle							

In the present study, the cDNA of PSP of pig and chick was sequenced and its amino acid sequences were determined. There is over 80% identity between PSP of the mammalian subjects, but PSP of the chick representing Aves shows only 70% identity to that of the mammals. Furthermore, the chick PSP differs from the mammalian PSP not only in the number of amino acid residues but also in the amino acid sequence. PSP are evolutionary and highly conserved among different forms of life. The difference in PSP between mammalians and Aves seems to cause the difference in evolution. PSP is one of the evolutionary indexes as well as insulin and hemoglobin.

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