

MOLECULAR CLONING OF THE cDNA CODING FOR PROLINE-RICH PROTEIN
(PRP): IDENTITY OF PRP AS C4b-BINDING PROTEIN

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Summary: Proline-rich protein (PRP) is a plasma protein with a high proportion of proline residues and possessing lipid-binding properties. In order to clarify its structure, a human liver cDNA library was screened using anti-PRP antiserum. Several overlapping phage cDNA clones were isolated and the total nucleotide sequence of the cDNA, 2178 bp in length, was analyzed. The amino acid composition of PRP deduced from the cDNA was essentially the same as that reported for PRP. In a homology search, the cDNA sequence was almost completely the same as the previously reported cDNA sequence of C4b-binding protein. Furthermore, the reported molecular weights of the two proteins under both reduced and unreduced conditions were quite alike. These findings indicate that PRP is identical with C4bp. © 1989 Academic Press, Inc.

Proline-rich protein (PRP) is a glycoprotein that was first isolated from lipoprotein-free plasma by absorption to lecithin-stabilized triglyceride emulsion (Intralipid) (1). PRP, which is also present in chylomicron, has been determined to have a molecular weight (MW) of 74,000 by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions, but in native plasma it behaves as a larger particle (1). The mean plasma concentration of PRP was reported to be 21.7 mg/dl and is closely related to serum cholesterol of lipoprotein and serum triglyceride (1). Recently, it was shown that PRP is an acute-phase reactant, and that its level rises in parallel with erythrocyte sedimentation rate (ESR) and C-reactive protein (2). Although little is known about its structure and function, amino acid analysis of PRP has shown a large proline content (1). In this study, we isolated and analyzed the cDNA for PRP and revealed that it is identical with that for C4b-binding protein (C4bp) (3, 4). Our present report also extends the previously reported nucleotide sequence of C4bp cDNA.

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ABBREVIATIONS: PRP, proline-rich-protein; C4bp, C4b-binding protein.

MATERIALS AND METHODS

PRP and antisera for PRP: Purification of PRP and production of its antiserum have been reported previously (1). Briefly, the dialyzed 1.21-density infranant fraction of plasma was mixed with 0.2% Intralipid and incubated at 37°C for 1 h. After centrifugation, the supernatant lipid layer was subjected to chromatography on a 4% agarose gel column. The purified emulsion particles were extracted with ethanol-diethylether, and re-subjected to chromatography on a 4% agarose gel column. The eluate was further purified on an alkaline 3.75% polyacrylamide gel. The PRP band was cut out and emulsified with Freund's complete adjuvant and injected intradermally into New Zealand albino rabbits. The injection was repeated three times at intervals of 2 weeks and the animal was bled 10 days after the last injection. The raised antiserum was preliminarily shown to give a single precipitation line against whole human serum.

Isolation of cDNA clones: A human liver cDNA library was purchased from Clontech Lab. Inc. (Palo Alto, CA) and screened with the PRP antiserum according to the method of Huynh *et al.*(5). The insert DNA of the positive clone (λ PRP2) was labeled with [32 P]CTP by random priming (6) and used as a probe to rescreen the same library by the nitrocellulose replica filter preparation technique (7). Filters were hybridized for 16 h at 42 °C in a buffer containing 50% formamide (8).

DNA Sequence Analysis: DNA sequencing was carried out by the chain-termination DNA sequencing method (9) with T7 DNA polymerase (Sequenase; United States Biochemical Corp., Cleveland, OH)(10).

Comparison of Nucleotide Sequences: The EMBL computer program was used for analysis of the nucleotide sequence data.

RESULTS

Approximately 40,000 phage clones from the human liver cDNA library were screened with rabbit anti-PRP antiserum and one positive cDNA clone was isolated and designated λ PRP2 (Fig.1). Using the insert DNA of λ PRP2 as a DNA probe, about 10,000 clones of the same library were rescreened and four overlapping additional cDNA clones (λ PRP4, λ PRP6, λ PRP7, λ PRP8) were isolated (Fig.1).

Nucleotide sequence analysis was performed on the five overlapping cDNA clones (Fig.1). The connected cDNA sequence, 2,178 bp in length, included all

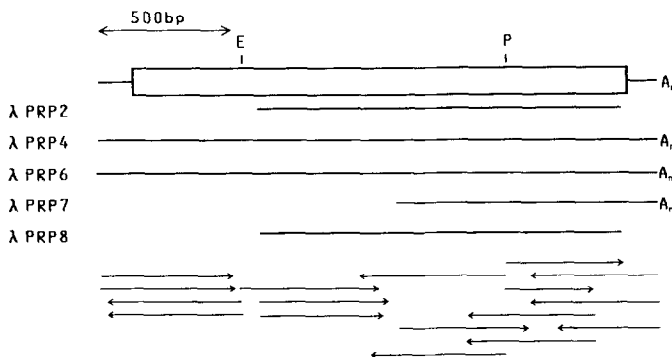


Fig. 1. Structure of human PRP cDNA. The coding region is represented by an open box. The isolated phage cDNA clones are represented by thick lines. The sequencing strategy is also shown. The restriction enzyme sites used in the preparation of the fragments for sequencing (E:EcoRI, P:PstI) are indicated.

AAA ACT GTG ATC TGG GGA GGA ACC AGG ACT ACA TAG ATC AAG GCA GTT TTC TTC TTT GAG	60	AGG CCG ACA AAA GAG GAT GTG TAT GTT GTT GGG ACT GTG TTA AGG TAC GGC TGT CAT CCT	1140
AAA CTA TCC GAG ATA TCA TCA TAG AGT GTT GTT GTT TTC CTC AAC TAC CAA AGA AAA ACA	120	Arg Pro Thr Lys Glu Asp Val Tyr Val Gly Thr Val Leu Arg Tyr Arg Cys His Pro	
TCA GGG AAG CAG CAG CAG CCG ATG CAC CCC CCA AAA ACT CCA TTT GGG GGT GTT CAT AGA AAA	180	GGC TAC AAA CCC ACT ACA GAT GAG CCG ACT CCG ACT GTT TGT CAG AAA AAT TTG AGA TGG	1200
Met His Pro Pro Lys Thr Pro Ser Gly Ala Leu His Arg Lys		Gly Tyr Lys Pro Thr Thr Asp Glu Pro Thr Thr Val Ile Cys Gln Lys Asn Leu Arg Trp	
AGG AAA ATG GGA GGC TGG CCC TTC TCC AGG CCG TGG AAA GTC TGT CAT CCA ATT CTC TTC	240	ACC CCA TAC CAA GGA TGT GAG GCG TTA TGT TGC CTT GAA CCA AAG CTA AAT AAT GGT GAA	1260
Arg Lys Met Ala Ala Trp Pro Phe Ser Arg Leu Trp Lys Val Ser Asp Pro Ile Leu Phe		Thr Pro Tyr Gln Gly Cys Glu Ala Leu Cys Cys Pro Glu Pro Lys Leu Asn Asn Gly Glu	
CAA ATG ACC TTG ATC GGT GGT CTT GTC TTT GGT GGT GGT GGT GGT GGT GGT GGT GGT GGT	300	ATC ACT CAA CAC AGG AAA AGT GGT GGT GGT GGT GGT GGT GGT GGT GGT GGT GGT GGT	1320
Gln Met Thr Leu Ile Ala Ala Leu Leu Pro Ala Val Leu Gly Asp Cys Gly Pro Pro Pro		Ile Thr Gln His Arg Lys Ser Arg Pro Ala Asn His Cys Val Tyr Phe Tyr Gly Asp Glu	
ACT TTA TCA TTT GGT GGC CCG ATG GAT ATT AGG TTG ACT GAG ACA CCG TTC AAA ACT GGA	360	ATT TCA TTT TCA TGT CAT GAG ACC AGT AGG TTT TCA GCT ATA TGC CAA GGA GAT GGC ACG	1380
Thr Leu Ser Phe Ala Ala Pro Met Asp Ile Thr Leu Thr Glu Thr Arg Phe Lys Thr Gly		Ile Ser Phe Ser Cys His Glu Thr Ser Arg Phe Ser Ala Ile Cys Gln Gly Asp Gly Thr	
ACT ACT GTG AAA TAC ACC TGC CTC GGT GGC TAC GTC AGA TCC CAT TCA ACT CAG AGC GTT	420	TGG AGT CCG CGA ACA CCA TCA TGT GGA GAC ATT TGC AAT TTT CTT CTT AAA ATT GGC CAT	1440
Thr Thr Leu Lys Tyr Thr Cys Leu Pro Gly Tyr Val Arg Ser His Ser Thr Gln Thr Leu		Trp Ser Pro Arg Thr Pro Ser Cys Gly Asp Ile Cys Asn Phe Pro Pro Lys Ile Ala His	
ACC TGT AAT TCT GAT GGC GAA TGG GTG TAT AAC ACC TTC TGT ACT TAC AAA CCA TGC AGA	480	GGG CAT TAT AAA CAA TGT AGT TCA TAC AGC TTT TTC AAA GAA GAG ATT ATA TAT GAA TGT	1500
Thr Cys Asn Ser Asp Gly Glu Trp Val Tyr Asn Thr Phe Cys Ile Tyr Arg Cys Arg		Gly His Tyr Lys Gln Ser Ser Ser Tyr Ser Phe Phe Lys Glu Glu Ile Ile Tyr Glu Cys	
CAC CCA GGA GAG TTA CTT AAT GGG CAA GTA GAG ATT AAG ACA GAT TTA TCT TTT GGA TCA	540	GAT AAA GGC TAC ATT CTG GTC GGA CAG GGG AAA CTC TGC GGT TAT TCA CAC TGG TCA	1560
His Pro Gly Glu Leu Arg Asn Gly Gln Val Glu Ile Leu Thr Asp Leu Ser Phe Gly Ser		Asp Lys Gly Tyr Ile Leu Val Gly Gln Ala Lys Leu Ser Cys Ser Tyr Ser His Trp Ser	
CAA ATA GAA TTC ACC TGT TCA GAA GGA TTT TTC TTA ATT GGC TCA ACC ACT AGT GGT TGT	600	GCT CCA GGC CCT CAA TGT AAA CCG GTG TGT GGT GGA AAA CCA GAA TTA GTG AAT GGA AGG TTC	1620
Gln Ile Glu Phe Ser Cys Ser Thr Glu Gly Phe Phe Leu Ile Gly Ser Thr Thr Ser Arg Cys		Ala Pro Ala Pro Gln Cys Lys Ala Leu Cys Arg Lys Pro Glu Leu Val Asn Glu Arg Leu	
GAA GTC CAA GAT AAG GGA GTT GGC TGG NAT CAT CTT CTC CAA CAA TGT GAA ATT GTC AAG	660	TCT GTG GAT AAG GAT CAG TAT GGT GGT CCA AAT GAT ACC ATC CAA TGT GAT TGT GGC	1680
Glu Val Gln Asp Arg Gly Val Gly Trp Ser His Pro Leu Pro Gln Cys Glu Ile Val Lys		Ser Val Asp Lys Asp Gln Tyr Val Glu Pro Glu Asn Val Thr Ile Gln Cys Asp Ser Gly	
TGT AAG CTT CCT CCA GAC ATC AGG AAT GGA AGG CAC AGC GGT GAA GAA AAT TTC TAC GCA	720	TAT GGT GTG GTT GGT CCG CAA AGT ACT ATC TGC TGT GCG AAC AGA ACC TGG TAC CCA GAG	1740
Cys Lys Pro Pro Pro Asp Ile Arg Asn Gly Arg His Ser Gly Glu Glu Asn Phe Tyr Ala		Tyr Gly Val Val Gly Pro Gln Ser Ile Thr Cys Ser Gly Asn Arg Thr Trp Tyr Pro Glu	
TAC GGC TTT TCT GTC ACC TAC AGC TGT GAC CCG CCG TTC TCA CTC TTG GGC CAT GGC TCC	780	GTG CCG AAG TGT GAG TGG GAG ACC CCG CAA GGC TGT GAA CAA GTG CTC ACA GGC AAA AGA	1800
Tyr Gly Phe Ser Val Thr Tyr Ser Cys Asp Pro Arg Phe Ser Leu Leu Gly His Ala Ser		Val Pro Lys Cys Glu Trp Glu Thr Pro Gln Gly Cys Glu Gln Val Leu Thr Gly Lys Arg	
ATT TCT TGC ACT GTG GAG AAT GAA ACA ATA GGC GTT TGG AGA CCA AGC CTT CTT ACT TGT	840	CTC ATG CAG TGT CTT CCA AAC CCA GAG GAT GTG AAA ATG GGC CTG GAG GTA TAT AAG CTG	1860
Ile Ser Cys Thr Val Glu Asn Glu Thr Ile Gly Val Trp Arg Pro Ser Pro Pro Thr Cys		Leu Met Gln Cys Leu Pro Asn Pro Glu Asp Val Lys Met Ala Leu Glu Val Tyr Lys Leu	
GAA AAA ATC ACC TGT CCG AAG CCA GAT GTT TCA CAT GGG GAA ATG GTC TGT GGA TTT GGA	900	TCT CTG GAA ATT GAA CAA CTG GAA CTA GAG AGA GAC AGC GGA AGA CAA TCC ACT TTG GAT	1920
Glu Lys Ile Thr Cys Arg Lys Pro Asp Val Ser His Gly Glu Met Val Ser Gly Phe Gly		Ser Leu Glu Ile Glu Gln Leu Glu Leu Gln Arg Asp Ser Ala Arg Gln Ser Thr Leu Asp	
CCG ATC TAT AAT TAC AAA GAC ACT ATT GTT TTG AAG TGC CAA AAA GGT TTT GTT CTC AGA	960	AAA GAA CTA TAA TTT TTC TCA AAA GAA GGA GGA AAA GGT GTC TTG GCT GGT GGC CTC TTG	1980
Pro Ile Tyr Asn Tyr Lys Lys Asp Thr Ile Val Phe Lys Cys Gln Lys Gly Phe Val Leu Arg		Lys Glu Leu	
GGG AGC AGT GTA ATT CAT TGT GAT GGT GAT AGC AAA TGG AAT GGT TCT CTT CTT GGT TGT	1020	CAA TTC AAT ACA GAT CAG TTT AGC AAA TGT ACT GTC AAT TTG GCA GTG ATA TTC ATC ATA	2040
Gly Ser Ser Val Ile His Cys Asp Ala Asp Ser Lys Trp Asn Pro Ser Pro Pro Ala Cys		ATA TAT ATC TAG AAA TGA TAA TTT GGT AAA GTT TAG TGC TTT GAG ATT GTG AAA TTA TTA	2100
GAG CCG AAT AGT TGT ATT AAT TTA CCA GAC ATT CCA CAT GGT TGC TGG GAA ACA TAT CCT	1080	ATC ATC CTC TGT GGT GGT CAT GTT TTT GGT TTT CAA CAC ACA AAG CAC AAA TTT TTT TTC	2160
Glu Pro Asn Ser Cys Ile Asn Leu Pro Asp Ile Pro His Ala Ser Trp Glu Thr Tyr Pro		GAT TAA AAA TGT ATG TAT A	2178

Fig. 2. The entire nucleotide sequence of PRP cDNA deduced from the sequences of the overlapping fragments shown in Fig.1. The sequences of the 5' and 3' untranslated regions which were not previously published for C4bp cDNA are underlined. The deduced amino acid sequence is shown below. The reported N-terminal amino acid (11) of C4bp is double-underlined. The nucleotides which were different from those in previous reports (3,4) are dotted. The used polyadenylation signal is thickly underlined.

of the 3' non-translated region as shown by the run of adenine residues at the 3' end of the cDNA (Fig.2). The position of the poly(A) tract was constant among three independent cDNA clones (λ PRP4, λ PRP6, λ PRP7), suggesting no variation in the length of the 3' non-translated region. The longest open reading frame was for 597 amino acids (AAs) and the first ATG codon was 139 nucleotides (nt) from the 5' end of the sequence. The length of the 3'non-translated region was 246 nt. The putative polyadenylation signal was ATTAAA located at residues 2,162-2,167 or 17 nt upstream from the poly(A) tract. The same kind of one base mutation of the commonest polyadenylation signal (AATAAA) has been reported to be valid in various genes (11).

Figure 2 also shows the deduced amino acid sequence of the protein encoded in the longest open reading frame starting at the ATG at nucleotide 139. When the amino acid composition of the deduced protein was compared with the previously reported amino acid analyses of PRP (1,2), they were basically

Table 1. Comparison of amino acid (AA) composition. A: AA composition deduced from the isolated cDNA sequence of PRP. B: Composition excluding the leader peptides. C: AA composition of PRP reported by Sata *et al.*(1). D: AA composition of PRP reported by Funakoshi *et al.*(2). Tryptophan residue is excluded in A and B, as it could not be detected by the analysis method used in the previous reports (1, 2).

mol/10 ³ mol of amino acids	Samples			
	A	B	C	D
Lysine	58	56	59	53
Histidine	29	28	27	27
Arginine	53	50	51	47
Aspartic acid	75	80	90	88
Threonine	70	72	72	69
Serine	87	89	91	98
Glutamic acid	106	113	127	122
Proline	89	85	89	86
Half-cystine	62	67	34	55
Glycine	70	72	87	86
Alanine	36	32	41	38
Valine	56	58	46	42
Methionine	12	7	6	10
Isoleucine	50	50	40	36
Leucine	67	59	64	62
Tyrosine	43	46	38	41
Phenylalanine	36	35	38	41

the same, although a signal peptide appeared to be included in our present data (Table 1).

When the isolated PRP cDNA sequence was compared with sequences in the EMBL Data Library, it shared almost complete homology with the cDNA sequence for C4b-binding protein (C4bp) (3,4). There were only three differences between our nucleotide sequence and the reported C4bp cDNA sequence, a T for C substitution at nucleotide 1,590, a G for A substitution at 1,644, and a T for TT substitution at 156. The first two substitutions do not change the coded amino acids, because they involve the third nucleotide of the codons. The last substitution of T for TT, which was revealed by repeated sequencing of this region in both directions using several different fragments (Fig.1), changes the frame of the leader peptide making the ATG codon at nucleotide 139 a candidate for the first methionine.

Our newly isolated cDNA sequence is longer than the previously reported C4bp cDNA sequences (3, 4). It adds 131 nucleotides to the 5'-end, and includes 86 more nucleotides of the 3'-untranslated region extending to the poly(A) tract. The N-terminal amino acid of the C4bp structural protein is reported to be asparagine, which is the 49th amino acid of our deduced polypeptide (12). This indicates that the leader peptide is as long as 48 amino acids. The amino acid composition of C4bp excluding its leader peptide was calculated (Table 1, column B), and found to be also very close to that already reported for PRP (1, 2).

DISCUSSION

PRP was first isolated in 1976 as a plasma protein showing affinity for lipid and containing large amount of proline (1). In spite of its appreciable concentration in plasma, little is known about its structure and function. In order to clarify the structure, we attempted to clone its cDNA. As the liver had been shown immunohistochemically to produce PRP (2), we screened a liver cDNA library to isolate PRP cDNA clones. The putative PRP cDNA was obtained using a λ gt11 expression vector system and a specific antiserum for PRP. In addition to immunological detection, the amino acid composition deduced from the cDNA was basically the same as that of PRP reported previously (Table 1). Furthermore, the MW of the protein estimated from the cDNA sequence was about the same as that of PRP. From these data we concluded that the isolated cDNA was for PRP.

On sequence comparison it was almost the same as the reported cDNA sequence for C4bp, indicating that PRP and C4bp are the same protein, but purified by different methods (1,13). In addition to their immunological relatedness, the reported molecular weights (MWs) of PRP and C4bp are quite alike. In previous reports (1,2), PRP showed a MW of 74,000 by SDS-PAGE but a much heavier MW of >1,000,000 (1) or 352,000 (2) by polyacrylamide gel electrophoresis (PAGE) under non-reduced conditions without (1) or with (2) urea. From these data, PRP was thought to exist in a polymerized form in plasma. On the other hand, C4bp was reported to have a MW of about 70,000 and 550,000 by PAGE under reduced and non-reduced conditions, respectively, and was thought to exist as a heptamer (14). As it is difficult to determine the exact MW of high-molecular-weight proteins by PAGE, the two proteins seem to have very similar MWs as a monomer and both exist in polymerized form in plasma. Furthermore, the mean concentrations of these two proteins in plasma were reported to be quite similar, 21.7 mg/dl for PRP (1) and about 20 mg/dl for C4bp (15). All these findings suggest that PRP is identical with C4bp.

The newly isolated cDNA was different from the previously reported C4bp cDNA sequence (3,4) at three nucleotides. The T for C substitution at nucleotide 1,590 and the G for A substitution at 1,644 do not change the coded amino acids because they involve the third nucleotide of each codon (Fig.2) and we considered that these are polymorphic sites. The same kinds of polymorphism have also been reported for C4bp cDNA previously (3). On the other hand, a T for TT substitution at nucleotide 156 will change the reading frame of the reported leader peptide (4) and make the ATG at 139 a candidate for the initiation codon. Though there are two other universal translation initiation codons 3'-downstream from this codon (at nucleotides 187 and 244), the ATG at 139 is most likely to be the point of initiation because it has

been reported that translation begins at the 5'-proximal AUG triplet in about 90% of eukaryotic mRNAs (16).

C4bp was first described in 1978 as a regulator of the classical pathway of the complement system. C4bp contains seven binding sites for C4b (14). It accelerates the dissociation of C2a from the C3 convertase (C4bC2a, EC 3.4.21.43) and also functions as a cofactor for enzyme factor I in the degradation of C4b (17, 18). C4bp also has a single binding site for the vitamin K-dependent protein S (19) and was recently demonstrated to be one of the regulators of the coagulation system (20, 21).

The identification of PRP and C4bp indicates that C4bp has a lipid binding capacity and that at least a proportion of it is present in chylomicron. C4bp, which is an important regulator of the complement system as well as of the coagulation system, may also be related to the metabolism of cholesterol, because the concentration of PRP is significantly correlated with that of cholesterol in lipoprotein of very low (VLDL) or low (LDL) density (1).

The PRP content of serum was reported to be related to the levels of CRP and ESR with or without inflammation or infection (2). Following acute myocardial infarction, the PRP level increases, and reaches a maximum on day 7, subsequently decreasing gradually to a normal level by day 20, just like the course of the CRP level (2). These findings also indicate that C4bp, which is the same as PRP, is one of the acute-phase reactants. The increase of C4bp during inflammation seems to be rational, as C4bp decreases C3 convertase activity and may limit the spread of inflammation.

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