

HAMSTER FEMALE PROTEIN

A New Pentraxin Structurally and Functionally Similar to C-reactive Protein and Amyloid P Component

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Female protein (FP)¹ is a fast alpha globulin present in generous amounts (1–2 mg/ml) in sera of normal female Syrian hamsters and not detectable by gel diffusion in sera of normal adult males (1). Serum FP in normal males is suppressed by testosterone as FP did appear in male hamsters after diethylstilbesterone treatment or castration and subsequently disappeared after exogenous testosterone administration (1). Other examples of sex hormone-controlled serum proteins have been documented in cockroaches (2) and chickens (3) where the protein was involved with egg production (4). The sex difference of mammalian serum proteins (Ss protein [5], IgM [6], various complement components [7, 8]) has not been related to any particular functional requirement unique for one sex. Similarly, we are not aware of a unique biological difference between male and female hamsters that could be functionally related to presence or absence of FP, and this protein did not appear analogous to any of the previously described sex-limited proteins in mammals. However, during the structural analysis of FP, some unusual properties were found which suggested the relationship of FP to a well known acute-phase protein, C-reactive protein (CRP). Accordingly, FP was studied to determine binding characteristics, appearance in electron microscopy, subunit structure, and primary amino acid sequence. In this report, FP is shown to be functionally, morphologically, and structurally similar to two related proteins, CRP and serum amyloid P component (SAP). In fact, FP appears to represent the CRP-SAP homologue in Syrian hamsters.

Materials and Methods

Animals. Random-bred Syrian hamsters were obtained from the National Institutes of Allergy and Infectious Diseases, (NIAID), Rocky Mountain Laboratory colony and New Zealand white rabbits were purchased locally.

¹ *Abbreviations used in this paper:* CRP, C-reactive protein; DTT, dithiothreitol; EDTA-E, EDTA column eluate; FP, female protein; HEA, hen egg albumin; IEP, immunoelectrophoresis; NFHS, normal female hamster serum; NHS, normal hamster serum; PAGE, polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline; PC, phosphorylcholine; PT, pass-through; PTH, phenylthiohydantoin; RGG, rabbit gamma globulin; SAP, serum amyloid P (component); SDG, sucrose density gradient; SDS-PAGE, sodium dodecyl sulfate-PAGE.

Antisera and Purified Proteins. Anti-whole normal hamster serum (NHS) was produced by inoculating a Targhee sheep with pooled normal male and female hamster serum in complete Freund's adjuvant. This sheep antiserum contained antibody to FP which was especially apparent on immunoelectrophoresis (IEP) because of low anti-albumin content. Antisera specific for FP were obtained by absorption as previously described (1) or utilized serum from rabbits inoculated with purified preparations of FP emulsified in complete Freund's adjuvant. Anti-B₇C was produced by injecting rabbits with washed zymozan after treatment with hamster serum (9). Anti-human amyloid P component purchased from Atlantic Antibodies, Westbrook, Mass., and generously supplied by Dr. Painter (University of Toronto, Toronto, Canada) were utilized. Anti-human CRP (Behring Diagnostics, Inc., Woodbury, N. Y.) and purified human CRP, rabbit CRP, and pneumococcal C-polysaccharide were obtained from Dr. H. Gewurz (Rush Medical College, Chicago, Ill.).

Serum Fractionation. Normal female hamster serum (NFHS; 4 ml) was filtered through a 2.5 × 90-cm column of Sephadex G-200 (Pharmacia Fine Chemicals, Uppsala, Sweden) with 1 M NaCl-Tris, pH 8.0 solution, and 2.5-ml fractions were collected. NFHS (0.3 ml) was analyzed on a 10-40% sucrose density gradient (SDG) spun at 35,000 rpm with SW50.1 rotor in a Beckman L2-65B ultracentrifuge (Beckman Instruments Inc., Fullerton, Calif.). DEAE-purified rabbit gamma globulin (RGG; Pentex Biochemicals, Kankakee, Ill.) and hens' egg albumin (HEA; ICN K and K Laboratories Inc., Plainview, N. Y.) were labeled with ¹³¹I or ¹²⁵I (ICN, Irving, Calif.) respectively by chloramine T (10) or KI (11) methods and incorporated with the sample as markers. Equal fractions recovered from the bottom of the tube, were assayed for radioactivity in dual-channel Nuclear Chicago automatic gamma counter and FP was quantified by ring diffusion analysis (1).

Affinity Chromatography of FP. FP was isolated by passing NFHS through a column containing Sepharose 2B conjugated by cyanogen bromide with specific rabbit anti-FP as previously described (1). Pure FP was obtained also by affinity chromatography using phosphorylcholine (PC) conjugated to Sepharose prepared according to the method of Chesebro and Metzger (12) and kindly supplied by Dr. B. Chesebro. In preparative use, NFHS (5 ml) was passed through a column containing 30 ml PC-Sepharose previously equilibrated with 0.5 mM CaCl₂, 0.1 M Tris buffer, pH 7.0. After thorough washing (400 ml) with the same buffer, FP was eluted either with 0.001 M phosphorylcholine (PC) (ICN Pharmaceuticals, Plainview, N. Y.) in the same CaCl₂-Tris buffer or with 0.1 M EDTA in 0.1 M Tris, pH 7.0. The eluate was concentrated by negative pressure and dialyzed against phosphate-buffered saline (PBS), pH 7.2. For comparative purposes, a 7-ml column of plain Sepharose (Sepharose 4B, Pharmacia, Fine Chemicals) and a 7-ml column of PC-Sepharose were tested for their relative yield of FP after addition of 1 ml NFHS, using similar buffer and elution scheme as presented above.

Analytical Techniques. Immunoelectrophoresis, simple gel diffusion, and ring diffusion quantification assays were done as before (1). Polyacrylamide gel electrophoresis (PAGE) (13) and sodium dodecyl sulfate (SDS-PAGE) (14) with 5, 7, or 10% gels used a Bio-Rad vertical slab gel apparatus (Bio-Rad Laboratories, Richmond, Calif.). Gels were stained with Coomassie Blue (15) and molecular weight-marker proteins were obtained from Boehringer Mannheim. Purified FP was labeled with ¹²⁵I-Bolton Hunter reagent (New England Nuclear, Boston, Mass.) and after disk SDS-PAGE, the gel was fractionated by Savant Autogeldiver (Savant Instruments, Inc., Hicksville, N. Y.).

Electron Microscopy. Electron microscopy was carried out on a Philips 300 electron microscope (Philips Electronic Instruments, Inc., Makwah, N. J.) at 80 Kv with a 70-μm objective aperture and a magnification of 27,000-40,000 times. Calibration was based upon photographs of indanthrene olive crystals (16). Micrographs were recorded sufficiently close to focus to make metal grain at the 2.0-nm level clearly resolved on the original plates. Solutions of protein, dialyzed exhaustively against 0.15 M ammonium acetate were sprayed at a concentration of ~0.1 mg/ml through a high-pressure spray gun at freshly cleaved mica. Preparations were either shadow cast with platinum by rotary shadowing as described previously (17) or negatively contrasted by the method of Valentine et al. (18).

In making the measurements from the micrographs, a number of entire fields was scored in order to reduce selectivity. From similarly prepared samples of other molecules of known size

and shape (17), a correction of 2.5 nm was applied to compensate for the distortion in thickness of the original particles by the cap of replicating metal.

Molecular Weight Determination of FP. Molecular weight determinations were done by the high-speed meniscus-depletion method (19) using Beckman Model E analytical ultracentrifuge equipped with Rayleigh interference optics.

A six-channel, externally loading cell with sapphire windows (20) was filled with protein at three initial concentrations (0.10, 0.30, and 0.72 mg/ml). The AN-E rotor was accelerated to 15,000 rpm at 5°C and at equilibrium, pictures were taken on Kodak Nuclear Medicine film or type IIG spectroscopic plates (Eastman Kodak Co., Rochester, N. Y.) at camera focus of 2/3 plane (19, 21). The molecular weight was calculated by linear regression analysis, from the equation

$$\text{mol wt} = \frac{2RT}{(1 - \bar{v}_p)\omega^2} \times 2.303 \left(\frac{d \log c}{dr^2} \right),$$

where R = gas constant; T = absolute T°; \bar{v} = partial specific volume of solute; ρ = density of solution; c = concentration of protein; r = radial distance; using a value of 0.728 cc/g for partial specific volume. To determine the molecular weight of FP subunits, the protein also was analyzed in 5 M guanidine hydrochloride, 20 mM sodium phosphate, pH 7.0, at two initial protein concentrations of 0.30 and 0.70 mg/ml.

Amino Acid Analysis. Two independent preparations of FP were analyzed. Aliquots of each preparation were hydrolyzed for 16, 24, and 72 h in vacuo in 6 N HCl, to obtain accurate values for labile and slowly released amino acids. Amino acid compositions were determined using a Durrum D-500 amino acid analyzer (Durrum Instrument Corp., Sunnyvale, Calif.).

Sequence Determination. A sample of 8 mg of FP was dissolved in 0.5 ml of 0.3 M Tris, pH 8.2, containing 6 M guanidine-HCl, reduced under N₂ with 0.02 M dithiothreitol (DTT) for 1 h at 0°C and alkylated with 0.042 M iodoacetamide at 0°C for 1 h. The protein was then dialyzed against 0.05 M sodium borate, pH 8.5 and succinylated with a large excess of succinic anhydride in a pH-stat at pH 10.5. The succinylated protein was deblocked (22), dialyzed against water, lyophilized and subjected to 25 cycles of the Edman degradation in a modified Beckman 890C sequencer using a 0.1 M quadrol program. Phenylthiohydantoin (PTH) amino acids were identified by liquid chromatography using the method of Gates et al. (23).

Results

Functional Studies of FP Binding. NFHS (1.0 ml) was passed through a 7-ml column of PC-Sepharose previously equilibrated with 0.5 mM CaCl₂ in 0.1 M Tris HCl, pH 7.0. After further washing (100 ml) with the same buffer, 0.1 M Na EDTA, pH 7.0 was added to the column. The collected pass-through and EDTA eluate volumes were concentrated to 1 ml, dialyzed against PBS, and tested for presence of FP. Fig. 1

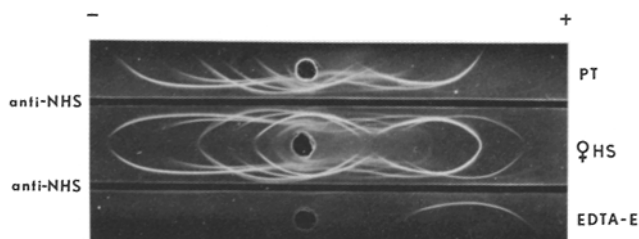


Fig. 1. Selective removal of FP by passage of NFHS through PC-Sepharose in presence of Ca⁺⁺. IEP comparing the original NFHS (center well, female hamster serum (♀ HS)) with that portion which passed through (PT, top well) the PC-Sepharose column and that portion which was subsequently eluted with EDTA (EDTA-E, bottom well). Notice the FP precipitin line is absent in PT and is the only detectable protein in EDTA eluate. Hamster albumin precipitin line not detected with this sheep anti-NHS (in troughs).

TABLE I
Differential Elution of FP and Other Proteins from PC- and Plain Sepharose Column

Elution scheme	PC-Sepharose		Plain Sepharose		Glass bead	
	FP	Other proteins	FP	Other proteins	FP	Other proteins
EDTA	+	+	+	+	0	0
PC	+	0	+	0	0	0
EDTA after PC	0	+	0	+		
PC after EDTA	0	0	0	0		

* +, present; 0, absent.

shows an IEP comparing the original serum in the center well, the pass-through of the PC column (PT) in the top well and the EDTA eluate of the column (EDTA-E) in the bottom well. When developed with sheep anti-NHS, the FP precipitin line was seen in the original serum, was singularly absent from the PT and was the only protein present in the EDTA eluate. Thus, FP binds to PC-Sepharose in a calcium dependent fashion. Analysis of EDTA eluates by simple gel diffusion and PAGE did reveal the presence of minor contaminants (Table I).

If PC were the binding ligand for FP, then free PC should elute bound FP from PC-Sepharose. NFHS was added to a PC-Sepharose column as before and after washing with CaCl_2 /Tris buffer, the column was eluted with 0.001 M PC in the same CaCl_2 /Tris buffer. FP was the only protein in the PC eluate by simple gel diffusion and PAGE analysis (Table I). Furthermore, no additional FP could be recovered from the column by subsequent elution with EDTA-Tris buffer, although this eluate contained small amounts of B_1C and, occasionally, albumin. Therefore, free PC in the presence of Ca^{++} eluted all the FP bound to PC-Sepharose and FP was the only protein in this eluate (Table I).

Next, NFHS was passed through a 7-ml plain Sepharose column under the same buffer conditions to test whether PC was indeed the binding ligand in PC-Sepharose chromatography. Small amounts of FP were recovered in the EDTA eluate (along with trace amounts of previously noted contaminants). As with the PC-Sepharose, all of the FP without contaminants could be eluted with 0.001 M PC in CaCl_2 /Tris buffer (Table I). To determine if the glass column itself was responsible for any FP binding, whole female serum was passed through a 100-ml column of glass beads under usual buffer conditions. No FP was lost from the PT or detected in the EDTA eluate. Thus, FP does bind to Sepharose in a Ca^{++} -dependent fashion and can be specifically eluted with free PC. A summary of these results is shown in Table I.

Further quantitative assays were done comparing FP yield with PC-Sepharose and plain Sepharose absorption under varying concentrations of Ca^{++} and PC. 1 ml of pooled NFHS was filtered through a 7-ml column of PC-Sepharose or plain Sepharose and, under similar conditions, the PT and eluted FP were collected, concentrated and quantified by ring-diffusion assay. The total recovered FP (PT plus eluate) averaged 70% with both columns and was similar at various Ca^{++} concentrations. The results, expressed as milligrams of FP in PC-eluate, are shown in Table II. At the optimum Ca^{++} concentration (0.5 mM), a comparable volume of PC-Sepharose bound ~15 times more FP than plain Sepharose (0.86 mg vs. 0.05 mg). The Ca^{++} dependence of binding was biphasic, with no binding at low concentrations (0.05 mM) and reduced

TABLE II
*Relative Yield of FP from PC-Sepharose and Plain Sepharose Columns by
 PC Elution in Various Concentrations of Ca⁺⁺*

Ca ⁺⁺	PC-Sepharose	Plain Sepharose
	<i>mg FP in PC-eluate</i>	
50 mM	0.34	0.09
	0.43	0.08
	0.19	0.05
5 mM	0.53	
0.5 mM	0.92	0.03
	0.97	0.02
	1.12	0.09
	0.53	0.05
0.25 mM	0.61	
	1.15	
0.05 mM	<0.01	<0.01
	<0.01	<0.01

* Conditions: columns (7 ml each) received 1 ml pooled NFHS that contained 1.5 mg FP/ml, and were eluted with PC (10^{-3} M, preceded by 10^{-4} M in some cases) and then EDTA. FP was never detected in the EDTA eluate when preceded by PC elution. Some columns were eluted with 10^{-4} M before 10^{-3} M PC and the respective amounts of eluted FP were quantified. In 50 mM Ca⁺⁺, 10^{-4} M PC did not elute FP from PC-Sepharose, although with plain Sepharose, equal amounts of FP were eluted with 10^{-4} M and subsequent 10^{-3} M PC (three columns each). In 0.5 mM Ca⁺⁺, distribution of FP in 10^{-4} M and subsequent 10^{-3} M PC elution from PC-Sepharose was 70-73% and 30-27%, respectively (two columns) whereas with plain Sepharose, all FP was eluted at 10^{-4} M PC (two columns).

binding at high concentrations (50 mM). A concentration of 10^{-3} M PC was sufficient to remove all FP, as subsequent EDTA eluates of all columns were consistently negative (data not shown). PC was partially effective at 10^{-4} M in recovery of FP, especially in 0.5 mM Ca⁺⁺, where all (plain Sepharose) or part (PC-Sepharose) of the FP was eluted. At 50 mM Ca⁺⁺, partial (plain Sepharose) or no (PC-Sepharose) elution was found at 10^{-4} M PC. These results suggest a greater affinity of FP for PC-Sepharose than plain Sepharose and, although Ca⁺⁺ is necessary, optimum binding to PC-Sepharose occurs with intermediate Ca⁺⁺ concentration (0.5 mM).

Gel Diffusion Tests with FP. CRP complexes in a Ca⁺⁺-dependent fashion with PC residues of pneumococcal C-polysaccharide (24, 25). However, when FP was tested on simple gel diffusion (5 mM Ca⁺⁺-agarose) against purified C-polysaccharide, no precipitin reaction was detected with various purified and unpurified preparations of FP. A satellite well of FP did not alter or interfere with the precipitin line produced by human CRP and C-polysaccharide. Rabbit CRP did produce a detectable precipitin line with this C-polysaccharide, although rabbit CRP reacts better with the less-degraded form of C-polysaccharide (26). Antigenic determinants common to FP and CRP or amyloid P component were not detected by gel diffusion assay as anti-CRP and anti-amyloid P component did not cross-react with FP and rabbit anti-FP did not precipitate CRP.

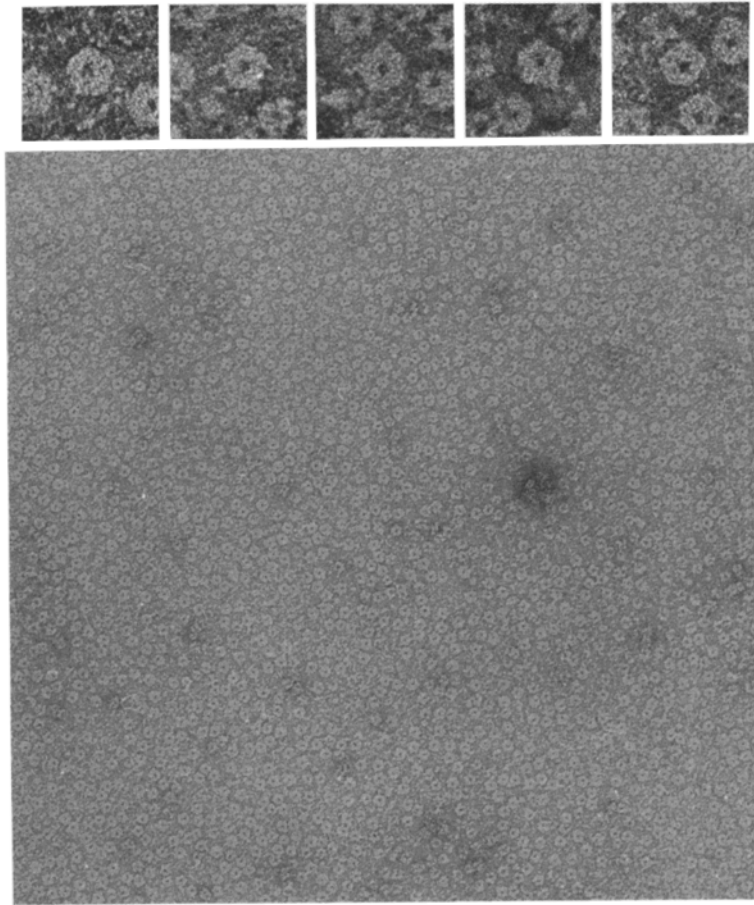


FIG. 2. Electron micrographs of hamster FP negatively contrasted with uranyl acetate. Field: $\times 134,784$; insets: $\times 494,208$.

Electron Microscopy. In negatively stained preparations, FP appears roughly as a toroidal or pentagonal object ~ 10 – 13 nm in diameter (Fig. 2). This dimension depends upon whether the diameter is measured face-to-face or vertex-to-vertex. The most frequent vertex-to-vertex measurements based on scoring of 200 particles averaged 12 – 13 nm with an SD of 1.5 nm. Measurements between sides based on 200 particles were slightly less and ranged from 10.5 to 12 nm with approximately the same standard deviation. Fig. 3 is a histogram showing the distribution of widths (face-to-face) for FP. Corrected width measurements from rotary shadow cast preparations were consistent with those from negatively stained material. In the majority of molecular images showing polyhedral symmetry, a pentameric structure was evident. In rare instances, a hexameric outline was seen.

Structure. Filtration of NFHS through Sephadex G-200 revealed the presence of FP in a single peak just after the 7S peak (Fig. 4). By using the gammaglobulin and albumin peaks as markers, the diffusion coefficient was calculated to be 4.4×10^7 (27). When analyzed by SDG ultracentrifugation, FP sedimented in a single 7.3S peak slightly faster than that of the 7S marker protein I^{125} -RGG (Fig. 5). The

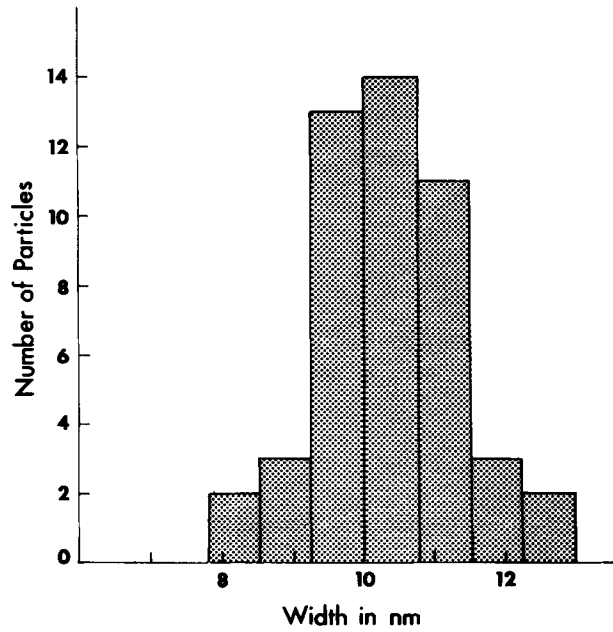


FIG. 3. Histogram of EM measurements showing the distribution of face-to-face diameter of FP.

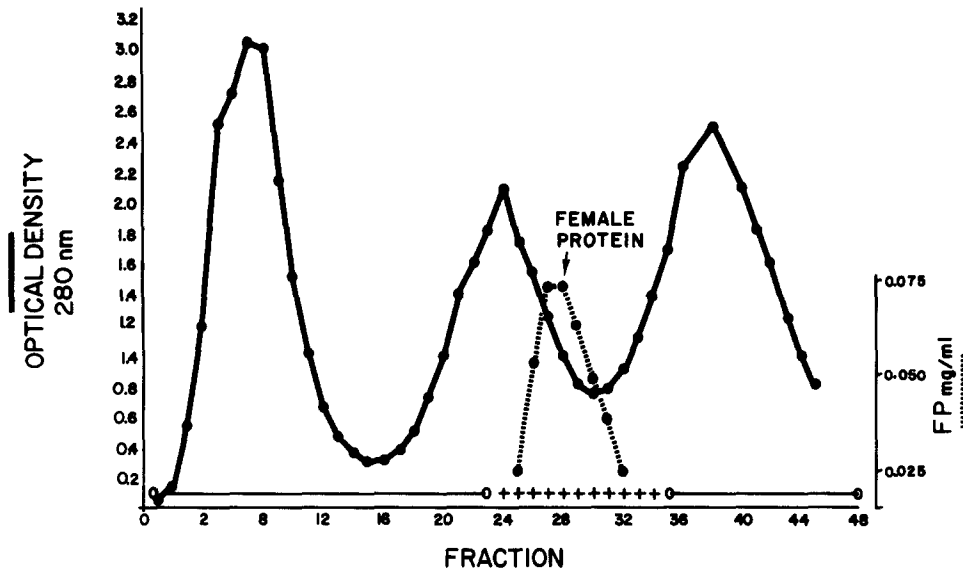


FIG. 4. Distribution of FP in Sephadex G-200 filtration of NFHS. FP detected by simple gel diffusion (+ = presence) on bottom line and quantified by ring diffusion (· · ·).

molecular size was estimated to be 150,000 by using the above values for diffusion coefficient and velocity, and assuming the partial specific volume of FP to be 0.73 (28).

In an effort to obtain more information concerning the size and homogeneity of FP, purified FP preparations obtained by affinity chromatography (anti-FP and PC

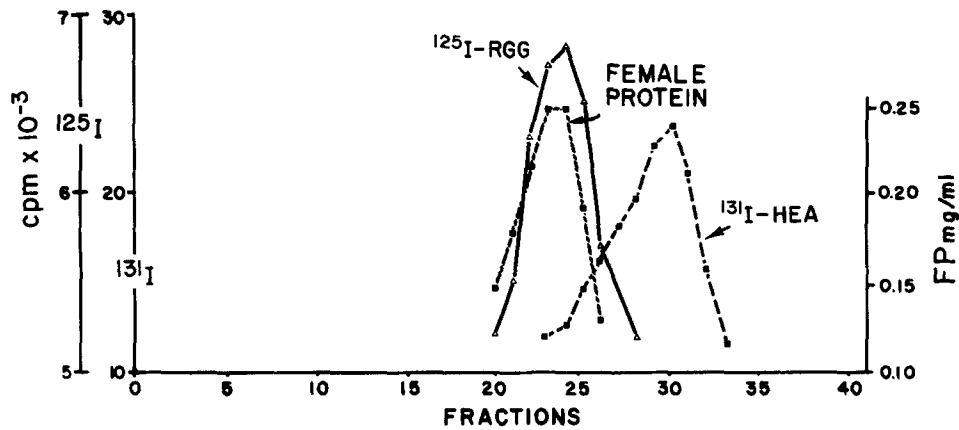


FIG. 5. Distribution of FP in SDS analysis (10–40%, 35,000 rpm \times 18 h) compared with ^{125}I -RGG and ^{131}I -HEA.

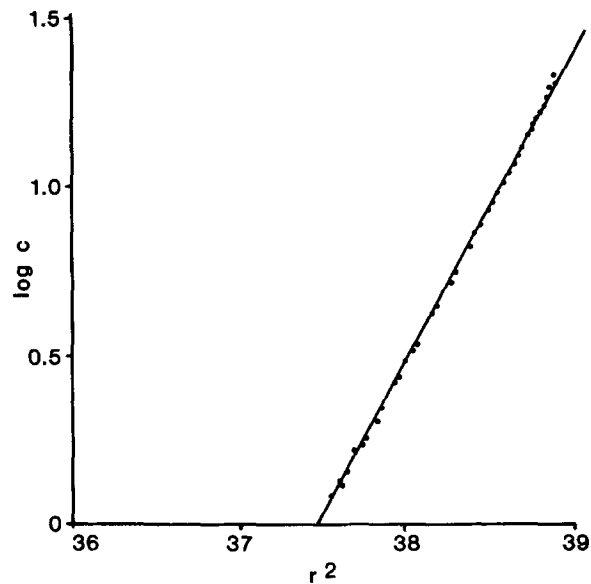


FIG. 6. High-speed sedimentation equilibrium values of FP. Log c vs. r^2 plot of values. Initial protein concentration: 0.72 mg/ml in 0.85% NaCl. Rotor speed: 15,000 rpm at 5°C.

affinity) were analyzed by the method of high-speed equilibrium centrifugation. Fig. 6 shows that the log C vs. r^2 plot was linear over the entire length of the solution column with a negligible curvature upward at the base of the cell, thus indicating a very homogenous, monodisperse preparation. The molecular weight calculated from these data was 151,000, in agreement with the value estimated by other methods.

Isolated FP obtained by affinity chromatography was analyzed by SDS-PAGE with 5, 7, and 10% gels. A consistent single band was detected of $\sim 30,000$ -mol wt size (Fig. 7) and a similar result was found with or without 2-mercaptoethanol. Other subunit structures were not detected with ^{125}I -labeled FP, as a single peak of radioactivity at $\sim 30,000$ -mol wt position was found when ^{125}I -FP was subjected to electrophoresis in disk SDS-PAGE and fractionated.

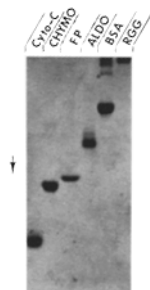


FIG. 7. SDS-PAGE analysis of FP compared with marker protein cytochrome C (Cyto-c; 11,700), chymotrypsinogen (CHYMO; 25,700), aldolase (ALDO; 40,000), bovine serum albumin (BSA; 68,000) and RGG (150,000). In presence of SDS with or without 2-mercaptoethanol, FP has monomer subunit size of $\approx 30,000$ mol wt.

TABLE III
Amino Acid Compositions of FP and Two CRP

	FP* (preparation 1)	FP* (preparation 2)	CRP*‡ (rabbit)	CRP.§ (human)
Asx	19.0	19.0	19.1	15
Thr	9.8	9.1	8.7	12
Ser	16.2	11.9	19.2	18
Glx	26.1	28.4	19.5	20
Pro	9.8	10.7	10.1	11
Gly	17.9	19.1	17.6	14
Ala	10.9	9.3	10.3	9
Cys	ND	ND	ND	2
Val	10.9	15.0	13.4	17
Met	2.6	2.2	3.8	2
Ile	8.2	8.4	8.4	9
Leu	15.0	15.4	16.7	14
Tyr	9.6	9.5	9.2	7
Phe	9.3	8.7	11.7	12
His	4.2	3.9	2.9	2
Lys	13.3	11.3	13.1	12
Arg	7.2	7.9	6.1	6
Trp	ND	ND	ND	5

* Composition based on number of residues/190 total residues.

‡ From reference (30).

§ Composition from complete sequence (29).

|| Not determined.

The subunit molecular weight also was calculated from high speed sedimentation equilibrium runs in 5 M guanidine-HCl. A value of 30,000 mol wt was obtained and did not change as the initial protein concentration was doubled (30,000 mol wt at 0.70 mg/ml) or when 1.0 mM DTT was included in the solvent system (28,200 mol wt). The above results then are consistent with a model of the native molecule consisting of a pentamer with five 30,000-mol wt subunits noncovalently assembled to form the parent molecule of 150,000 mol wt.

Amino Acid Composition and Sequence Analysis. Table III shows the amino acid compositions for two preparations of FP compared with the published composition for human (29) and rabbit (30) CRP. A significant similarity can be seen.

TABLE IV
NH₂-Terminal Amino Acid Sequence of FP Compared with Those of Amyloid P Component, Human GRP, and Rabbit GRP

Refer- ence	Position number																										
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	
FP	—	PCA	X	D	L	S	G	K	V	F	V	F	P	R	Q	S	E	T	D	Y	V	N	L	I	X	X	L
Clt	(31)	H	T	—	—	—	—	—	—	—	—	—	E	—	V	—	H	—	—	—	—	—	—	—	T	P	—
AP component	(32)	[]	X	—	—	—	—	—	—	—	—	—	E	—	V	—	Y	—	H	—	—	—	—	—	T	P	—
Human GRP	(29)	—	T	—	M	—	R	—	A	—	—	—	K	E	—	D	—	S	—	—	S	—	K	A	P	—	—
Rabbit GRP	(31)	[]	A	G	V	M	H	K	—	A	—	—	K	E	—	D	B	S	—	S	—	S	—	B	—	G	—

X, no identification; [], deletion (no residue present); line indicates homology to top sequence; Two letters (e.g., H^Y) indicate mixed sequence found at that step.
 A = Ala; B = Asx; D = Asp; E = Glu; F = Phe; G = Gly; H = His; I = Ile; K = Lys; L = Leu; M = Met; N = Asn; P = Pro; Q = Gln; R = Arg; S = Ser; T = Thr;
 V = Val; Y = Tyr.

Two attempts to sequence unmodified FP yielded no PTH derivatives, indicating that the protein was blocked. Enzymatic deblocking with pyroglutamate amino peptidase permitted the determination of 23 of the first 26 residues from the NH₂ terminus. Table IV shows the FP sequence compared to the published amino terminal sequences of C1t (31), amyloid P component (32), human CRP (29) and rabbit CRP (31) (C1t is now known to be amyloid P component [33]). The homology of FP and amyloid P component is apparent, as their sequences were identical except at the very beginning and at residues 14, 16, and 19. Thus, of 23 hamster FP residues, 19 sequences were identical to amyloid P component (83%). The homology between amyloid P component and CRP has been previously established with identity at $\approx 50\%$ of NH₂-terminal residues (31, 32).

Discussion

Hamster FP is a model example of a sex-limited serum protein, as it is expressed prominently in the female and testosterone suppressed in the male. However, the binding specificity, morphology, and structure of FP were found to be remarkably similar to CRP and SAP, two related proteins that are normally present in low serum concentration and not known to be under sex-hormone control. Human CRP is the classic acute-phase reactant, which can nonspecifically increase almost 1000-fold in serum level after a variety of inflammatory stimulæ. Yet, CRP does react in a specific, Ca⁺⁺-dependent fashion with C-polysaccharide of pneumococcus, a characteristic which led to the discovery of CRP by Tillet and Francis in 1930 (34). Subsequently, numerous investigations have defined the structure of CRP (29, 35), its binding properties (24, 36), its synthesis and kinetics (37-39), and have demonstrated related proteins in other animals (26, 40). Although the biological function of CRP remains an enigma, numerous biological activities have been shown including activation of complement (41, 42), interaction with T lymphocytes (43) and platelets (44), and enhancement of phagocytosis (45). Another related protein, called amyloid P component (31, 32), is found in all forms of human amyloid (46). SAP component is found in microgram amounts in normal human and mouse serum, but only in mice does SAP increase as a typical acute-phase reactant after inflammatory stimulæ (47). CRP and SAP both have a unique polygonal structure by electron microscopy, which prompted their designation as pentraxins (31), and comparison of amino-terminal primary structures has established their homologous relationship (31, 32).

FP of Syrian hamsters is another member of this interesting family of pentraxins, which actually represents an unusual protein configuration (48). The electron micrographs indicate that in both morphology and dimensions, FP is very similar to CRP and SAP. The pentameric outline indicates the presence of five subunits, which was confirmed by the fact that the molecular weight (150,000) is five times the subunit molecular weight (30,000). Thus, the noncovalent assemblage of FP subunits is similar to CRP (35) and SAP (32), although the subunit size of FP is somewhat larger than CRP and SAP (23,000). Also, the parent molecule of FP (150,000) is larger than CRP (120,000-140,000) but smaller than SAP (9.5S), which characteristically forms doublets of pentamers (49).

FP binds to both PC and agarose (Sephacrose) in a calcium-dependent fashion and thus displays the Ca⁺⁺-dependent-binding specificities previously described for CRP (PC-binding specificity) (24, 25) and SAP (agarose-binding specificity) (50). The 15

times greater elution yields of FP after binding to PC-Sepharose vs. plain Sepharose would indicate a greater affinity to PC ligand than to agarose. The elution by free PC of pure FP bound to either PC or agarose would indicate a predominant specificity for the PC ligand. CRP reacts with the cell membrane components lecithin and sphingomyelin (42) and the capacity of CRP to affect various cellular functions may be directed toward these choline phosphatides. Whether FP alters cellular functions in a fashion similar to CRP is currently under investigation.

FP resembles CRP and SAP in the noncovalent binding of its monomer subunits. In fact, this observation was the initial clue that this sex-limited protein of Syrian hamsters might be related to such a disparate family of pentraxins such as CRP and SAP. The amino-terminal sequence data show a clear homology between FP and SAP in which 19 of 23 identifiable residues (83%) were identical. Larger differences were seen between FP and two CRP, but SAP and CRP have been shown to be homologous (31, 32). Thus, CRP, SAP, and FP appear to have evolved from the same ancestral gene, although FP superficially appears to have little in common with the other two acute-phase proteins primarily because (a) FP is such a conspicuous serum protein (≈ 2 mg/ml) in NFHS, and (b) the synthesis or at least serum levels of FP are under hormonal control (testosterone suppression). Studies in humans have shown no apparent difference of CRP levels between sexes, although SAP levels were somewhat higher in normal males (43 μ g/ml) vs. females (33 μ g/ml) (51). In the mouse, where SAP is an acute-phase reactant, comparable levels were detected in stimulated male and female mice (47). Hamster FP does have a semblance of acute-phase reactivity, as male hamsters (but not females) do manifest a moderate increase of FP after nonspecific inflammatory stimulæ ([1]; and our unpublished observations).

The abundance of FP in female hamsters suggests that this pentraxin has especial functional significance in this species, and was evolved for a peculiar biological requirement. If so, elucidating the biological role of FP in hamsters may provide insight into the function of pentraxins in other species. The high levels of FP in females will be particularly interesting if FP does have the biological activities of CRP, such as complement fixation and/or lymphocyte and platelet modulation. If so, the normal female Syrian hamster would represent an animal in constant acute-phase activation and should normally demonstrate the biological benefits/detriments that acute-phase reactants confer to man and the mouse. In this case, one should expect obvious sex differences in biological responsiveness to inflammatory disease when comparing female and male Syrian hamsters. To our knowledge, such differences have not been described. Similarly, sex differences in amyloid accumulation in hamsters has not been published, although the similarity of FP to SAP would make it a likely candidate for incorporation into hamster amyloid. In hamsters, this gene apparently came under hormonal control in a common ancestor of the Syrian (*Mesocricetus auratus*) and Kurdistan (*Mesocricetus brandti*) hamster, as both species have similar FP, whereas it has not been detected in normal serum of Chinese, Armenian, European, and Djzungarian hamsters (52). Perhaps FP is present in low concentration in these latter hamsters and acts in a more typical acute-phase pattern. In Syrian hamsters, FP appears to be the sole expression of the ancestral CRP gene, as other CRP-SAP-like proteins so far have not been detected in acutely stressed female or male hamsters (our unpublished observations).

For approximately 50 years, the functional significance of CRP has eluded inves-

tigators although a wide variety of biological effects has been discovered and another homologous protein (SAP) has been defined. The present report indicates the existence of other CRP-SAP homologs which superficially bear little resemblance to these acute-phase reactants and suggests that a wide diversity of proteins may be found within this family.

Summary

Female protein (FP), a serum protein present in normal female hamsters was found to be similar to acute-phase reactant, C-reactive protein (CRP) and serum amyloid P component (SAP) in the following ways: (a) hamster FP complexed with phosphorylcholine (PC) in a Ca^{++} -dependent fashion as shown by its isolation from serum by affinity chromatography with PC-Sepharose and selective elution with free PC or EDTA; (b) electron microscopy of FP indicated a pentameric structure similar in size and appearance to other pentraxins; (c) the parent molecule of FP (150,000 mol wt) was composed of five noncovalently assembled subunits of 30,000 mol wt; and (d) the amino acid analysis and terminal NH_2 sequence of FP clearly showed homology with SAP-CRP. Although FP evolved from an ancestral gene common to SAP and CRP, and shares functional, morphological and structural properties with these acute-phase proteins, the biological homology of FP appears quite diverse as this protein is a prominent serum constituent (1–2 mg/ml) of normal female hamsters and under hormonal control (testosterone suppression) in males.

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